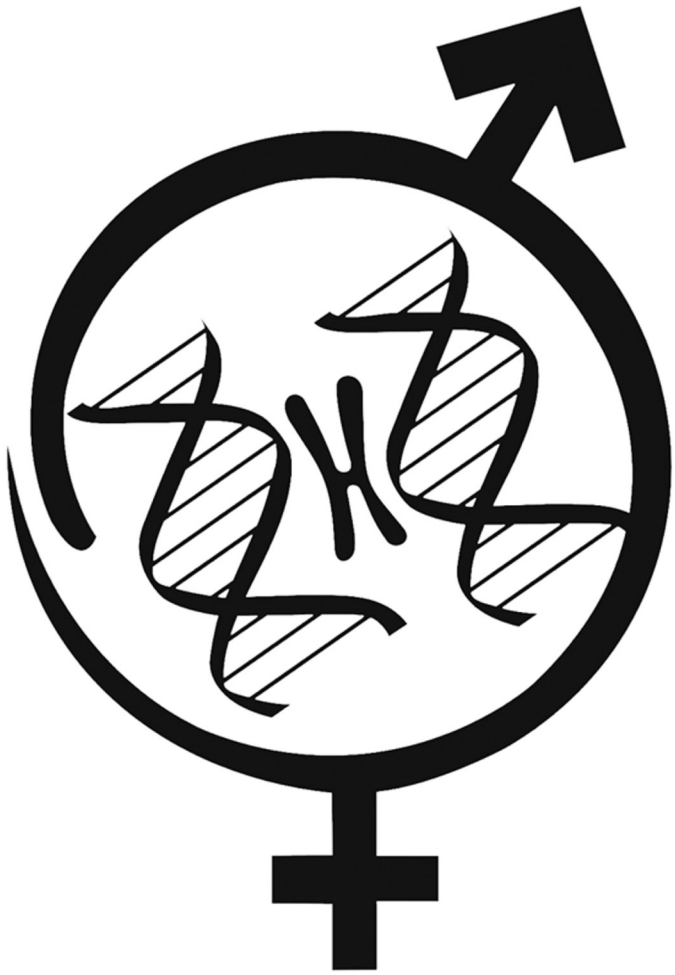
Abstracts of

Royan International Hybrid Twin Congress

27th Hybrid Congress on Reproductive Biomedicine
2-3 September 2026

21st Seminar on Nursing and Midwifery
2-3 September 2026



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Abstracts of the
27th Hybrid Congress on Reproductive Biomedicine
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Contents

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Roohbani Sh.
Sabbaghiyan M.
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Shajarehpour L.
Shakeri Y.
Shirvanizadeh F.
Taheri H.
Zarbaksh M.
Zarezadeh N.

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Azam Dalman

Dear Colleagues, Esteemed Professors, and Researchers,
On behalf of the Scientific Committee, it is my great honor to welcome you to the **27th International Congress on Reproductive Biomedicine**, which will be held from **September 2-3, 2026 in Tehran, Iran.**
Given the rapid advancements in fields such as female and male infertility, Assisted Reproductive Technologies (ART), reproductive genetics, and embryology, the need for scientific synergy and knowledge exchange among specialists is more essential than ever. To this end, the Scientific Committee is dedicated to providing a dynamic platform for sharing the latest achievements.
Our comprehensive scientific program will feature a diverse range of key topics and specialized sessions, including:
• Novel Technologies in ART
• Male Infertility: Basic and Clinical Aspects
• Cell and Tissue Engineering in Reproductive Biology
• Reproductive Genetics
• Endometrial Disorder & Ovulatory Dysfunction
• Maternal and Perinatal Outcomes after Assisted Reproductive Technology (Panel Discussion)
• Animal Biotechnology
To facilitate maximum participation from specialists worldwide, this congress will be held in a hybrid format (both in-person and virtual). We cordially invite all gynecologists, urologists, embryologists, geneticists, and researchers to enrich this outstanding event by submitting their valuable research papers and actively participating in our scientific community.
We eagerly look forward to welcoming you, whether in person or online.

Best regards,
Azam Dalman, Ph.D. Chairperson
27th Royan International Congress on Reproductive Biomedicine, 2026

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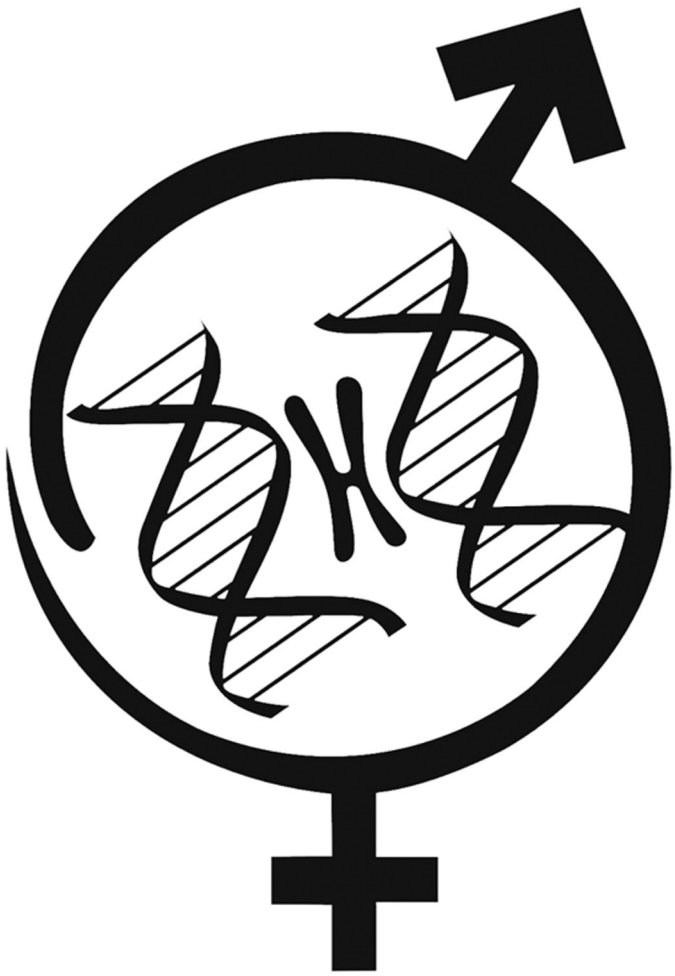

Royan Institute

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Invited Speakers

I-1: Role of the Testicular Cells Metabolome on *In vitro* Gametogenesis

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There are evidences of the role of the metabolic co-dependence of the germ cells and their neighboring nursing cells during their development inside gonads. Moreover, several studies in different species have indicated that these nursing cells in germ cells niche such as testicular cells, cumulus cells and granulosa cells secrete the factors in culture medium which has supportive effect on *in vitro* maturation (IVM), and also *in vitro* gametogenesis (IVG) using different sources of stem cells.
Recently we have reported that testicular cells conditioned medium play a critical role to support IVM in mice and IVG from human embryonic stem cells (hESCs) which confirmed previous studies using different cells source in different species.
Moreover, we have assessed the shift of metabolites' concentrations during days of differentiation of hESCs to germ cells induced by testicular cells conditioned medium. This new finding can pave novel insights on role of metabolites on gamete development and help infertility treatments in the future.

I-2: Application of Imaging Tools in Diagnosis of Endometrial Disorders in Infertile Women

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Endometrial assessment is an integral component of the imaging evaluation of women with infertility, as abnormalities of the endometrium and uterine cavity may adversely affect implantation and reproductive outcomes. Transvaginal ultrasonography (TVUS) represents the first-line imaging modality, providing assessment of endometrial thickness, echogenicity, morphology, and associated myometrial abnormalities. Three-dimensional transvaginal ultrasonography (3D-TVUS) offers multiplanar and coronal visualization of the uterine cavity, enabling more comprehensive evaluation of endometrial contour and subtle structural abnormalities. Saline infusion sonohysterography (SIS) further enhances delineation of intracavitary pathology and can distinguish focal lesions from diffuse endometrial abnormalities. The major abnormalities encountered include endometrial polyps, submucosal leiomyomas, intrauterine adhesions, endometrial hyperplasia, and congenital uterine anomalies. Endometrial polyps and submucosal leiomyomas are particularly relevant because of their potential to distort the cavity and interfere with implantation. Intrauterine adhesions may result in partial or complete cavity obliteration and impaired endometrial regeneration. Endometrial hyperplasia represents an important diffuse endometrial abnormality requiring appropriate characterization and, when indicated, histopathological correlation. Accurate characterization of these abnormalities is essential for differentiating clinically relevant pathology from incidental findings and for guiding further evaluation, including hysteroscopy. A systematic imaging-based approach can therefore improve the detection of potentially correctable uterine factors contributing to infertility and facilitate individualized management. This presentation reviews the current role of ultrasound-based imaging in the assessment of the endometrium, with emphasis on imaging techniques, characteristic findings, and their clinical implications in infertile women.

I-3: Treatments in hypogonadotropic hypogonadism

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Male hypogonadism is a clinical syndrome which is associated with decreased testicular function and production of androgens and/or impaired sperm production. This may be caused by impaired testicular function (hypergonadotropic hypogonadism or primary hypogonadism) or because of inadequate stimulation of the testes by the hypothalamic-pituitary axis (hypogonadotropic hypogonadism or secondary hypogonadism). In patients with secondary hypogonadism, both fertility and testosterone normalisation can be theoretically achieved with adequate treatment. Pre-Pubertal-Onset: As for the type of gonadotropin treatment, it is usual to commence hCG first and titrate the dose to achieve testosterone levels within the normal physiological range. However, FSH can be given first or in combination with hCG.
(EAU2026). Post-pubertal onset: hCG alone is usually required first to stimulate spermatogenesis. Semen analysis should be performed every three months to assess response, unless conception has taken place. If there is a failure of stimulation of spermatogenesis, FSH can be added. Combination therapy with FSH and hCG can be administered from the start of treatment (EAU2026). Treatment with Pulsatile GnRH is not currently approved in the U.S. or Europe. (AUA/ASRM2024). Investigational Treatment include: Kisspeptin, Neurokinin B (NKB), KISS1R agonists, NKB / NK3R modulation, Gene therapy
and Growth Hormone (GH). Kisspeptin and neurokinin B (NKB) play a key role in several physiological processes including in puberty, adult reproductive function including the menstrual cycle, as well as mediating the symptoms of menopause. Infundibular kisspeptin neurons, which coexpress NKB, regulate the activity of gonadotropin-releasing hormone (GnRH) neurons and thus the physiological pulsatile secretion of GnRH from the hypothalamus. kisspeptin is located upstream of GnRH in the HPG axis. Consequently, it may theoretically represent a highly physiological approach for the treatment of certain forms of hypogonadotropic hypogonadism (HH) in the future. Kisspeptin → GnRH neuron Stimulation → pulsatile GnRH → LH/FSH → Testicular function. Growth hormone (GH) therapy currently has no role in the treatment of hypogonadotropic hypogonadism. GH has a role only when the patient also has true growth hormone deficiency (GHD). Conclusion: For patients with hypogonadotropic hypogonadism, treatment with hCG, FSH, and a combination of hCG and FSH is currently accepted. Treatment with pulsatile GnRH is accepted in the EAU guideline but not in

the AUA/ASRM 2024 guideline.

I-4: Decoding the Zona Pellucida: Unimagined Features Revealed by Protein-Targeting Reverse Genetics

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Karl Ernst von Baer was the first to describe the zona pellucida surrounding human oocytes in 1827. Ever since has the zona been known as quintessential extracellular structure of mammalian oocytes. It is composed of glycoproteins (ZP1, ZP2, ZP3, ZP4 in humans; ZP1, ZP2, ZP3 in mice) that are synthesized only during oogenesis and compartmentalized into the secretory pathway. Once secreted and assembled around the oocyte, ZP proteins are tasked with encasing the oocyte in the ovarian follicle, mediating sperm-oocyte interaction while avoiding polyspermy, and preventing premature contact between the embryo and the oviductal epithelium after fertilization. However, sometimes it takes a second look and with different techniques to realize that not all was as it first seemed. It all began when we detected ZP2 and ZP3 proteins in blastocyst-stage mouse embryos, by mass spectrometry, even though we had previously removed the zona with acidic Tyrode's solution (PMID 31527703). We confirmed the observation with the orthogonal method of immunofluorescence, using monoclonal antibodies that have been proven to be absolutely specific for ZP proteins for the past 40 years. Moreover, the intra-embryonic ZP2 and ZP3 proteins were detected not only in the secretory pathway, but also in the cytosol, based on non-colocalization with markers of the endoplasmic reticulum and Golgi apparatus (PMID 37930049). This cytosolic location is consistent with that of a ZP3 variant described in human ovarian tumors, unable to be secreted as it lacks the signal peptide (‘ZP3 cancer’; PMID 38125942). To shed light on the functional significance of intra-embryonic ZP2 and ZP3 we performed protein knockdown using the method of ‘Trim-Away’ (PMID 29153837) either on its own or in combination with morpholino (PMID 14654215). The former degrades the protein of interest via the proteasome pathway, while the latter prevents mRNA translation and thereby addresses a possible concern of protein replenishment. Off-target effects were discounted by confirming that the monoclonal antibodies produced a single band in immunoblotting of oocyte lysates, and that the morpholinos did not bind anything but Zp mRNA, according to BLAST search. The knockdowns were partial, probably because of the considerable amount of substrate, and because only a fraction of the total is cytosolic and thereby accessible for 'Trim-Away'. Knockdown of ZP3 resulted in zygotic arrest (PMID 37930049). Knockdown of ZP2 resulted in mouse embryo retardation at the morula stage; and resultant blastocysts presented a reduction in the size of the extra-embryonic compartments (trophectoderm, primitive endoderm) as well as reduction in the rates of post-implantation development (https://www.biorxiv.org/content/10.64898/2025.12.12.692802v1). These phenotypes were not observed upon targeting of an irrelevant protein or mRNA sequence (GFP mock control). Transcriptome (RNA-seq) and proteome (mass spec) analysis of the ZP2 and ZP3 knockdown embryos compared to mock controls hinted at the possible underlying mechanisms: failure of zygotes to regulate genes involved in embryonic genome activation, in the case of ZP3, and failure to regulate genes involved in morula-to-blastocyst transition, in the case of ZP2. Taken together, these findings call into question the traditional model that ZP proteins function solely in the extracellular space and accompany embryogenesis as passive bystanders: on the contrary, ZP proteins seem to participate actively in the intracellular processes of early embryogenesis. Future research will need to clarify how ZP protein can possibly fold outside the secretory compartment, and whether our observations in mice hold true also in humans.

I-5: PEGylated Fibrin for Engineered Ovary Fabrication: Advancing Fertility Preservation in Cancer Patients

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Advances in cancer treatment have significantly improved survival rates among young female patients. However, the preservation of fertility remains a major clinical challenge. While ovarian tissue cryopreservation followed by transplantation is an established strategy for fertility preservation in cancer patients, it is not recommended for patients with malignancies associated with a high risk of ovarian metastasis due to the potential reintroduction of malignant cells. For these patients, the development of a transplantable engineered ovary represents a promising alternative strategy. A critical component of engineered ovary fabrication is the selection of a biomaterial capable of recreating the ovarian microenvironment while supporting follicle survival, growth, and function. Among the biomaterials investigated, fibrin has emerged as one of the most widely used matrices because of its biocompatibility and intrinsic bioactivity. However, its rapid degradation and limited mechanical stability compromise long-term structural support for encapsulated ovarian cells and follicles, restricting its translational potential. To address these limitations, our research has focused on the development of PEGylated fibrin hydrogels as a next-generation biomaterial for ovarian tissue engineering. In an initial study, we demonstrated that PEGylation significantly enhanced fibrin stability while maintaining a supportive environment for human ovarian stromal cells, improving cell viability, proliferation, and scaffold stability *in vitro*. Building upon these findings, we subsequently employed a biomechanical design strategy to tailor the hydrogel formulation to mimic the Young’s modulus of the human ovarian cortex at reproductive age. This mechanically tailored PEGylated fibrin matrix successfully supported the survival and growth of isolated human preantral follicles, preserved follicular architecture, and maintained essential cell–cell communications. More recently, we have extended this work by investigating the *in vivo* performance of PEGylated fibrin–based ovarian constructs. Preliminary findings indicate that PEGylated fibrin constructs are a biocompatible and supportive platform for the *in vivo* grafting of human ovarian stromal cells. The implanted grafts induced a controlled foreign body response, with limited fibrosis, moderate immune cell infiltration, low late apoptosis, and preservation of stromal cell viability during the first week after transplantation. Overall, these studies demonstrate the potential of PEGylated fibrin as a biomimetic platform for engineered ovary fabrication. By improving scaffold stability, reproducing the biomechanical properties of the ovarian cortex, and supporting both *in vitro* follicle development and early *in vivo* graft

performance, this strategy represents an important step toward the clinical translation of transplantable engineered ovaries for fertility preservation in cancer patients who are not candidates for ovarian tissue transplantation.

I-6: Premature Ovarian Insufficiency: Diagnosis, Causes and Management

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Premature ovarian insufficiency (POI) known as a significant challenge in women's health, that defined as the cessation of normal ovarian function before the age of 40. Diagnosis of POI is based on the presence of spontaneous amenorrhea or irregular menstrual cycles for at least 4 months combined with an elevated Follicle Stimulating Hormone (FSH) concentration greater than 25 IU/L. A single FSH assessment combined with the characteristic clinical picture is now considered sufficient for POI diagnosis. Recent studies show a higher prevalence of POI compared to previous studies and emphasize that POI is not a rare phenomenon, affecting approximately 3.5 to 3.7 percent of women. The etiology of POI is heterogeneous and multifactorial, although the underlying cause remains unknown in approximately 75-90% of cases. The etiology of POI is particularly important to determine the individual and their relative risk.
Beyond immediate symptoms, the resulting estrogen deficiency poses long-term health risks, including emotional distress, increased susceptibility to cardiovascular disease, osteoporosis, and reduced life expectancy.
Clinical management of POI is shifting toward a multidisciplinary and patient-centered approach that prioritizes early diagnosis, psychological support, early fertility counseling and standardized Hormone Therapy. HT is confirmed as the gold standard for protecting cardiovascular, bone, and cognitive health until the natural age of menopause. Some experimental interventions like mesenchymal stem cell (MSC)-derived exosomes and ovarian PRP therapy, show promise in restoring ovarian function in preclinical models but remain outside standard clinical recommendations.

I-7: 3D MicroPhysilogical Systems in Reproduction Microfluidic Systems for Cell Biology and Translation into Practice

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Microfluidics has emerged as a transformative technology at the interface of biology and medicine, enabling precise manipulation of cells and fluids at physiologically relevant scales and unlocking new opportunities ranging from single-cell analysis to complex multicellular systems. In this seminar, I will discuss the remarkable role of microfluidic technologies in advancing biomedical discovery and their translation into clinically relevant applications, particularly in cancer liquid biopsy and assisted reproduction. In the context of oncology, I will highlight our work on label-free microfluidic platforms for the enrichment and analysis of circulating tumor cells (CTCs), enabling non-invasive cancer diagnostics, real-time disease monitoring, and improved understanding of metastasis. In the realm of assisted reproductive technologies (ART), I will highlight our recent work on the development of novel biomimetic technologies for sperm selection. To complement these advances, we have developed and clinically validated AI-powered sperm identification and tracking tools, including SpermSearchAI, trained on thousands of clinical cases to support real-time selection of viable sperm from challenging surgical samples such as mTESE. Overall, this seminar will demonstrate how the convergence of microfluidics and AI is reshaping modern medicine, bridging fundamental science with impactful clinical translation in both cancer diagnostics and reproductive biology.

I-8: Chronic Endometritis: Hysteroscopic vs. Biopsy-Based Diagnosis in Recurrent Implantation Failure

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Endometrial receptivity abnormalities are known as one of the possible causes of recurrent implantation failure (RIF) in IVF. Chronic endometritis (CE) is an important factor of uterine receptivity observed up to half of patients with repeated implantation failure (RIF) in IVF. It is an unusual inflammatory condition characterized by endometrial plasmacyte infiltration traditionally not been a concern in clinical practice because it is usually asymptomatic or seldom manifests with subtle symptoms such as abnormal uterine bleeding, pelvic pain, dyspareunia, and leucorrhea (CE). Confirming CE resolution before proceeding with IVF and CE therapy may improve IVF outcome in patients suffering from RIF. The combination of hysteroscopy and histopathology was used to diagnose CE in these patients. New hysteroscopic scoring systems along with novel techniques and methods has improved diagnostic sensitivity and specificity of CE. New evidences explore that antibiotic treatment may improve the pregnancy outcomes of RIF patients in subsequent IVF cycles if the condition of CE is confirmed cured in a control biopsy afterwards. Therefore, the association between CE and adverse reproductive outcomes stresses the necessity for comprehensive diagnostic and therapeutic strategies to optimize fertility outcomes.

I-9: Implementing the ICMART Registry Framework at Royan Institute: A Step Toward a National ART Registry in Iran

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The establishment of standardized assisted reproductive technology (ART) registries has become a global priority for monitoring clinical outcomes, improving quality of care, enhancing

patient safety, and supporting evidence-based healthcare policy. By using uniform definitions and harmonized data collection methods, ART registries enable benchmarking among fertility centers, facilitate national and international comparisons, and provide reliable evidence for clinical research and health planning. The International Committee for Monitoring Assisted Reproductive Technologies (ICMART) has developed internationally accepted terminology, reporting standards, and data collection tools that form the foundation of global ART surveillance. This lecture introduces the ICMART registry framework and its key components, including the International Glossary on Infertility and Fertility Care, the ICMART Toolbox, the structure of national and regional registries, and the principles underlying the ICMART World Reports. The presentation highlights how standardized definitions and reporting methodologies contribute to the generation of reliable, comparable, and clinically meaningful ART data across countries. Rather than presenting institutional outcome data, this lecture introduces Royan Institute's initiative to implement an ICMART-based registry framework as a practical first step toward establishing a national ART registry in Iran. The presentation aims to demonstrate the feasibility of adopting internationally standardized terminology and data collection methods within the Iranian ART community and to encourage collaboration among fertility centers in developing a unified national registry. Such a registry would provide a robust infrastructure for quality improvement, multicenter research, healthcare planning, and future participation in global ART monitoring through ICMART.

I-10: How Does Genetic Abnormality Could Cause Reproductive Problem?

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Human infertility, in both male and female, could be result from a heterogeneous genetic basis include chromosomal abnormalities to single-gene and epigenetic changes. Studies from the Abortion research center in Yazd Reproductive Sciences Institute show these genetic variants disrupt stages of gametogenesis. In male infertility, exome/genome sequencing in consanguineous families with oligoasthenotratozoospermia and non-obstructive azoospermia (NOA) identified candidate variants involving multiple functional pathways, sperm flagellar structure (DNAH family genes, AKAP4, CFAP45), ribosomal translation, ubiquitination and protein stability (RNF220), and DNA repair/homologous recombination (RAD54L), along with transcriptional regulators of meiosis and spermiogenesis (SOX30), suggesting that disruption of any stage of spermatogenesis can independently cause NOA. In female infertility, exome sequencing in several families with primary ovarian insufficiency (POI) showed positive findings with high yield, including novel oligogenic contributions from genes in the synaptonemal complex (SYCP1) and germ cell fate (NANOS2), along with variants affecting follicular survival (BNC1), granulosa cell/oocyte pathways (ALOX12B, BMP15), and GnRH signaling (WDR11), with recessive, dominant, and X-linked inheritance patterns. Beyond coding variants, alterations in the microRNAs miR-146a and miR-196a2 were studied in idiopathic recurrent miscarriage, demonstrating how dysregulation can compromise implantation without chromosomal structural disease. These findings demonstrate a spectrum of genetic abnormality, from chromosomal rearrangements to single-gene and oligogenic meiotic/structural defects to microRNA level alterations, that disrupt spermatogenesis, oogenesis, embryo, fetal and ovarian reserve (POI, puberty arrest) and pregnancy maintenance, supporting integrated exome/genome-based genetic screening in cases of idiopathic and familial infertility.

Keywords: Reproductive Problem, Whole Exome Sequencing, Azoospermia, Oligo asthenotratozoospermia, Primary Ovarian Insufficiency, MicroRNA

I-11: Exploring the role of the maternal-effect Padi6 gene in early mouse embryogenesis.

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The maternal-effect protein PADI6, which is part of the subcortical maternal complex, is involved in proper spindle assembly, organelle distribution, ribosome storage, and cytoplasmic lattice organization in mouse oocytes. In humans, variants of PADI6 are associated with female infertility and multilocus imprinting disturbance in offspring. Recently, it was demonstrated that PADI6 plays a role in the storage and cytoplasmic localization of epigenetic factors, including UHRF1 and DNMT1. Moreover, maternal PADI6 depletion leads to defective epigenetic reprogramming and zygotic genome activation but not to an imprinting defect in two-cell mouse embryos. By employing combined single-blastocyst RNA-seq/BS-seq and immunostaining validation in the embryos derived from Padi6P620A-mutant oocytes, we investigated the role of Padi6 in late preimplantation development. We demonstrated that embryos that overcame the two-cell stage block had a dramatic reduction in UHRF1 and DNMT1 protein levels, and whole-genome hypomethylation, including most imprinted loci and repetitive elements, at the blastocyst stage. Furthermore, these maternal mutant embryos showed deregulation of inner cell mass markers and defective blastocyst implantation, but no effect on trophoblast differentiation.
Our results demonstrate that maternal PADI6 is a key regulator of epigenetic factors required to maintain repressive marks in late preimplantation mouse embryos. Its deficiency results in genomic imprinting defects that closely resemble those found in human patients and provide a mechanistic explanation for MLID caused by maternal PADI6 variants. Furthermore, the impairment of blastocyst implantation capacity, likely due to dysregulation of inner cell mass differentiation, provides new mechanistic insights into the control of female fertility and embryo development exerted by PADI6.

I-12: Management of Poor Ovarian Response: Pharmacologic and Non-Pharmacologic Options Antagonist vs Agonist, Adjuvants

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Poor ovarian response (POR) has long been considered one of the most challenging scenarios in assisted reproductive medicine. However, the concept of POR has evolved from a primarily quantitative definition toward a more individualized assessment of reproductive prognosis. The Bologna criteria provided an important step toward standardizing the diagnosis of POR, while the POSEIDON classification subsequently introduced a more clinically relevant framework by incorporating patient age, ovarian reserve, and ovarian response to stimulation.
This presentation will explore how this evolution has influenced the management of patients with low prognosis undergoing IVF. We will first examine what we currently know about ovarian response and the limitations of conventional definitions, before discussing the transition from Bologna to POSEIDON and its implications for treatment selection.
The evidence behind commonly used strategies will then be critically reviewed, including pretreatment approaches, pituitary suppression, controlled ovarian stimulation protocols, gonadotropin dosing, the potential role of LH activity, and different approaches to final oocyte maturation and triggering.
Finally, we will address the growing use of supplements and adjunctive therapies, including androgens, DHEA, growth hormone, and Coenzyme Q10. Rather than focusing solely on increasing the number of oocytes retrieved, the discussion will emphasize whether these interventions translate into meaningful improvements in embryo competence and cumulative reproductive outcomes

I-13: Advancing Ovarian Tissue Purging with Nanophotodynamic Therapy

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Despite remarkable improvements in the survival of children with leukemia, fertility preservation remains a major clinical challenge. In 2022, an estimated 64,600 children younger than 15 years were newly diagnosed with leukemia worldwide, making leukemia the most common childhood cancer. Ovarian tissue cryopreservation is currently the only established option for preserving fertility in prepubertal girls undergoing gonadotoxic cancer treatment. However, transplantation of cryopreserved ovarian tissue is contraindicated in leukemia patients because of the risk of reintroducing residual malignant cells that may have infiltrated the ovarian tissue before its collection. To overcome this limitation, we developed a nanophotodynamic therapy (NanoPDT)-based approach for the *ex vivo* purging of leukemic cells from ovarian tissue before transplantation. By integrating photodynamic therapy with nanotechnology, we have designed and optimized a selective purging strategy targeting acute myeloid leukemia cells while preserving the structural integrity, viability, and developmental potential of ovarian follicles. Our work focuses on identifying treatment conditions that maximize leukemic cell elimination without compromising ovarian tissue quality. Because human ovarian biopsies are exceptionally scarce and precious, yet numerous questions remain regarding treatment optimization, safety, long-term tissue function, and biological responses, we have in parallel developed 3D tumor models as a complementary experimental platform. These models provide a reproducible system for investigating treatment efficacy while reducing reliance on limited patient-derived ovarian tissue. In parallel, our current research aims to elucidate the molecular mechanisms underlying the different forms of cell death induced by NanoPDT and to understand the basis of its remarkable selectivity toward leukemic cells while sparing healthy ovarian tissue. This approach represents a promising step toward enabling the safe transplantation of cryopreserved ovarian tissue in leukemia survivors, with the potential to expand fertility restoration options for young patients at high risk of ovarian contamination.

I-14: Adenomyosis-Associated Endometrial Dysfunction: Impact on Implantation and IVF Live Births

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Uterine adenomyosis is a disorder in which endometrial glands and stroma are present within the myometrium, resulting in hypertrophy of the surrounding myometrium. Patients with symptomatic adenomyosis will often present with uterine enlargement, abnormal uterine bleeding (AUB), and painful menses. Adenomyosis may also coexist with uterine leiomyoma and/or endometriosis.
The diagnosis is now made by imaging-based criteria using transvaginal ultrasonography (TVUS) and/or magnetic resonance imaging (MRI) which has resulted in a greater understanding of the disease.
Adenomyosis may be associated with infertility; In a meta-analysis of 11 cohort studies including 2054 patients undergoing *in vitro* fertilization (IVF), those with adenomyosis (25 percent) versus without adenomyosis had lower rates of implantation, clinical pregnancy per cycle, clinical pregnancy per embryo transfer, ongoing pregnancy, and live birth. The type of adenomyosis (ie, focal or diffuse) may also impact fertility. Diffuse adenomyosis did not appear to be associated with infertility.

I-15: Reproductive Aging in Iran across Four Decades: From Population Trends to the Genetics of Early Menopause

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A quarter-century of prospective follow-up within the Tehran Lipid and Glucose Study (TLGS) cohort has unveiled a significant secular trend in reproductive aging, characterized by a progressive decline in the age of natural menopause (ANM) and a twofold increase in the prevalence of both early menopause and primary ovarian insufficiency (POI) over the past four decades. This shifting demographic landscape cannot be fully explained by constitutional factors alone; rather, it reflects a complex interplay of rapid socioeconomic transitions, altered lifestyle patterns, unique nutritional shifts, chronic environmental stressors, and cardiometabolic pressures that have characterized the re-

gion's recent history. Despite these prominent epidemiological shifts and their profound implications for postmenopausal cardiovascular and metabolic health, the underlying genetic architecture governing the rate of follicular depletion in Iranian women remains critically understudied. Furthermore, global reproductive genome-wide association studies (GWAS) continue to exhibit a profound ancestral bias toward European cohorts, leaving a crucial gap in our understanding of genetic susceptibility in Middle Eastern populations. Our GWAS study seeks to bridge this gap by investigating the genetic determinants of early menopause and ovarian reserve in Iranian women through a discovery cohort of 3,421 women (276 cases of early menopause vs. 3,145 controls). Candidate variants were validated in an independent confirmation cohort of 1,015 women stratified by ovarian reserve (208 poor vs. 807 normal). Over 9 million genetic variants were analyzed using Genome-wide Complex Trait Analysis (GCTA), followed by bioinformatic annotation and epigenomic evaluation. We identified a novel locus associated with early menopause: the rs9943588 variant, which significantly increased the risk of early menopause in the discovery cohort (OR=1.93; $P=2.54\times10^{-8}$). This association was replicated in the confirmation cohort, where the same variant was associated with a 35% increased risk of poor ovarian reserve (OR=1.35; P<0.0001). This intronic variant is located in the Polypeptide N-Acetylgalactosaminyltransferase 18 (GALNT18) gene on chromosome 11 and represents a novel finding in Iranian women. Epigenomic analysis suggested that rs9943588 potentially alters a regulatory motif for the ETS transcription factor family.

I-16: Reprogramming Life: The Role of Artificial Intelligence in Reproductive Technologies and Cellular Engineering

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Artificial intelligence is changing reproductive medicine by helping to combine complex data from embryology, clinical studies, molecular biology, and immunology. Besides assessing embryos and evaluating sperm and egg quality, AI can offer a better understanding of the maternal environment in patients with recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL). By spotting important patterns across immune and reproductive factors, AI can help provide more accurate patient grouping and tailored reproductive care. The combination of artificial intelligence and reproductive immunology may shift the focus from just selecting the "best" embryo to understanding how the embryo interacts with its maternal environment. This shift is an important step toward more personalized, evidence-based reproductive medicine.

I-17: Cytotoxicity Thresholds and Early Nanomechanical Biomarkers of Spermatozoal Injury Induced by a Panel of Chemical Agents

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Background: Nanomechanical characterization by atomic force microscopy (AFM) can reveal early, sensitive signatures of cell injury — changes in membrane stiffness and surface topology — that may precede conventional viability or motility loss, making it a promising tool for reproductive toxicology. However, AFM of spermatozoa has so far been restricted almost entirely to fixed or dried cells, since the continuous motility of live, functional spermatozoa precludes stable probe-based imaging; genuine, physiologically relevant nanomechanical signatures of toxicant-induced injury in live gametes have therefore never been captured. A further obstacle is that spermatozoa are a non-standard cell type — terminally differentiated, non-adherent, and short-lived *in vitro* (3–6 h) — for which cytotoxicity assays developed for adherent cell lines are not directly applicable. Aim: To establish the methodological foundation required for nanomechanical AFM analysis of live human spermatozoa, by (1) developing a method to immobilize live spermatozoa while preserving their viability and structural integrity, and (2) adapting and validating a suspension-compatible cytotoxicity assay to define sublethal exposure thresholds for a panel of model toxicants.
Materials and Methods: Cryopreserved spermatozoa from approximately 20 healthy donors were processed per WHO guidelines and selected for motility by centrifugation. GelMA-LAP (2.5–10%) and chemically crosslinked 4% HA-EGDE hydrogels were evaluated as substrates for centrifugal immobilization (300×g, 5 min) of live spermatozoa; viability was assessed by calcein-AM/PI staining. In parallel, the Alamar Blue (resazurin reduction) assay was optimized and validated for suspension cultures (3-h exposure; working concentration 5×10^6 cells/mL) and used to determine half-maximal inhibitory concentrations (IC_{50}) for eight agents with distinct mechanisms of action: Triton X- 100, SDS, H_2O_2, NaOCl, iodine, sodium azide, antimycin A, and acrylamide.
Result: Among the hydrogels, 4% HA-EGDE immobilized live spermatozoa while preserving viability comparable to gel-free controls (50.4% vs. 56.4%), outperforming all GelMA-LAP formulations (14.7–32.9%) — offering a viable route to stable AFM imaging of live, undamaged spermatozoa. In parallel, IC_{50} values for the eight agents spanned more than three orders of magnitude (0.153–568.9 mM), establishing a clear sensitivity hierarchy: detergents and oxidative agents (Triton X-100, iodine, SDS, H_2O_2, NaOCl) were the most potent, in the same range as the Complex III inhibitor antimycin A (0.15–2.5 mM), whereas the Complex IV inhibitor sodium azide required a nearly 150-fold higher concentration (289.7 mM), and acrylamide was the least potent agent tested (568.9 mM).
Conclusion: We established a hydrogel-based immobilization method that keeps live spermatozoa intact and viable, and validated a suspension-compatible cytotoxicity assay defining sublethal exposure thresholds for eight mechanistically distinct agents. Together, these results provide the methodological foundation — a viable immobilization platform and defined sublethal concentrations — required for our next step: nanomechanical characterization of toxicant-induced injury in live human spermatozoa by AFM.
Our research was supported by the Russian Science Foundation (RSF), grant No. 24-45-20007.
Keywords: Spermatozoa, Cytotoxicity, Alamar Blue, IC_{50}, Hydrogel Immobilization, Atomic Force Microscopy

I-18: Genetic Landscape of Premature Ovarian Insufficiency: Integrating Whole Exome Sequencing of an Iranian Cohort with Global Gene–Disease Evidence

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Premature ovarian insufficiency (POI) affects approximately 3.5% of women under 40 years and contributes substantially to female infertility. Although many cases remain idiopathic, accumulating evidence supports an important genetic contribution. Whole-exome sequencing (WES) has accelerated identification of POI-associated genes. However, gene–disease evidence varies considerably, highlighting the need to distinguish established genes from emerging candidates for reliable interpretation and clinically meaningful genetic testing.
We performed WES in 39 Iranian women with genetic susceptibility for idiopathic POI, and assessed variant pathogenicity according to ACMG criteria. We also critically evaluated genetic evidence from global high-throughput sequencing studies and developed an evidence-stratified gene panel. Gene–disease validity was assessed using an adapted ClinGen framework integrating genetic and experimental evidence.
WES identified 31 pathogenic/likely pathogenic variants in 23 patients of Iranian cohort. The implicated genes involved DNA repair, mitochondrial function, meiotic regulation, and follicular development, highlighting the complex genetic architecture of POI. In global evaluation, of 415 articles screened, 135 studies met the eligibility criteria (105 case reports and 30 cohorts). The 30 WES-based cohorts included 3,156 women and identified 413 genes: 20 with definitive, 28 with moderate, and 357 with limited evidence. The case reports independently identified 66 unique genes, 47 overlapping with the cohort-derived panel. In the Iranian cohort, BMP15, BRCA2, ERCC6, POLG, HFM1, and NR5A1 were classified as definitive, ZSWIM7 and ANKRD31 as moderate, and nine other genes as limited POI genes using our evidence-scoring framework.
Limited evidence does not necessarily exclude biological or clinical relevance, but warrants caution when attributing causality. Improved molecular diagnosis may facilitate genetic counseling, reproductive planning, and personalized management.
Keywords: Premature ovarian insufficiency, Iran, Whole-exome sequencing, Gene–disease relationship, Gene panel

I-19: One Carbon Metabolism and Oocyte Competence: From The Bovine Model to Translational Research

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In both humans and bovines, oocyte maturation and embryo development strictly depend of the performance of the one carbon metabolism (OCM), the pivotal biochemical pathway providing nucleic acids synthesis, methylation capacity for DNA and histones epigenetic programming and GSH and Hydrogen sulphide (H 2 S) to regulate the redox metabolism. The OCM, in turn, is directly regulated by the dietary feed of key micronutrients. We investigated the effect of micronutrient supplementation in the in-vitro manipulation of bovine oocytes to better understand how to maximize the benefit of such feed. In a first study [1] we used immature bovine oocyte-cumulus complexes for standard in vitro maturation compared to the add-on of 8 key micronutrients feeding the OCM. We used only physiologic substances, where possible in their pre-activated form (i.e. methylfolate and methylcobalamine), at concentrations calculated to replicate those expected after a dietary exposure. Supplemented oocytes exerted improved mitochondrial mass and DNMT3a protein expression and reduced DNA fragmentation with strongly increased DNA methylation in female pronucleus of zygotes, although maturation rate and fertilization rate were not different from controls. Surprisingly, the blastocyst rate of supplemented oocytes was double than controls reflecting the fingerprint of a 24-hour supplementation in the critical phase of final maturation. This prompted us to suggest the existence of an OCM metabolic check point that limits the progression of oocytes to blastocyst post-fertilization. However, two questions raised: 1) Does the same supplementation beyond oocyte maturation, i.e. during in-vitro culture, further improve the yield? 2) Is the increased number of blastocysts just a cosmetic effect pushing to the blastocyst stage oocytes that remain of reduced quality? To this aim we performed a second study [2] using the same metabolic enhancers to extend the supplementation to the embryo culture phase and to check the in-vivo developmental potential of blastocysts obtained after supplementation of the maturation phase in-vitro. The supplementation during IVM resulted again in a doubled blastocyst rate whereas extending the supplementation to the embryo culture did not improve the outcome. Thus, if the oocyte is not metabolically primed it cannot be rescued at time of embryo culture, i.e. we must feed the mothers. After single embryo transfer, the supplemented blastocysts showed a trend for increased pregnancy rate suggesting that they were at least as competent as non-supplemented ones and that the higher number of blastocysts would translate into a real expansion of the livestock. Bovine and human reproductive physiology, including oocyte aneuploidy and mosaicism rates, follicle sizes, single-ovulation tendencies, and preimplantation embryo mechanics, are very similar. Thus, bovine oocytes are also a proxy for the investigation of human reproduction. In human assisted reproduction technologies (ART) the main limit to blastocyst expansion is oocyte aneuploidy, which increases with the ageing of the mothers and has no known remedy. A defective OCM may impact the chromosome segregation in 2 ways: The lack or delayed methylation of the recombination hot spots and/or; The energetic deficit due to shortage of mitochondrial GSH resulting in mitochondrial dysfunction. Both defects may result in chromosome segregation errors and embryo aneuploidy. A translational study is now ongoing in women of advanced reproductive age supplemented by the same micronutrients for 3 months before stimulation for ART. Propensity score matching will be used to select non-supplemented controls running the procedures in the same period to compare their blastulation rates and aneuploidy rates. Pending the results from the translational study, beyond its role for livestock management, animal biotechnology further confirms the potential to provide invaluable inputs for human research.

I-20: Folate Form Matters: Rethinking Folic Acid and 5-Methyltetrahydrofolate in Oocyte and Embryo Development

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Folate-dependent one-carbon metabolism is essential for nucleotide synthesis, methylation reactions, homocysteine remethylation, redox homeostasis, and normal gamete and embryo development. Although folic acid (FA) supplementation has an established role in preventing folate-responsive developmental abnormalities, accumulating evidence suggests that reproductive outcomes may depend not only on folate availability, but also on folate form, dose, and metabolic capacity. Recent studies indicate that reduced folate availability particularly compromises embryonic development in the MTHFR 677TT background, while human pharmacokinetic data show greater bioavailability of 5-methyltetrahydrofolate (5-MTHF) than FA and less accumulation of unmetabolized folic acid.

Against this background, we investigated the differential effects of FA and 5-MTHF on mouse oocyte competence and embryo development in vitro and in vivo. Cumulus–oocyte complexes were exposed to increasing concentrations of FA or 5-MTHF during in vitro maturation, followed by assessment of meiotic maturation, spindle integrity, fertilization, and blastocyst development. In parallel, juvenile female mice received high-dose FA or 5-MTHF for five weeks before reproductive assessment.

High FA concentrations impaired oocyte maturation, spindle organization, fertilization, and blastocyst formation, whereas comparable 5-MTHF exposure did not reproduce these detrimental effects and improved several developmental outcomes. In vivo, excessive FA increased embryo resorption, while 5-MTHF improved implantation-related outcomes and fetal growth. Despite comparable circulating folate levels, high-dose FA markedly increased blood formaldehyde, a genotoxic one-carbon metabolite associated with redox disruption, whereas 5-MTHF reduced formaldehyde accumulation.

Together with emerging evidence on MTHFR-dependent folate requirements, unmetabolized FA, and differential folate bioavailability, these findings challenge the assumption that all folate forms are biologically equivalent in reproductive settings. They support a more precise approach to folate supplementation that considers dose, chemical form, metabolic genotype, and reproductive context. Further human studies are warranted to determine whether 5-MTHF offers specific advantages in preconception care and assisted reproduction.

Keywords: Folic Acid, 5-Methyltetrahydrofolate, One-Carbon Metabolism, Oocyte, Embryo Development, MTHFR, Formaldehyde, Reproductive Health

Oral Presentation

Andrology

O-1: Effect of Human Umbilical Cord Blood Serum on Sperm Quality Following Vitrification

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Background: Cryopreservation of human sperm is a fundamental technique in assisted reproductive technologies; however, optimizing cryoprotective media remains a challenge. This study aimed to develop a novel sperm vitrification medium supplemented with different concentrations of human umbilical cord blood serum (hUCBs) and to evaluate its effects on post-thaw sperm quality parameters.
Materials and Methods: This experimental study included semen samples from 20 normozoospermic healthy volunteers (20–35 years). Following initial evaluation according to WHO guidelines, samples were divided into five groups: control (standard vitrification medium) and experimental groups supplemented with 5%, 10%, 15%, or 20% human umbilical cord blood serum (hUCBs). Samples were vitrified using the direct drop method and thawed after one week. Post-thaw sperm motility, plasma membrane integrity, DNA integrity, and viability were evaluated using standard laboratory assays
Results: A progressive, dose-dependent improvement in post-thaw sperm quality parameters was observed with increasing concentrations of hUCBs. All experimental groups demonstrated improved motility, membrane integrity, DNA integrity, and viability compared to the control group. The most pronounced enhancement in all evaluated parameters was observed in the 20% hUCBs group, which showed the highest post-thaw sperm functional quality.
Conclusion: Supplementation of sperm vitrification medium with human umbilical cord blood serum significantly improves post-thaw sperm quality in a dose-dependent manner. The 20% concentration demonstrated the most effective results, suggesting that hUCBs may represent a promising additive for improving sperm cryopreservation outcomes in assisted reproductive technologies.
Keywords: Vitrification, Sperm, Umbilical Cord Blood Serum, Male infertility.

O-2: Plasma Rich in Growth Factors as a Supplement for Short-Term *In Vitro* Culture of Human Fetal Testicular Tissue

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Background: Fertility preservation for prepubertal boys is clinically complex, particularly because widely used xenogeneic supplements such as Knockout Serum Replacement (KSR) raise safety and translational concerns. Plasma Rich in Growth Factors (PRGF), a platelet-derived product, may provide a xeno-free alternative. This study aimed to determine whether PRGF can more effectively support short-term *in vitro* maintenance of human fetal testicular tissue compared with KSR
Materials and Methods: Immature testicular tissue was obtained from three 26-week-old human fetuses (n=3 donors). Tissue fragments were cultured for 15 days in media containing 5%, 10%, or 20% PRGF, while medium supplemented with 10% KSR served as the control. Testicular architecture was evaluated by Hematoxylin and Eosin (H&E) staining, and immunofluorescence was used to quantify marker expression for germ cells, Sertoli cells, steroidogenic activity, proliferation, and apoptosis. Data were analyzed using a mixed effects model followed by post hoc multiple comparisons, with statistical significance set at $P<0.05$.
Results: After 15 days of culture, supplementation with 5% PRGF preserved seminiferous tubule morphology more effectively than 10% KSR and higher PRGF concentrations ($P<0.001$). The 5% PRGF group showed the highest number of $PLZF^+$ (pro) spermatogonia (13.22 ± 1.56 cells/tubule), exceeding both the 10% KSR group (11.00 ± 1.65; $P<0.05$) and the 10% and 20% PRGF groups ($P<0.0001$). Total germ cell counts ($OCT4^+/DDX4^+$) were likewise greater with 5% PRGF than with 10% KSR (13.56 ± 2.60 vs. 8.78 ± 1.39 cells/tubule; $P<0.001$). In addition, 5% PRGF increased $SOX9^+$ Sertoli cells, steroidogenic acute regulatory protein (STAR) immunoreactivity, and Ki 67^+ proliferating cells, while reducing total Caspase 3 immunoreactivity ($P<0.05$).
Conclusion: PRGF functions as a promising xeno-free supplement for short-term *in vitro* culture of human fetal testicular tissue, supporting superior preservation of seminiferous tubule structure and key cellular populations compared with KSR. These data justify further long-term functional studies.
Keywords: Platelet-Rich Plasma, Spermatogenesis, Fertility Preservation, Organ Culture Techniques.

Embryology

O-3: Improvement of Human Oocyte *In vitro* Maturation via The Two-Phase CAPA-IVM System

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Background: To mimic natural meiotic arrest *in vivo*, a biphasic IVM system (CAPA-IVM) has been developed. By allowing adequate time for cytoplasmic maturation before extrusion of the second polar body, CAPA IVM improves both the maturation rate and oocyte quality compared with standard IVM. Patients with polycystic ovary syndrome have a higher incidence of oxidative stress, therefore, supplementation of IVM media with antioxidant can enhance oocyte maturation. This study aimed to evaluate the effect of glutathione supplementation as an antioxidant on human oocyte maturation in two-phase CAPA-IVM system.
Materials and Methods: Immature cumulus-oocyte-complexes (COCs) were aspirated from small follicles (<11 mm) of polycystic ovarian morphology (PCOM) patients undergoing infertility treatment in Royan infertility treatment center. Retrieval was performed 42–46 hours after the final ovulation trigger under ultrasound guidance. Collected COCs were incubated for 24 hours in a SAGE 1-Step™ supplemented with 1mIU/mL recombinant FSH, 5 ng/mL insulin, 10nM estradiol, 10 mg/mL human serum albumin, and 25 nM CNP at 37°C in 6% CO_2. Then, they were cultured for additional 24-30 hours in SAGE 1-Step™ supplemented with insulin, estradiol, and 100 mIU/mL rFSH, with or without supplementation of 12.5 mM glutathione ethyl ester (Control and Glutathione group) at 37°C in 6% CO_2. After IVM period, oocytes were denuded, and the nuclear status of the oocytes were assessed. Afterward the MII oocytes were collected for gene expression analysis (*CCNB1, MARF1, BRCA2, NLRP5, CDC2, CDC25*). Data were analyzed using GraphPad Prism and the Mann–Whitney U test.
Results: In the Control group, 39 patients (113 COCs) and in the Glutathione group 25 patients (109 COCs) were evaluated. After 48-54 hours of IVM, the proportion of MII oocytes was significantly higher in the Glutathione group (81.65% ± 4.9) compared to the control group (66.37% ± 4.9) (P0.05). Moreover, the *CCNB1, MARF1, CDC25*, and BRCA2 gene expressions were significantly downregulated in the Glutathione group.
Conclusion: In this preliminary study of PCOM patients, it was observed that glutathione supplementation of the two-phase CAPA-IVM system significantly improves human oocyte nuclear maturation. Moreover, as BRCA2 is involved in the mechanism for repairing double-stranded DNA breaks, its downregulation in the glutathione group indicates genomic stability maintenance in MII oocyte in this group. Further analysis is needed to clarify the protective mechanism of glutathione during IVM and subsequent fertilization.
Keywords: *In Vitro* Maturation (IVM), Biphasic Culture, Human Oocyte, Glutathione, PCOM

O-4: Effect of Empagliflozin on Oxidative Stress and Oocyte Quality in Women with Polycystic Ovary Syndrome Undergoing Intracytoplasmic Sperm Injection (ICSI)

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine metabolic disorder associated with increased oxidative stress, which may impair oocyte quality and maturation (1,2,3). Empagliflozin (EMPA), a sodium–glucose cotransporter 2 (SGLT2) inhibitor used for type 2 diabetes, has demonstrated antioxidant properties (4). Considering the metabolic similarities between PCOS and type 2 diabetes, this study investigated the effects of EMPA on oxidative stress and oocyte quality in women with PCOS.
Materials and Methods: Forty women with PCOS were randomly assigned to two groups and received either empagliflozin (25 mg) or placebo for eight weeks. Treatment started two months before the ovulation cycle and continued until oocyte retrieval. Retrieved oocytes were assessed for quality and maturation. Serum and follicular fluid MDA and TAC levels were measured using ELISA. Data were analyzed with an independent t test, with significance set at P0.05.
Results: The mean total number of retrieved Oocytes (COC) did not differ significantly between the EMPA group and the placebo group (P>0.05). However, the mean number of mature oocytes (MII) and fertilized oocytes (2PN) was significantly higher in the EMPA group compared with the placebo group (P=0.01), while the mean number of immature oocytes (MI+GV) showed a significant decrease (P0.01). In addition, the mean level of MDA in serum (P=0.01) and follicular fluid (P=0.05) showed a significant reduction after EMPA administration, whereas the mean TAC level in serum (P=0.01) and follicular fluid (P=0.05) demonstrated a significant increase following EMPA treatment.
Conclusion: Empagliflozin improved oxidative stress status and oocyte quality in patients with PCOS, as evidenced by reduced MDA levels, increased TAC, and a higher number of mature and fertilized oocytes. These findings suggest that EMPA may enhance oocyte maturation and fertilization outcomes in PCOS patients.
Keywords: Polycystic Ovary Syndrome, Empagliflozin, Oxidative Stress, Oocytes Quality

O-5: Kisspeptin's Protective Mechanism Against Human Ovarian Vitrification Damage: Regulation of ROS-Mediated Apoptosis

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Background: Kisspeptin's is characterized as a neuropeptide with a pivotal function in female and male infertility, and its antioxidant properties have been demonstrated. In this study, the effects of Kisspeptin's on the improvement of the Vitrification and thawing results of human ovarian tissues were investigated
Materials and Methods: In this work, 12 ovaries from patients who underwent hysterectomy were collected laparoscopically, and then 32 samples from each of their tissues were taken. Haematoxylin and eosin (H&E) staining was performed to check the normality of the ovarian tissue and, subsequently, the samples were allocated randomly into four groups, including: (1) fresh (control), (2) Vitrification, (3) vitrified + 1 μM kisspeptin, and (4) vitrified + 10 μM kisspeptin groups. After Vitrification, thawing, and tissue culture processes, H&E staining for tis-

sue quality assessment, terminal deoxynucleotidyl transferase dUTP nick end labelling assay for apoptosis evaluation, and malondialdehyde (MDA), superoxide dismutase (SOD), and ferric reducing ability of plasma tests for oxidative stress appraisal were carried out.
Results: Our histological results showed incoherency of ovarian tissue morphology in the Vitrification group compared with other groups. Other findings implicated increased apoptosis rate and MDA concentration and reduced SOD activity and total antioxidant capacity (TAC) in the Vitrification group compared with the control group ($P<0.05$). Moreover, decreased apoptosis rate and MDA concentration, and increased TAC and SOD function were observed in the Vitrification with kisspeptin groups (1 μM and 10 μM) compared with the vitrified group ($P<0.05$).
Conclusion: Our reports express that kisspeptin is an effective agent to overcome the negative effects of Vitrification by regulating reactive oxygen species-related apoptotic processes.
Keywords: Apoptosis, Human Ovarian Cryopreservation, Kisspeptin's, Oxidative Stress, Vitrification.

O-6: Enzymatic Method Superior to Mechanical Dissociation for Recovering Viable Cells from Vitrified Human Ovarian Cortex

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Background: The isolation of viable human ovarian cells (HOCs) is a key method in reproductive biology, fertility preservation, and regenerative medicine. This study aimed to compare the efficiency of mechanical and enzymatic dissociation methods for recovering viable cells from vitrified human ovarian cortical fragments.
Materials and Methods: Ovarian tissue samples were obtained from three patients (mean age 40.67 ± 5.73 years) undergoing oophorectomy and cryopreserved using vitrification. After one week of storage in liquid nitrogen, samples were thawed and subjected to either mechanical scraping or enzymatic digestion using collagenase 1.5 mg/mL.Cell yield and viability were assessed using a Neubauer chamber and flow cytometry with Annexin V/Propidium Iodide staining, respectively.
Results: The enzymatic method resulted in a significantly higher cell yield ($30.7 \pm 4 \times 10^6$ cells) compared to the mechanical method ($22.7 \pm 2 \times 10^6$ cells, $P< 0.05$). Moreover, cell viability was markedly improved in the enzymatic group (64.1 ± 6.4 %) versus the mechanical group (11.5 ± 8 %, $P<0.05$). These findings indicate that enzymatic dissociation is a more efficient approach for obtaining higher numbers of viable human ovarian cells from vitrified tissue.
Conclusion: This study provides practical insights for optimizing cell isolation protocols in oncofertility and ovarian tissue engineering. Further studies with larger sample sizes and functional characterization of isolated cells are warranted.
Keywords: Human Ovarian Cells, Cell Isolation, Enzymatic Digestion, Mechanical Dissociation, Vitrification

Investigating the Effect of Equilibration Time on the Toxicity and Quality of Vitrified Mature Mouse Oocytes

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Background: Oocyte vitrification is a widely utilized assisted reproductive technique for women seeking to postpone fertility for medical or social reasons. However, a significant challenge in this process is the inherent toxicity of cryoprotectant agents (CPAs). This study aimed to reduce the toxicity of CPAs by shortening the exposure time during the equilibration stage of vitrification.
Materials and Methods: Mature NMRI mouse oocytes obtained via *in vitro* maturation (IVM) were exposed to an equilibration solution (ES) containing 7.5% dimethyl sulfoxide (DMSO) and 7.5% ethylene glycol (EG), followed by a vitrification solution (VS) containing 15% DMSO, 15% EG, and 0.5 M sucrose for 1 minute. Oocytes were randomly divided into three groups: (1) control (12 minutes in ES), (2) experimental group 1 (2 minutes in ES), and (3) experimental group 2 (5 minutes in ES). Following at least 24 hours of vitrification, oocytes were warmed using the same protocol. Data were analyzed by one-way ANOVA, with statistical significance set at $P<0.05$.
Results: No significant differences were observed in the survival rates of the control (85.45%), 2-minute (81.63%), and 5-minute (90.74%) groups ($P>0.05$). However, oxidative stress levels were significantly lower in both experimental groups compared to the control ($P<0.05$). Specifically, oxidative stress was reduced by 47.15% in the 2-minute group and by 36.64% in the 5-minute group. Regarding mitochondrial membrane potential, no significant variations were detected among the study groups ($P>0.05$).
Conclusion: Overall, this study demonstrated that shortening the equilibration duration during oocyte vitrification significantly mitigates CPA-induced oxidative stress without compromising survival rate and mitochondrial membrane potential. Specifically, reducing the equilibration stage to 2 minutes offers a safer and more efficient strategy for clinical oocyte vitrification by minimizing oxidative injury while preserving mitochondrial integrity.
Keywords: Oocyte Vitrification, Cryoprotectant Toxicity, Equilibration Time, Oxidative Stress.

O-7: Multimodal Deep Learning for Automated Blastocyst Grading and Clinical Outcome Prediction: A Multiplicative Cascade Framework with Asymmetric Loss and Label-Shift Correction

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Background: Manual Gardner blastocyst grading suffers from high inter-observer variability (Cohen's κ=0.40–0.65), while conventional artificial intelligence (AI) models analyse static images in isolation, ignoring clinical covariates and hierarchical biological relationships. This study introduces a unified multimodal deep-learning framework that automates all three Gardner grading components — expansion, inner cell mass (ICM), and trophectoderm (TE) — and sequentially predicts longitudinal clinical *in vitro* fertilization (IVF) outcomes under biological constraints.
Materials and Methods: An EVA-02 Vision Transformer (ViT-Large, 448×448 px) was trained on 2,044 day-5/6 blastocyst images from 802 patients. A cross-attention module fused image features with 15 clinical covariates. Three sequential binary outcome heads were structured into a multiplicative cascade enforcing that each milestone probability could not exceed the prior one. An asymmetric loss function prevented minority-class collapse in skewed outcome targets. A post-hoc expectation-maximisation (EM) label-shift correction algorithm overcame institutional distribution shifts between training annotators and an independent gold-standard consensus panel (n=298). Clinical interpretability was validated via linear concept probes and spatial attention mapping.
Results: On the independent consensus test set, the base model achieved expert-level Cohen's κ values of 0.804 for expansion, 0.785 for ICM, and 0.765 for TE — exceeding human inter-rater thresholds. Unsupervised EM label-shift correction significantly improved agreement to κ=0.852, 0.821, and 0.832, respectively, with the largest gain for trophectoderm grading (κ improvement: 0.067, P=0.001). The multiplicative cascade achieved clinically informative areas under the receiver-operating-characteristic curve (AUROCs) of 0.668, 0.608, and 0.574 for biochemical pregnancy, clinical heart activity, and live birth. Monte Carlo dropout uncertainty analysis confirmed well-calibrated predictions. Spatial attention maps verified alignment with central embryonic poles and peripheral trophectoderm boundaries; concept probes identified blastocoel volume, ICM compaction, and cell density as key predictive features.
Conclusion: To our knowledge, this is among the first clinical AI frameworks to simultaneously deliver expert-level morphological grading and logically consistent, calibrated pregnancy milestone predictions, while dynamically adapting to deployment-site label shifts without manual re-annotation. By addressing a critical standardisation gap in embryo selection, this framework offers a robust and interpretable clinical decision-support tool to optimise elective single-embryo transfer policies.
Keywords: Artificial Intelligence, Blastocyst Grading, Multimodal Fusion, IVF Outcome Prediction.

Genetics

O-8: WGCNA-Based Identification of Endometrial Hub Genes and Biomarker Candidates in Unexplained Recurrent Pregnancy Loss

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Background: Unexplained recurrent pregnancy loss (URPL) is one of the most challenging reproductive disorders affecting women worldwide and is associated with significant physical, emotional, and psychological consequences for affected couples. Despite advances in clinical diagnostics and reproductive medicine, the underlying molecular mechanisms responsible for many cases of URPL remain poorly understood. Increasing evidence suggests that alterations in endometrial receptivity, immune regulation, hormonal signaling, and cellular metabolism may contribute to implantation failure and pregnancy loss. Therefore, identification of key genes, molecular pathways, and reliable biomarkers involved in URPL could improve early diagnosis, risk prediction, and targeted therapeutic strategies.
Materials and Methods: In the present study, integrated bioinformatic and weighted gene co-expression network analysis (WGCNA) approaches were employed to investigate molecular alterations associated with URPL in endometrial tissue. Microarray data from the GSE165004 dataset, including 48 endometrial samples (24 women with URPL and 24 fertile controls), were obtained from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were identified using the limma package in R software, followed by WGCNA to determine disease-associated modules and hub genes. Functional enrichment analyses and protein–protein interaction (PPI) network construction were further performed using STRING and Cytoscape platforms.
Results: A total of 1,041 DEGs were identified, including 480 upregulated and 561 downregulated genes in URPL samples compared with fertile controls. WGCNA analysis identified the paleturquoise module as one of the most significantly disease-associated modules. Among the identified hub genes, *SUMO1P3, ATP5L2, SNRPE, LOC647979, ANKRD12*, XLOC_l2_003065, *PGAM4, FKSG2, C11orf30,* and *PSIP1* showed strong associations with URPL. Notably, SUMO1P3, ATP5L2, and SNRPE demonstrated the highest connectivity scores and significant downregulation in patients with URPL. SUMO1P3, a long non-coding RNA (lncRNA), may play an important role in endometrial receptivity and embryo implantation. ATP5L2 is involved in ATP synthesis and cellular energy metabolism, while SNRPE participates in RNA processing and regulation of hormone receptor-related pathways. Dysregulation of these genes may impair endometrial function and contribute to embryo implantation failure and recurrent pregnancy loss.
Conclusion: Overall, the findings of this study suggest that disruption of endometrial regulatory networks and molecular signaling pathways plays a critical role in the pathogenesis of URPL. The identified hub genes may serve as potential diagnostic biomarkers and promising therapeutic targets for improving pregnancy outcomes in women with unexplained recurrent pregnancy loss.
Keywords: Unexplained Recurrent Pregnancy Loss (URPL), Weighted Gene Co-Expression Network Analysis (WGCNA), Endometrial Receptivity, Hub Genes, Biomarkers

Poster Presentation

Andrology

P-1: Protective effect of resveratrol on methamphetamine-induced reproductive toxicity in male rats

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Background: Methamphetamine (METH) is a potent central nervous system stimulant. METH use is associated with oxidative stress, inflammation, and tissue damage in various organs. Resveratrol is known as a natural polyphenol with antioxidant and anti-inflammatory properties. The aim of this study was to investigate the protective effect of resveratrol on METH-induced damage in male rats.
Materials and Methods: In this experimental study, 32 adult male rats were randomly divided into four groups: control, METH, resveratrol, and METH + resveratrol. METH and resveratrol were administered daily for 28 days. At the end of the period, oxidative stress indices, sperm parameters and testosterone levels were evaluated.
Results: METH administration significantly increased lipid peroxidation and decreased antioxidant enzyme activity. Sperm count, motility, and viability, as well as testosterone levels, were also significantly reduced in the METH group. Concomitant administration of resveratrol improved antioxidant indices, increased sperm quality and improved testosterone levels ($P<0.05$).
Conclusion: The results of this study indicate that resveratrol has a significant protective effect against METH-induced toxicity by reducing oxidative stress and inhibiting cellular damage, and can be considered as a potential agent for reducing reproductive complications caused by METH use.
Keywords: Resveratrol, Methamphetamine, Oxidative Stress, Male fertility

P-2: Artificial Intelligence for Clinical Decision-Making in Male Infertility: A Systematic Review of Current Applications and Performance

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Background: Male infertility remains a major contributor to couple infertility worldwide, and its evaluation and management are often complex, operator-dependent, and time-consuming. Artificial intelligence (AI) has recently emerged as a promising tool to enhance diagnostic accuracy, standardize semen analysis, and support clinical decision-making in male infertility care. This systematic review aimed to synthesize current evidence on AI applications in the clinical management of male infertility and to highlight existing gaps and future directions.
Materials and Methods: This systematic review was conducted in accordance with PRISMA 2020 guidelines. PubMed and Web of Science were systematically searched using a predefined search strategy combining AI-related and male infertility-related terms. Original studies evaluating AI-based approaches in the diagnosis, evaluation, or treatment-related decision-making of male infertility were considered eligible. After duplicate removal, titles and abstracts were screened independently by two reviewers, followed by full-text assessment of potentially relevant articles. A total of 69 studies met the inclusion criteria and were synthesized narratively using a standardized data extraction template.
Results: The included studies demonstrated diverse AI applications across the male infertility care pathway, including automated semen analysis, sperm quality and DNA fragmentation assessment, prediction of infertility risk, hypogonadism-related evaluation, and outcome prediction in assisted reproductive technology settings. Machine learning and deep learning models were most frequently employed and often showed improved diagnostic or predictive performance, greater objectivity, and enhanced efficiency compared with conventional methods. However, the evidence base was characterized by heterogeneous study designs, limited sample sizes, lack of external validation, and variability in performance reporting, which collectively restrict immediate clinical implementation.
Conclusion: AI holds substantial potential to transform the clinical management of male infertility, particularly in standardizing semen analysis and enabling data-driven risk stratification and treatment planning. Nonetheless, robust multicenter studies with transparent reporting, external validation, and clinically meaningful endpoints are urgently needed to bridge the gap between experimental models and routine andrology practice. Establishing standardized frameworks for AI development and evaluation in male infertility will be crucial for safe and effective clinical translation.
Keywords: Artifical Intelligence, Male Infertility, Machine Learning, Deep Learning, Clinical Decision Making,

P-3: Effects of Platelet Derived Products on *In Vitro* Spermatogenesis: A Systematic Review

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Background: Platelet derived products, including Platelet Rich Plasma (PRP) and Plasma Rich in Growth Factors (PRGF), are emerging as therapeutic candidates for tissue regeneration.

However, their efficacy in male reproductive medicine remains debated. This systematic review evaluates the effects of platelet-derived products on *in vitro* spermatogenesis models to determine their potential as culture additives.

Materials and Methods: A comprehensive systematic search was performed across MEDLINE/PubMed, Cochrane Library, Scopus, ScienceDirect, and Web of Science. The search covered literature up to March 2025 using terms: ("Spermatogenesis" OR "Spermatogonial stem cell" OR "SSC" OR "*In vitro*") AND ("Platelet-rich plasma" OR "PRP" OR "Plasma rich in growth factors" OR "PRGF"). Following PRISMA guidelines, 4 experimental studies meeting strict inclusion criteria were analyzed to assess the impact of these derivatives on germ cell viability and differentiation.

Results: Findings from the four included studies demonstrated variable effects of platelet-derived products on *in vitro* spermatogenesis models. PRGF supplementation (5%) in immature mouse testicular cultures enhanced production of flagellated sperm and upregulated germ cell markers such as PLZF, SYCP3, ACRBP, and Ki67, while reducing Bax expression—indicating improved germ cell survival. Consistent results were observed with PRP-enriched three-dimensional (3D) spermatogonial stem cell (SSC) cultures, where increased viability and expression of pre-meiotic (GFRA1) and post-meiotic (PRM2) genes were reported. However, in comparative assessments, media containing 5% KnockOut Serum Replacement (KSR) + 5% PRGF induced significant central necrosis and disorganization within seminiferous tubules, coupled with increased Bax expression ($P< 0.05$) and diminished Ki67 signals ($P< 0.001$) versus controls, suggesting impaired differentiation and heightened apoptotic activity.

Conclusion: This systematic review highlights that while PRP and PRGF possess intrinsic regenerative potential for male reproductive tissues, their therapeutic efficacy *in vitro* is highly dependent on concentration and culture environment. Our findings demonstrate a "biphasic-like" effect, where optimized PRP application enhances spermatogonial proliferation; however, suboptimal supplementation ratios may paradoxically induce testicular necrosis and apoptotic signaling. Future research must prioritize the standardization of dosage and culture protocols to translate these findings into viable clinical strategies for male infertility.

Keywords: Platelet-Rich Plasma, Spermatogenesis, *In Vitro* Techniques, Testis

P-4: Targeting Toll-like Receptors 7 and 8 for Sperm Sex Selection: A Systematic Review

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Background: Sex selection before fertilization has gained considerable attention in reproductive medicine and animal biotechnology due to its applications in preventing sex-linked diseases and improving livestock productivity. Flow cytometry is currently considered the standard method for sperm sexing; however, its high cost, technical complexity, and potential adverse effects on sperm quality have limited its widespread use. Recently, Toll-like receptor 7 and 8 (TLR7/8) ligands such as resiquimod (R848) have been proposed as novel alternatives for sperm sex selection through selective suppression of X-bearing sperm motility. Therefore, this study aims to systematically review and meta-analyze the available evidence regarding the effects of TLR7/8 ligands on sperm sex selection and embryo sex ratio.

Materials and Methods: A systematic search was conducted in PubMed, Scopus, Web of Science and Google Scholar to identify relevant studies investigating the role of TLR7/8 ligands in X/Y sperm separation. Eligible experimental studies was selected according to predefined inclusion and exclusion criteria following PRISMA guidelines. Extracted data included species, sample size, type and concentration of ligand, incubation time, sperm separation method, sperm motility, viability, membrane integrity, and embryo sex outcomes.

Results: Based on database searchs, 1,070 manuscripts were identified. After removing dublicate studies, the data were extracted from 15 eligible studies. Activation of TLR7/8 by R848 appeared to selectively reduce the motility of X-bearing sperm in murine, bovine and canine species, thereby enriching Y-bearing sperm in the upper fraction of the culture medium. There was no negative effect of TLR agonists on sperm membrane integrity and embryo quality. The sex ratio of embryos derived from the enriched upper fraction showed a significant shift toward male (XY) offspring across the majority of analyzed studies.

Conclusion: This systematic review and meta-analysis will provide a comprehensive evaluation of the efficacy and safety of TLR7/8 ligand-based sperm sexing methods and may support the development of simpler, less invasive, and more cost-effective alternatives to conventional sperm sex selection techniques.

Keywords: Sperm Sexing, TLR7/8 Agonists, Sex Ratio

P-5: Adipose MSC-Derived Extracellular Vesicles Improve Azoospermia via NF-κB reducing inflammation and oxidative stress

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Background: Azoospermia is strongly associated with inflammation and oxidative stress. This study evaluated the thera-

peutic potential of extracellular vesicles derived from adipose mesenchymal stem cells (MSC-EVs) in a mouse model of azoospermia, with a focus on modulation of inflammation and oxidative responses.
Materials and Methods: Mesenchymal stem cells were isolated from adipose tissue of NMRI mice and characterized by flow cytometry and differentiation assays. MSC-derived extracellular vesicles were purified using ultracentrifugation and characterized by dynamic light scattering transmission electron microscopy, and western blotting. Azoospermia was induced using busulfan and confirmed by histological staining. EVs were administered via sub-tunica albuginea injection. Gene expression levels inflammatory biomarkers were assessed by quantitative real-time PCR. Oxidative stress markers and antioxidant indices alongwith inflammatory cytokines were quantified by ELISA.
Results: Histological evaluation indicated improved testicular architecture and partial recovery of spermatogenesis after EV therapy. MSC-EV treatment significantly reduced oxidative stress markers and increased antioxidant capacity compared with the untreated azoospermic group. In parallel, EV administration led to significant downregulation of inflammatory mediators.
Conclusion: MSC-derived extracellular vesicles attenuate inflammation and oxidative stress in busulfan-induced azoospermia which promote restoration of testicular structure. These findings highlight the therapeutic potential of MSC-EVs as a cell-free approach for male infertility.
Keywords: Spermatogenesis Recovery, Oxidative Stress Biomarkers, Pro-Inflammatory Cytokines, Nonobstructive Azoospermia

P-6: Sperm Small Non-Coding RNAs as Functional Biomarkers of Male Infertility: A Systematic Review and Thematic Synthesis of Clinical Evidence up to 2026

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Background: Conventional semen analysis fails to explain ~30% of male infertility. Sperm-borne small non-coding RNAs (sncRNAs: miRNAs, piRNAs, tsRNAs) are emerging epigenetic biomarkers, but clinical evidence remains fragmented. This systematic review synthesised evidence linking sperm sncRNA profiles to male infertility phenotypes and Assisted Reproductive Technology (ART) outcomes.
Materials and Methods: Following PRISMA 2020, we searched PubMed, Scopus, Web of Science, and EMBASE (inception–February 2026) without language restrictions. Eligible studies compared sperm sncRNA expression between infertile and fertile men. Two reviewers independently screened, extracted data, and assessed risk of bias (QUADAS-2, ROBINS-I). Thematic synthesis was structured using the ACCE framework (Analytical validity, Clinical validity, Clinical utility, Ethical, Legal, and Social Implications (ELSI)).
Results: From 1,243 records, 34 studies (3,812 infertile men, 1,756 fertile controls) were included. For analytical validity, 28 studies reported sensitivities of 0.65–0.91 and specificities of 0.60–0.88. For clinical validity, a panel (piR-31068, miR-191-5p, tsRNA-Gly-GCC) was repeatedly associated with fertilisation rate and live-birth probability across independent studies. Clinical utility was limited: only 12 studies assessed ART as a primary outcome, and none addressed ELSI. Recurrent themes: miR-34c/449a/21 downregulation in oligo-/azoospermia targeting BCL2/E2F1/DAZL; miR-122/210 upregulation in asthenozoospermia linked to mitochondrial dysfunction. Panel heterogeneity was extreme—no two panels identical—risking inconsistent patient classification. Only four studies were prospective; 24 of 28 diagnostic accuracy studies had high/unclear risk of bias (mostly retrospective, unblinded). External validation, piRNA/tsRNA representation (n=6 studies), and long-term offspring safety data were critically lacking.
Conclusion: Sperm sncRNAs show promise as non-invasive biomarkers beyond conventional semen parameters, but current evidence is constrained by methodological heterogeneity, bias, and absent clinical utility/ELSI research. Multicentre prospective studies with harmonised protocols, blinding, external validation, and long-term offspring follow-up are urgently needed.
Keywords: Sperm sncRNA, Male Infertility, Biomarker, Systematic Review

P-7: Oxidative Stress-Associated Downregulation of Sperm ACTL7A Expression in Infertile Men with Varicocele

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Background: ACTL7A is a testis-specific cytoskeletal protein that plays an essential role in spermiogenesis, acrosome formation, sperm head shaping, and fertilization-related processes. Given the vulnerability of spermatozoa to oxidative and thermal stress, varicocele may impair ACTL7A expression and consequently affect sperm structural and functional integrity. This study aimed to evaluate ACTL7A mRNA and protein expression in spermatozoa from infertile men with varicocele and to investigate their association with oxidative stress, chromatin packaging, and DNA integrity.
Materials and Methods: Semen samples were obtained from infertile men with varicocele (n=35) and fertile controls (n=35). Standard sperm parameters were evaluated according to the WHO 2021 guidelines. Intracellular reactive oxygen species and lipid peroxidation were assessed using DCFH-DA and BODIPY probes, respectively. Chromatin packaging status was evaluated by Chromomycin A3 and Aniline Blue staining, while sperm DNA integrity was assessed using the Sperm Chromatin Structure Assay. ACTL7A mRNA and protein expression levels were determined by quantitative real-time PCR and western blotting. To compare the data, an independent t-test was used for comparing the means between the two groups, with a significance level set at $P<0.05$ for all analyses.
Results: Compared with fertile controls, infertile men with varicocele showed significantly impaired sperm parameters, elevated oxidative stress, increased lipid peroxidation, higher chromatin immaturity, and greater sperm DNA fragmentation, $P<0.01$. ACTL7A mRNA and protein expression levels were significantly reduced in the varicocele group, $P<0.05$. Moreo-

ver, ACTL7A mRNA expression was inversely correlated with markers of defective chromatin packaging, including protamine deficiency and residual histone retention, P<0.05.
Conclusion: Sperm ACTL7A mRNA and protein expression are significantly reduced in infertile men with varicocele and are associated with oxidative stress, impaired chromatin remodeling, and sperm DNA damage. These findings suggest that ACTL7A may serve as a potential molecular marker of varicocele-related testicular dysfunction and sperm quality impairment. Further studies are warranted to determine whether varicocele severity, disease duration, antioxidant therapy, or varicocelectomy can modulate ACTL7A expression and improve reproductive outcomes.
Keywords: Varicocele, ACTL7A, Sperm DNA Fragmentation, Chromatin Packaging, Oxidative Stress

P-8: Effect of Tuberose (Polianthes tuberosa) Flower Extract on Post-Thaw Quality of Rooster Sperm

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Background: The aim of this study was to evaluate the impact of different concentrations of tuberose (Polianthes tuberosa) flower extract on the post-thaw quality of rooster sperm.
Materials and Methods: Healthy adult roosters were used and semen samples were collected by abdominal massage. Immediately after collection, ejaculatory volume, concentration, color, mass motility, and individual motility were evaluated. Only samples of acceptable initial quality were used in the experiment. The selected ejaculates were pooled and diluted with a standard poultry semen extender containing cryoprotectant to reduce individual variation. The diluted semen was divided into four experimental groups; a control group without the extract and three treatment groups with the addition of 25, 50 and 100 µg/mL of tuberose flower extract. Samples were equilibrated at 4 °C, frozen and stored in liquid nitrogen until evaluation.
Results: Routine laboratory methods were used to evaluate sperm quality parameters (total motility, progressive motility, viability, plasma membrane integrity, and abnormal morphology) after thawing. The results showed that the addition of tuberose flower extract to the semen extender did not significantly influence the post-thaw sperm quality parameters in comparison to the control group (motility: 45%) (P>0.05). Although there were slight numerical differences in viability and membrane integrity, these differences were not statistically significant. The lack of a significant effect may be attributed to the concentration range used, the chemical composition of the extract or possible interactions between plant compounds and semen extender components. Moreover, rooster spermatozoa are very sensitive to oxidative and cold stress and not all the plant extracts can provide enough protection during cryopreservation.
Conclusion: In conclusion, the addition of tuberose flower extract at concentrations of 25, 50 and 100 µg/mL did not significantly improve post-thaw quality of rooster sperm. Therefore, in the present study conditions, tuberose extract was not effective as a cryoprotective supplement for freezing rooster semen.
Keywords: Tuberose, Rooster, Cryopreservation, Sperm

P-9: Paternal Age, Semen Quality, and Sperm DNA Fragmentation in an Infertile Male Cohort: A TUNEL and SCSA Study

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Background: Advancing paternal age may be associated with impaired spermatogenesis, altered semen quality, and reduced sperm genomic integrity. Although conventional semen analysis remains central to male infertility evaluation, it may not fully reflect sperm DNA damage. This study aimed to compare sperm DNA fragmentation (SDF) according to paternal age and semen-quality categories using two complementary assays.
Materials and Methods: In this retrospective cross-sectional study, semen samples from 6,000 infertile men were analyzed according to the World Health Organization 2021 guidelines. Conventional semen parameters, including semen volume, sperm concentration, total sperm count, motility, and morphology, were assessed using standard criteria. SDF was evaluated using terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) and sperm chromatin structure assay (SCSA). Participants were categorized by age into ≤40 and >40 years and by semen-quality abnormalities into normozoospermia, teratozoospermia, asthenozoospermia, asthenoteratozoospermia, oligoasthenoteratozoospermia, oligoasthenozoospermia, oligoteratozoospermia, and oligozoospermia. Age-group comparisons were performed using the independent-samples t-test. Semen-quality subgroup comparisons were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. A P<0.05 was considered statistically significant.
Results: The mean age of participants was 38.1 ± 6.4 years. Overall mean SDF levels were 11.9 ± 7.5% by TUNEL and 20.3 ± 8.9% by SCSA. Men aged >40 years showed significantly higher SDF levels and lower total motility compared with men aged ≤40 years. In subgroup analysis, SDF levels differed significantly across semen-quality categories. Groups with motility-related abnormalities, particularly asthenozoospermia and asthenoteratozoospermia, showed higher DNA damage than normozoospermic and isolated oligozoospermic groups. The asthenoteratozoospermia group showed the highest SDF levels in both TUNEL and SCSA assessments.
Conclusion: SDF was higher in infertile men older than 40 years and in semen abnormality groups involving impaired motility, especially asthenoteratozoospermia. These findings suggest that advancing paternal age and motility-related semen defects are associated with reduced sperm genomic integrity. Assessment of SDF using TUNEL and SCSA may provide clinically useful information beyond conventional semen analysis in infertile men.
Keywords: Sperm DNA Fragmentation, TUNEL, SCSA, Paternal Age, Semen Quality

P-10: Impaired Sperm Chromatin Integrity in Varicocele-Related Infertility: DNA Fragmentation, Protamine Deficiency, and Semen Parameters

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Background: Varicocele is a prevalent and treatable cause of male infertility, often associated with oxidative stress and compromised sperm chromatin integrity. This study aims to evaluate semen parameters, sperm DNA fragmentation, and protamine deficiency in infertile men with varicocele and to investigate their impact on semen quality.
Materials and Methods: This retrospective study was conducted at the Pooyesh and Rooyesh Fertility and Infertility Center in Isfahan, Iran, from 2021 to 2023. A total of 450 men were included: 300 infertile men with clinically diagnosed varicocele and 150 fertile controls who had fathered at least one child. Semen samples were analyzed according to the WHO Laboratory Manual, 6th edition. Sperm DNA fragmentation was assessed using the TUNEL assay, and protamine deficiency was evaluated by Chromomycin A3 staining. Statistical analyses were performed using SPSS version 20. Between-group comparisons were conducted using the independent-samples t-test, and correlations were assessed using Spearman's rank correlation test. Graphs were generated using GraphPad Prism, and statistical significance was defined as $p \leq 0.05$.
Results: Men with varicocele showed significantly lower sperm concentration, total sperm count, motility, progressive motility, and semen volume compared to fertile controls ($P<0.001$). Abnormal sperm morphology was also notably higher in the varicocele group ($P<0.001$). Sperm DNA fragmentation and protamine deficiency were both significantly elevated in the varicocele group ($P<0.001$). Protamine deficiency negatively correlated with most sperm parameters, while positively correlating with sperm DNA fragmentation ($P<0.01$) in infertile men with varicocele. No such correlation was found in the fertile group.
Conclusion: Infertility associated with varicocele is characterized by compromised sperm chromatin integrity, including increased DNA fragmentation and protamine deficiency, which are closely linked to reduced semen quality.
Keywords: Varicocele, Male Infertility, Sperm DNA Fragmentation, Protamine Deficiency, Semen Analysis

P-11: Effect of Male Aging on Sperm DNA Fragmentation and Conventional Semen Parameters in Infertile Men

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Background: Conventional semen analysis remains the cornerstone of male fertility evaluation; however, it provides limited information about sperm genomic integrity. Sperm DNA fragmentation (SDF) is increasingly recognized as an important biomarker of male reproductive potential and may rise with advancing paternal age due to oxidative stress, defective chromatin packaging, and impaired DNA repair. This study investigated the association between paternal age, sperm DNA damage, and conventional semen parameters in infertile men.
Materials and Methods: This analytical cross-sectional study included 1,000 infertile men aged 20–60 years who attended Pooyesh and Rooyesh Infertility Centers. Participants were categorized into three age groups: <30, 30–40, and >40 years. Semen volume, sperm concentration, motility, viability, and morphology were assessed according to the World Health Organization 2021 guidelines. Sperm DNA fragmentation was measured using the Sperm Chromatin Structure Assay (SCSA). Statistical analyses included analysis of variance, correlation tests, and post hoc comparisons, as appropriate.
Results: Conventional semen parameters did not differ significantly among the age groups. In contrast, sperm DNA fragmentation increased significantly with advancing paternal age ($P<0.001$). Men older than 40 years showed the highest SDF levels. Higher SDF was significantly associated with lower sperm concentration, total and progressive motility, viability, and normal morphology (all $P<0.001$). Correlation analysis demonstrated a significant positive relationship between paternal age and SDF ($r=0.232$; $P<0.001$). SDF also showed weak but statistically significant negative correlations with most conventional semen parameters.
Conclusion: Sperm DNA fragmentation increases with paternal age, even when routine semen parameters remain largely unchanged. These findings suggest that conventional semen analysis alone may underestimate age-related sperm dysfunction. Incorporating sperm DNA integrity testing into routine fertility assessment, particularly in older men, may improve the evaluation of male reproductive potential and guide clinical decision-making.
Keywords: Sperm DNA Fragmentation, Paternal Age, Semen Quality, Oxidative Stress, SCSA

P-12: Comparative Analysis of ACTL7A Expression, Localization, and Sperm Function in Fertile and Infertile Men

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Background: Testis-specific actin-like 7A (ACTL7A) is an acrosome/perinuclear theca-associated protein that may contribute to sperm function and male infertility. This exploratory study evaluated ACTL7A transcript expression in a clinical as-

sisted reproductive technology setting and performed preliminary protein-level assessment in a limited subset of samples. Associations between ACTL7A expression, sperm functional parameters, chromatin integrity, and clinical outcomes were also examined.

Materials and Methods: This preliminary cross-sectional case–control study included 27 men, comprising 11 infertile candidates undergoing intracytoplasmic sperm injection and 16 fertile candidates undergoing preimplantation genetic testing. ACTL7A mRNA expression was quantified in all samples using quantitative reverse transcription polymerase chain reaction. ACTL7A protein expression was assessed by Western blot in a feasibility-limited subset of samples, including three participants from each group, and was interpreted as preliminary. Protein localization was evaluated by immunostaining. Conventional semen parameters and sperm functional assays were performed, including sperm chromatin structure assay for DNA fragmentation, aniline blue staining for histone retention, and chromomycin A3 staining for protamine deficiency. Clinical reproductive outcomes were recorded.

Results: ACTL7A mRNA expression was significantly lower in infertile men than in fertile controls (P=0.02). Preliminary Western blot analysis also showed reduced ACTL7A protein expression in the infertile group (P=0.01). Infertile men had lower sperm viability (P=0.04), higher histone retention (P=0.02), and a non-significant trend toward increased protamine deficiency (P=0.07). ACTL7A expression correlated positively with sperm viability (r=0.40, P=0.04) and negatively with DNA fragmentation (r=−0.43, P=0.02). No significant association was observed with fertilization rate or embryo quality. Receiver operating characteristic analysis showed moderate discriminatory ability for ACTL7A expression (AUC=0.727, P=0.048), with 62.5% sensitivity and 81.8% specificity.

Conclusion: Reduced ACTL7A expression is associated with impaired sperm viability and chromatin integrity, supporting its potential role as an exploratory research biomarker of male infertility.

Keywords: ACTL7A, Protamine Deficiency, Histone Retention, DNA Fragmentation, ICSI

P-13: Supplementation of buckks semen extender with mitochondrial -targeted and untargeted antioxidants: Improvement in quality parameters

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Background: High levels of polyunsaturated fatty acids (PUFAs) in buck sperm make it vulnerable during the cooling process, which decrease the potential quality of semen. An approach to preserve sperm quality during cooling storage is to enhance the chilled sperm medium by supplementing suitable antioxidants. This study aimed to evaluate the effects of mitochondria-targeted antioxidants (MitoQ and Mito-TEMPO) and untargeted antioxidants (superoxide dismutase and CoQ10) on buck sperm quality during cooling process.

Materials and Methods: Semen samples were collected, diluted in an extender and divided in to eight groups. First group saved as the control, while other groups were supplemented with: superoxide dismutase (100U/ml), CoQ10(1uM), MitoQ at two concentrations (50 and 100nM),Mito-TEMPO at concentrations (50and 100uM) and combination of MitoQ and Mito-TEMPO (50/50uM). samples were then cooled to 5 C for up to 50 hours, and sperm total motility, progressive motility and viability were investigated at 0,25 and 50 hours of cooling storage.

Results: The results showed no significant differences (P>0.05) among the groups at time 0. However, after cooling, the group supplemented with MitoQ and Mito-TEMPO (MQ/MT) exhibited the highest levels of total motility, progressive motility and viability. (P ≤0.05).

Conclusion: In conclusion, supplementing buck sperm cooling extender with a combination of 50nM MitoQ and 50 uM Mito-TEMPO, as powerful mitochondria-targeted antioxidants, is an effective strategy to preserve sperm quality during cooling storage.

Keywords: Cooling, Mito-TEMPO, MitoQ, Goat, Spermatozoa

P-14: The Role of Hypoxia-Inducible Factors (HIFs) and Associated Biomarkers in Varicocele-Associated Impaired Spermatogenesis: An Updated Systematic Review and Meta-Analysis

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Background: Varicocele is a common and potentially correctable cause of male infertility, yet the molecular mechanisms linking venous dysfunction to impaired spermatogenesis remain incompletely defined. Hypoxia-inducible factor (HIF) signaling may represent a central pathway connecting testicular hypoxia, oxidative stress, and sperm dysfunction. Background: To systematically evaluate evidence on HIF-1α and related hypoxia-associated biomarkers in men with varicocele compared with controls.

Materials and Methods: This systematic review and meta-analysis followed PRISMA guidance, with PROSPERO registration (CRD420251147242). PubMed, Scopus, ScienceDirect, and Google Scholar were searched up to October 2025. Eligible human case–control studies assessed hypoxia-related biomarkers in testicular tissue, sperm, seminal plasma, or venous samples from men with varicocele and controls. Standardized mean differences (SMDs) with 95% confidence intervals (CIs) were pooled using inverse-variance methods. Heterogeneity, subgroup effects, risk of bias, sensitivity, and small-study effects were assessed.

Results: Eight case–control studies, including 308 participants (175 varicocele cases and 133 controls), were included. Ten comparisons covering HIF-1α, VEGF, BCL2, metallothionein, and p53 were synthesized. Overall, hypoxia-related marker levels were significantly higher in men with varicocele. The random-effects model showed a pooled SMD of 6.17 (95% CI:

4.33–8.01; P<0.00001), with very high heterogeneity (I^2=96%). HIF-1α showed the strongest evidence base, being increased across five studies. Evidence for VEGF and other markers was limited by fewer studies and substantial heterogeneity.
Conclusion: Varicocele is associated with activation of hypoxia/HIF-related molecular pathways, supporting a mechanistic role for hypoxia in impaired spermatogenesis. However, standardized, prospective studies are required for validation.
Keywords: Varicocele,Hypoxia-Inducible Factor 1, alpha Subunit, Hypoxia, Spermatogenesis

P-15: Asthenozoospermia: Compensatory SGMS2 Rise in Ceramide Axis SGMS2–SMPD1–ASAH1

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Background: Asthenozoospermia, characterized by reduced sperm motility, is a major cause of male infertility. Sperm motility depends largely on membrane integrity, proper mitochondrial function, and the ability to cope with oxidative stress. Because sphingolipids, particularly ceramide, play key roles in cellular survival and function, this study examined whether altered expression of genes involved in ceramide metabolism is associated with asthenozoospermia. To address this, the expression levels of three key genes SGMS2, SMPD1, and ASAH1 were compared in spermatozoa from asthenozoospermic men and normozoospermic controls.
Materials and Methods: Publicly available datasets from the Human Protein Atlas, *GTEx Portal, STRING, NCBI GEO*, and *CellxGene* were initially analyzed to validate the biological context of the target genes, focusing on their expression dynamics during spermatogenesis, retention in mature spermatozoa, subcellular compartment context, and predicted protein–protein interactions. Subsequently, semen samples were collected from 25 asthenozoospermic men and 25 normozoospermic controls. Total RNA was extracted from washed spermatozoa, and gene expression levels were quantified using RT-qPCR. Differences between the two groups were analyzed using an independent t-test.
Results: Bioinformatic analyses confirmed that all three genes are expressed during spermatogenesis and their transcripts are retained in mature human spermatozoa. Protein interaction networks supported their involvement in maintaining the ceramide–sphingomyelin balance, with ASAH1 and SMPD1 showing association with lysosome-related pathways, and SGMS2 showing association with the plasma membrane and Golgi apparatus. RT-qPCR analysis revealed a significant upregulation of SGMS2 expression in asthenozoospermic samples (13.5-fold, P<0.0001). SMPD1 expression was also significantly elevated (4.8-fold, p=0.016). In contrast, ASAH1expression showed a 60% reduction, which did not reach statistical significance (P=0.11).
Conclusion: These findings suggest that ceramide metabolism is altered in asthenozoospermic spermatozoa. The upregulation of SMPD1 may enhance ceramide production, while the downward trend in ASAH1 expression could compromise its degradation. Concurrently, the marked upregulation of SGMS2 might reflect a compensatory regulatory response aimed at limiting detrimental ceramide accumulation to support sperm integrity. Taken together, alterations in the SGMS2–SMPD1–ASAH1 axis may contribute to reduced sperm motility and warrant further functional investigation.
Keywords: SGMS2, SMPD1, ASAH1, Asthenozoospermia, Sphingolipid, Bioinformatics

P-16: The Effect of Theobromine on Sperm Biochemical Factors in Asthenozoospermic Men During the Freeze–Thaw Process

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Background: Human sperm cryopreservation is considered an essential component of infertility treatment centers, andrology laboratories, and sperm banks. However, this technique induces oxidative stress and can cause significant damage to sperm quality. The addition of antioxidants to the freezing medium may effectively reduce these adverse effects. Theobromine, a plant alkaloid derived from methylxanthine, possesses notable antioxidant properties. In this study, the effects of theobromine on the biochemical factors of frozen sperm in asthenozoospermic men were investigated.
Materials and Methods: Semen samples were collected from 30 asthenozoospermic men and divided into three groups: Control (fresh), Freeze (treated with cryoprotectant alone), and Freeze + Theobromine (treated with cryoprotectant and 10 mmol/L theobromine). The freezing groups were cryopreserved using a human sperm freezing medium and a rapid freezing method. For each sample, total antioxidant capacity (TAC), malondialdehyde (MDA), TNF-α, and interleukin-10 (IL-10) levels were measured using commercial ELISA kits according to the manufacturer's instructions. Data were analyzed using the Repeated Measures Analysis method, with the level of statistical significance set at P<0.05 for all compared groups.
Results: The mean TAC and the level of IL 10 were significantly decreased in the freeze group compared with the control group (P<0.001), whereas the levels of TNF α and MDA showed a significant increase (P<0.001). Furthermore, in the freeze + theobromine group, compared with the freeze group, the mean levels of TAC and IL 10 significantly increased (P<0.001), while the mean levels of TNF α and MDA showed a significant decrease (P<0.001).
Conclusion: Our data indicated that sperm cryopreservation induces oxidative stress and inflammatory changes in asthenozoospermic samples. The addition of theobromine to the freezing medium improved antioxidant status and reduced inflammatory and oxidative markers. Therefore, theobromine may act as a protective antioxidant during sperm cryopreservation.
Keywords: Asthenozoospermia, Sperm Cryopreservation, Theobromine, Oxidative Stress, Antioxidant Capacity

P-17: Vestibular Varicosities Mimicking a Reproductive Tract Mass in an Aged Mare: A Diagnostic Pitfall

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Background: Vestibular varicosities are commonly observed in aged multiparous mares but may be misinterpreted as reproductive tract masses or hematomas during clinical examination. This case report describes a 24-year-old Arabian-Barb mare referred for vulvar bleeding.
Materials and Methods: General physical examination was unremarkable. During transrectal palpation, a firm, elongated structure adjacent to the cervix raised suspicion of a uterine or cervical mass. However, transrectal ultrasonography of the uterus and ovaries revealed no abnormalities. Further evaluation using vaginal speculum examination identified multiple engorged venous structures at the vestibulovaginal junction.
Results: The findings were consistent with vestibular varicosities, explaining the observed hemorrhage. No evidence of intrauterine pathology was detected.
Conclusion: This case highlights the importance of systematic examination of the lower reproductive tract in mares presenting with vulvar bleeding, particularly in aged pluriparous animals. Vestibular varicosities can closely mimic more severe reproductive conditions during palpation and may lead to misdiagnosis if vaginal examination is omitted. Recognition of this condition is essential to avoid unnecessary interventions and to establish appropriate clinical management.
Keywords: Mare, Vestibular Varicosities, Vulvar Bleeding, Reproductive Examination

P-18: Effects of Platelet-rich plasma on Oxidative Stress and Sperm Parameters in STZ Induced Diabetes in Male Rats

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Background: Chronic hyperglycemia induced by diabetes can lead to the function impairment of male reproductive system and infertility. Platelet-rich plasma (PRP) is considered in various medical fields. PRP, an autologous blood-derived product concentrated with growth factors and cytokines, has attracted considerable attention as a regenerative therapeutic agent owing to its angiogenic, anti-apoptotic, and proliferative capabilities. This study investigated the effect of PRP on sperm quality, levels of testosterone and gonadotropins, and testicular oxidative stress parameters in STZ-diabetic rats.
Materials and Methods: Thirty-two male Sprague-Dawley rats were divided into four groups including Control, PRP, Diabetes and Diabetes + PRP groups. The groups were injected with 80 µl of normal saline or PRP under the scrotum every two weeks for 60 days. Sperm parameters, serum levels of glucose, testosterone, and gonadotropins, testicular levels of malondialdehyde (MDA) and glutathione (GSH) levels, as well as glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities were measured.
Results: Sperm count, motility, and viability, serum testosterone and luteinizing hormone (LH) levels, and testicular levels of antioxidants were reduced and testicular levels of malondialdehyde (MDA) were increased in diabetic rats. PRP improved hyperglycemia and recovered sperm count, motility and oxidative parameters in STZ-diabetic rats.
Conclusion: PRP, due to its growth factors and antioxidant activity, can have a potential protective effect against diabetes-induced reproductive disorders and it is also able to positively affects testicular function and steroidogenesis.
Keywords: Diabetes Mellitus, Platelet-rich Plasma (PRP), Sperm Parameters, Oxidative Stress, Testicular Function

P-19: The effect of loud noise stress on fertility in male rats

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Background: Anxiety is important factor affecting the reproductive system. Exposure to loud noise as an environmental stressor can affect reproductive function by activating the hypothalamic-pituitary-adrenal axis and increasing oxidative stress. The aim of this study was to investigate the effect of loud noise-induced anxiety on fertility indices in male rats.
Materials and Methods: In this experimental study, 30 adult male rats were randomly divided into two groups: control and loud noise exposure. Animals in the experimental group were exposed to noise with an intensity of 90 to 100 decibels daily for 4 weeks. At the end of the period, anxiety behaviors were assessed using behavioral tests. Sperm count, motility, viability, and morphology of sperms were also examined.
Results: The results showed that exposure to loud noise significantly increased anxiety-like behaviors in rats. Also, sperm count and motility levels in the noise-exposed group were significantly reduced compared to the control group ($P<0.05$). The percentage of abnormal sperm also increased.
Conclusion: Anxiety caused by long-term exposure to loud noise can reduce sperm quality and increased oxidative stress. These findings indicate the negative impact of noise pollution on male reproductive health and the need to pay attention to environmental stressors.
Keywords: Anxiety, Loud Noise, Wistar Rats, Sperm

P-20: Effect of Methamphetamine on Sperm Motility and Morphology in Male Rats

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Background: Methamphetamine (METH) is a central nervous system stimulant whose use has increased in recent years. Harmful effects on various body systems including the reproductive system have been reported in METH users. The aim of this study was to investigate the effect of METH on fertility indices in male rats.
Materials and Methods: This study was conducted experimentally on 30 adult male Wistar rats. The animals were randomly divided into three groups: control, sham, and METH-treated. METH was administered daily at a dose of 5 mg/kg for 21 days. At the end of the period, fertility indices including sperm count, motility, and morphology, sex hormone levels, were evaluated. The data were analyzed using appropriate statistical tests.
Results: The results showed that METH administration significantly reduced sperm count and motility compared to the control group ($P<0.05$). The percentage of sperm with abnormal morphology also increased. Hormonal studies showed a decrease in serum testosterone levels in the methamphetamine group.
Conclusion: METH use can affect male fertility by disrupting spermatogenesis and hormonal changes. These findings emphasize the need to raise awareness about the reproductive side effects of METH use.
Keywords: Methamphetamoin, Male Fertility, Sperm, Wistar Rat

P-21: Ameliorative Effects of Vitamin C on Sperm Quality and DNA Integrity in Cadmium-Induced Reproductive Toxicity in Adult Male Rats

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Background: Cadmium (Cd) is a pervasive environmental and industrial toxicant with a long biological half-life and is widely recognized for its detrimental effects on the male reproductive system. It is strongly associated with male infertility through the disruption of spermatogenesis, induction of oxidative stress, and consequent oxidative DNA damage in spermatozoa. Cd can accumulate in the testicular tissue, impair antioxidant defense mechanisms, and alter cellular homeostasis. Despite growing evidence of Cd-induced reproductive toxicity, limited data exist regarding the protective effects of antioxidants, particularly on functional sperm parameters and genomic integrity. Therefore, this study aimed to investigate the impact of Vitamin C (Vit C) on sperm quality and DNA fragmentation in Cd-exposed adult male Wistar rats.
Materials and Methods: In this experimental study, 24 adult male rats were randomly assigned to four groups (n=6 per group) following a two-week acclimatization period. Group I (control) received normal saline. Group II received $CdCl_2$ (2 mg/kg/day, intraperitoneally). Group III received $CdCl_2$ (2 mg/kg/day, intraperitoneally) in combination with Vit C (250 mg/kg/day, orally by gavage), and Group IV received Vit C alone (250 mg/kg/day, orally by gavage) for 28 consecutive days. Sperm samples were evaluated for their count, motility, viability, and DNA fragmentation. Motility and count were assessed using a Neubauer chamber under a light microscope. Sperm viability was determined using eosin-nigrosin staining. DNA integrity was evaluated using acridine orange staining under fluorescence microscopy, distinguishing double-stranded (green) from single-stranded (yellow-red) DNA.
Results: Cd exposure resulted in a significant decline in sperm count, motility, and viability compared to the control group ($P<0.05$), accompanied by a significant increase in sperm DNA fragmentation. Co-administration of Vit C significantly improved sperm motility and viability compared to the Cd group ($P<0.05$). Although modest improvements in sperm count and reductions in DNA fragmentation were observed in the Vit C-treated groups, these changes were not statistically significant.
Conclusion: In conclusion, Cd-induced reproductive toxicity is strongly associated with impaired sperm function and increased DNA damage. Vitamin C partially mitigates these adverse effects, particularly by enhancing sperm motility and viability. These findings emphasize the importance of antioxidant-based strategies for preserving male fertility under environmental toxicant exposure.
Keywords: Cadmium, Vitamin C, Testicular Toxicity, Male Infertility, Rat

P-22: Protective Effects of Vitamin C Against Cadmium-Induced Testicular Toxicity in Adult Male Wistar Rats

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Background: Cadmium (Cd) is a widespread environmental and industrial pollutant with a long biological half-life and a high tendency for bioaccumulation in various organs, including the testes. Increasing evidence indicates that Cd exposure is associated with impaired spermatogenesis, hormonal imbalance, and structural damage to testicular tissue, primarily through the induction of oxidative stress and disruption of the antioxidant defense system. Vitamin C (Vit C), a potent water-soluble antioxidant, has been shown to scavenge free radicals and protect cellular components against oxidative damage. Therefore, this study aimed to evaluate the protective role of Vit C against Cd-induced testicular toxicity in adult male Wistar rats.
Materials and Methods: In this experimental study, 24 male rats were randomly divided into four groups (n=6 per group) after a two-week acclimatization period. Group I (control) received normal saline for 28 consecutive days. Group II received cadmium chloride ($CdCl_2$) at a dose of 2 mg/kg/day via intraperitoneal injection. Group III received $CdCl_2$ (2 mg/kg/day, intraperitoneally) in combination with Vit C (250 mg/kg/day, orally via gavage), whereas Group IV received Vit C alone (250 mg/kg/day, orally) for 28 days. One day after the final administration, the animals were euthanized, and testicular tissues were collected for biochemical and histological analyses.
Results: Cd exposure resulted in a significant reduction in body and testis weights compared to the control group ($P<0.001$).

Histological examination revealed marked degeneration of seminiferous tubules, reduced germinal cell layers, decreased Leydig cell population, and thinning of the seminiferous epithelium in the Cd-treated group. In contrast, co-administration of Vit C significantly preserved the testicular architecture and improved the histomorphometric parameters. Biochemically, Cd significantly increased malondialdehyde (MDA) levels ($P<0.05$). Treatment with Vit C significantly reduced MDA levels. These findings suggest that oxidative damage plays a central role in Cd-induced reproductive toxicity.
Conclusion: In conclusion, Vitamin C exerts a significant protective effect against Cd-induced testicular toxicity by enhancing antioxidant defense mechanisms, reducing lipid peroxidation, and maintaining tissue integrity. These results highlight the therapeutic potential of antioxidant supplementation in mitigating heavy metal-induced reproductive damage and may provide insights into future reproductive health interventions.
Keywords: Cadmium, Vitamin C, Testicular Toxicity, Oxidative Stress, Wistar Rats

P-23: Evaluation of the Effects of Eugenol on Sperm Parameters and Testicular Tissue Structure in Male Rats Exposed to Doxorubicin

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Background: Cancer is a lethal disease characterized by uncontrolled cellular proliferation and abnormal cell cycle progression. Chemotherapy is considered one of the most common treatment strategies for cancer; however, its use is not free from adverse effects and may exert harmful impacts on various body tissues. Doxorubicin is a chemotherapeutic drug widely employed in the treatment of numerous cancers. Nevertheless, it induces toxicity in non-target tissues, including testicular tissue. Accordingly, the occurrence of infertility in men following treatment with this drug represents a serious threat. Eugenol, as an antioxidant, has been shown to be effective against the toxic effects of chemotherapeutic agents. Therefore, the present study investigated the effects of eugenol on sperm parameters and testicular histological structure in male rats exposed to doxorubicin.
Materials and Methods: 25 male Wistar rats, approximately 8 weeks old, were randomly divided into five groups: control, doxorubicin, eugenol, doxorubicin followed by eugenol, and concurrent administration of doxorubicin and eugenol. At the end of the experiment, following testicular weighing, the right testis was transferred to a fixative solution for histological evaluations. The right epididymis was also isolated and used for the assessment of sperm parameters.
Results: The results showed that testicular weight in the doxorubicin group was significantly reduced compared to the control group ($P \leq 0.01$), whereas eugenol administration led to an increase in testicular weight. In addition, the number of testicular cells (Sertoli cells, primary spermatocytes, spermatogonia, spermatids, and Leydig cells) was significantly decreased following doxorubicin injection ($P \leq 0.001$), while a significant increase was observed in the DE(H) group after eugenol treatment. Furthermore, doxorubicin administration resulted in a reduction in sperm count and motility, along with an increase in the proportion of dead and abnormal sperm compared to the control group ($P \leq 0.001$). These parameters were improved following eugenol administration in both the DE and DE(H) groups.
Conclusion: Eugenol can mitigate the adverse effects of doxorubicin chemotherapy on testicular tissue, sperm parameters and may be considered as a plant-derived compound in reducing the toxicity of chemotherapeutic drugs.
Keywords: Doxorubicin, Testis, Sperm, Eugenol

P-24: Impact of Chronic Ritalin Use on Sperm Parameters: An Evaluation in Psychiatric Outpatients

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Background: Some studies have shown that Ritalin can interfere with the growth and development of the reproductive system and can also have a serious and harmful effect on sperm parameters, so we decided to conduct studies in this field on the human sample.
Materials and Methods: In a case–control study, 100 adult men aged 21–31 years with hyperactivity were divided into two groups of 50 past users and 50 current users and, 50 patients who had not used Ritalin before were included as the control group. Data were analyzed using SPSS software, version 20. Analysis of variance, Pearson correlation coefficient, and regression analysis was used to assess the correlation between variables.
Results: The results also showed that there was a statistically significant difference between the current users and the control group in terms of sperm count, abnormality, and motility ($P<0.47$). Comparison of the user group in the past and the control group showed that there was no statistically significant difference in terms of sperm count ($P<0.59$), but there was a significant difference in terms of sperm motility and abnormality between the two groups ($P<0.001$).
Conclusion: The present study showed that long-term use of Ritalin can have negative effects on sperm parameters in humans.
Keywords: Ritalin, Sperm, Psychiatric, Motility, Semen Quality

P-25: Interferon-Associated Inflammatory Signatures in Sertoli Cell-Only Syndrome: Integrative Transcriptomic Analysis and Peripheral Blood Validation of IFI6

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Background: Sertoli cell-only syndrome (SCOS), histologically termed germ cell aplasia, represents the most severe form of non-obstructive azoospermia and is characterized by complete germ cell depletion within seminiferous tubules. Although spermatogenic failure defines SCOS, its molecular basis remains poorly understood. Emerging evidence suggests that immune and inflammatory pathways contribute to the SCOS testicular microenvironment. This study aimed to characterize interferon-associated inflammatory signatures in SCOS and determine whether these alterations are reflected in peripheral blood.
Materials and Methods: Three testicular microarray datasets (GSE45887, GSE45885, GSE145467) and one RNA-seq dataset (GSE224929) were analyzed to identify differentially expressed genes (DEGs) in SCOS. Functional enrichment and protein–protein interaction analyses were performed to identify inflammatory pathways and hub genes. Top hub genes were evaluated in an independent single-cell testicular RNA-seq dataset using pseudobulk expression analysis. Immune infiltration patterns were assessed using CIBERSORT. Based on network centrality and single-cell expression patterns, IFI6 was selected for peripheral blood validation. IFI6 mRNA expression was quantified by RT-qPCR in peripheral blood samples from 10 SCOS patients and 5 controls.
Results: A total of 214 overlapping DEGs were identified across datasets ($|log2FC| > 1$, adjusted $p < 0.05$). Enrichment analysis demonstrated significant activation of interferon-associated and innate immune pathways, including interferon and interferon-gamma signaling. Protein interaction analysis identified IFI6 as a central hub gene within the most significant interaction module. Independent single-cell analysis confirmed marked upregulation of major hub genes in SCOS, with IFI6 showing the highest patient-to-control expression ratio. Immune infiltration analysis revealed increased CD8 T cells, activated NK cells, monocytes, and mast cells in SCOS samples. Peripheral blood RT-qPCR further demonstrated significant upregulation of IFI6 in SCOS patients compared with controls.
Conclusion: This integrative multi-cohort transcriptomic study identified prominent interferon-associated inflammatory signatures and altered immune infiltration patterns in SCOS. Peripheral blood upregulation of IFI6 suggests a systemic correlate of the testicular interferon signature and supports further investigation of IFI6 as a potential non-invasive biomarker of SCOS-associated immune dysregulation.
Keywords: Sertoli Cell-only Syndrome, Non-obstructive Azoospermia, Interferon Signaling, Transcriptomic Analysis

P-26: Isolation and Characterization of Extracellular Vesicles Derived from Human Sertoli Cells in Non-Obstructive Azoospermia

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Background: Extracellular vehicles (EVs) are critical mediators of intercellular communication, carrying bioactive molecules that reflect the physiological state and origin of their parent cells. Sertoli cells, which are essential for maintaining testicular homeostasis and supporting spermatogenesis, actively secrete EVs that may regulate germ cell development. However, EVs derived from human Sertoli cells (HSerCs), particularly in the context of non-obstructive azoospermia (NOA), remain poorly characterized.
Materials and Methods: In this study, Sertoli cells were isolated from testicular biopsy samples obtained from NOA patients using a differential plating technique. The identity and purity of the isolated cells were confirmed through immunocytofluorescence analysis of specific markers, including Vimentin, anti-Müllerian hormone (AMH), GATA4, and SOX9. Extracellular vesicles were subsequently isolated from conditioned media collected from HSerCs at passages 3 to 5 using differential ultracentrifugation. The isolated EVs were characterized using Western blotting, transmission electron microscopy (TEM), dynamic light scattering (DLS), and bicinchoninic acid (BCA) protein assay.
Results: Sertoli cells represented the predominant cell population by passage 2 and maintained stable growth through passage 5. The isolated EVs were positive for established EV markers CD63 and TSG101 and negative for calnexin, confirming minimal cellular contamination. DLS analysis revealed a heterogeneous size distribution with a Z-average diameter of 345.74 nm and a polydispersity index of 0.27, while number-based analysis indicated that over 94% of particles had a diameter of approximately 113 nm, consistent with small EVs. TEM confirmed the characteristic vesicular morphology. The protein concentration of EV samples was measured at 35 μg/mL.
Conclusion: Overall, this study successfully establishes a reliable approach for isolating and characterizing EVs from HSerCs in NOA patients, providing a foundation for future investigations into their functional roles in impaired spermatogenesis and their potential applications in male infertility diagnosis and therapy.
Keywords: Human Sertoli Cells, Extracellular Vesicles, Non-obstructive Azoospermia, Spermatogenesis

P-27: Integrative Transcriptomic and Experimental Validation Identifies Cell-Cycle and Immune-Related Hub Genes in Non-Obstructive Azoospermia

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Background: Male infertility is a significant public health concern, with non-obstructive azoospermia (NOA) representing the most severe clinical manifestation. Because testicular biopsy is invasive, it is very important to find reliable noninvasive biomarkers. The aim of this study was to identify significant apoptosis- and immune-related genes associated with NOA and evaluate their diagnostic potential in peripheral blood.
Materials and Methods: An integrated bioinformatics analysis was performed on three testicular microarray datasets (GSE145467, GSE45885, and GSE45887). Differentially expressed genes (DEGs) were identified using adjusted $P<0.05$ and $|\log2FC| > 1$. Functional enrichment and protein–protein interaction (PPI) network analyses were conducted to identify key pathways and hub genes. Among the identified genes, *TNFSF10* and *EIF2AK2* were selected for experimental validation. Their expression levels were assessed in peripheral blood samples from NOA patients, obstructive azoospermia (OA) patients, and healthy controls using quantitative real-time PCR.
Results: A total of 590 commonly upregulated and 45 commonly downregulated genes were identified in NOA. Functional enrichment analysis revealed significant involvement of pathways related to cell cycle regulation, cilium assembly, apoptosis, and immune response. PPI network analysis identified several hub genes, including *PTTG1*, *SPAG5*, *PLK1*, *SMAD2*, *TNFSF10*, and *EIF2AK2*. Experimental validation demonstrated that *TNFSF10* and *EIF2AK2* were significantly upregulated in the peripheral blood of NOA patients compared to OA patients and healthy controls. In contrast, both genes were downregulated in testicular tissue. Additionally, NF-κB expression in peripheral blood showed no significant change.
Conclusion: These findings suggest that *TNFSF10* and *EIF2AK2* may serve as promising noninvasive blood-based biomarkers for distinguishing NOA from OA. The observed dual expression pattern highlights the interplay between local testicular dysfunction and systemic immune activation. Integrating bioinformatics analysis with experimental validation provides deeper insight into the molecular mechanisms of NOA and may contribute to the development of targeted diagnostic and therapeutic strategies in male infertility.
Keywords: Male Infertility, Non-obstructive Azoospermia, TNFSF10, EIF2AK2

P-28: Influence of Radiofrequency Electromagnetic Radiation on Spermatogenesis and Sperm Function in Rodent Models: A Systematic Review

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Background: Infertility is increasing worldwide and has been associated with several lifestyle and environmental exposures, including radiofrequency electromagnetic radiation (RF-EMR). Because digital devices are often carried close to the body, possible effects on male reproduction remain a public health concern.
Materials and Methods: This systematic review addressed the PECO question: how does RF-EMR exposure affect male fertility in rodent models? Studies published up to December 2025 were searched in PubMed, Google Scholar, SpringerLink, ScienceDirect, and Scopus (PROSPERO: CRD42023417536). From 4,220 records, screening and updated searches yielded 88 eligible studies. Methodological quality and risk of bias were assessed using SYRCLE's Risk of Bias tool.
Results: Included studies used multiple rat and mouse strains exposed to 800–24,000 MHz RF-EMR, with reported specific absorption rates of 0.014–34 W/kg. Exposure durations varied and were mainly subchronic. Evaluated outcomes included sperm parameters, oxidative stress biomarkers, lipid peroxidation, DNA damage, apoptosis, testicular histopathology, and hormonal indices. Oxidative and reproductive alterations were reported in the included studies; however, findings were heterogeneous and limited by uncertainties in allocation, housing, blinding, dosimetry, and outcome assessment.
Conclusion: Current evidence suggests biologically plausible signals but does not support a uniform causal interpretation. Standardized, well-controlled studies are needed with comprehensive, clearly defined reproductive and fertility endpoints.
Keywords: Radiofrequency Electromagnetic Radiation, Male Fertility, Sperm, Rodents, Oxidative Stress

P-29: Selenium Nanoparticles Combined with Date Palm Pollen Upregulate Spermatogenesis-Related Genes (Sycp3, Tnp1, CatSper1) and Reduce Testicular Oxidative Stress in Mice

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Background: Nutritional interventions that reduce oxidative stress and enhance spermatogenesis gene expression are of interest in male infertility. Selenium nanoparticles (SeNPs) and date palm pollen (DPP) individually show pro-fertility effects, but their synergistic molecular action is unknown. This study investigated whether SeNPs combined with DPP improve expression of key spermatogenesis-related genes in a murine model.
Materials and Methods: Materials and Methods: Thirty adult male NMRI mice were divided into five groups (n=6 each): control (distilled water vehicle), SeNPs alone (0.2 mg/kg/day), DPP alone (200 mg/kg/day based on prior dose-response data), SeNPs+DPP (same doses), and positive control (vitamin E, 100 mg/kg/day). All treatments were given orally by gavage for 35

days (one complete spermatogenic cycle). On day 36 (24h after last gavage), testicular tissues and epididymal sperm were collected. Germ cell-enriched fractions were isolated prior to RNA extraction. Real-time PCR measured expression of Sycp3 (Synaptonemal Complex Protein 3, a meiosis marker), Tnp1 (Transition Nuclear Protein 1, a spermiogenesis marker), and CatSper1 (Cation Channel of Sperm, involved in motility and fertilization). Relative expression was calculated using the 2^{-} ΔΔCt method with β-actin as housekeeping gene. Testicular malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured colorimetrically. Sperm count, motility (CASA), and morphology were assessed. One-way ANOVA with Tukey's post hoc test was used ($P<0.05$).

Results: The SeNPs+DPP group significantly upregulated all three genes compared to control and single-treatment groups: Sycp3 (4.2-fold, $P<0.001$), Tnp1 (3.8-fold, <0.001), and CatSper1 (3.1-fold, $P<0.01$). MDA decreased by 58% ($P<0.001$) and TAC increased by 72% ($P<0.001$) in the combination group versus control. Sperm parameters improved: count (+85%), progressive motility (+92%), and normal morphology (+64%; all $P<0.001$). No hepatotoxicity was observed (ALT/AST within normal range).

Conclusion: The combination of SeNPs and date palm pollen upregulates meiotic and post-meiotic genes (Sycp3, Tnp1, CatSper1) while reducing testicular oxidative stress. A limitation is that fertility outcomes (litter size) were not assessed. This nutritional intervention may be a promising low-toxicity strategy for male infertility, pending live birth studies.

Keywords: oxidative stress, male infertility, Selenium nanoparticles, date palm pollen, spermatogenesis

P-30: Effects of Sumatriptan on Testosterone Levels, Spermatogenesis, and Oxidative Stress in Adult Male NMRIs

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Background: Sumatriptan is a selective serotonin 5-HT1B/1D receptor agonist widely used in the treatment of migraine. While its neurological effects are well characterized, limited information is available regarding its potential impact on male reproductive function. Given the involvement of serotonergic signaling in the regulation of the hypothalamic–pituitary–gonadal axis and testicular function, investigating the reproductive effects of sumatriptan may provide insight into its potential impact beyond neurological targets.

Materials and Methods: Twenty-four adult male mice were randomly divided into four groups: sham, control, and two sumatriptan-treated groups receiving 0.1 or 0.5 mg/kg/day orally for 28 consecutive days. Serum testosterone levels were measured using enzyme-linked immunosorbent assay (ELISA). Sperm parameters, including sperm count, motility, viability, and morphology, were assessed using standard laboratory methods. Testicular oxidative stress status was evaluated by measuring malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity. Histological examination of testicular tissue was performed to assess germ cell populations and somatic cell counts.

Results: Sumatriptan administration significantly increased serum testosterone levels and sperm count compared with control and sham groups. Testicular MDA levels were significantly reduced, while SOD activity was increased in sumatriptan-treated animals, indicating an improvement in antioxidant status. Histological analysis revealed increased numbers of primary spermatocytes and spermatids, particularly in the high-dose group. No significant differences were observed in sperm motility, viability, morphology, or Sertoli cell number among the experimental groups.

Conclusion: These findings suggest that sumatriptan may modulate male reproductive function in mice, potentially through endocrine regulation and attenuation of oxidative stress, without inducing adverse effects on sperm quality or testicular structure.

Keywords: Sumatriptan, Serotonin, Spermatogenesis, Male fertility, Oxidative stress

P-31: Evaluating the comparative efficacy of mitochondria-targeted (MitoQ and Mito-TEMPO) and untargeted (SOD and CoQ10) antioxidants on buck's sperm quality during the cooling storage period

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Background: Effective management of superior male small ruminants can be enhanced through the use of artificial insemination with chilled semen. This method functionality protects sperm under cold environment by alleviating sperm metabolism. However, high levels of polyunsaturated fatty acids (PUFAs) in the buck sperm plasma membrane can generate reactive oxygen species (ROS), making the sperm more vulnerable to oxidative stress, which decrease the potential quality of semen. An approach to preserve sperm quality during cooling storage is to enhance the chilled sperm medium by supplementing suitable antioxidants. The present study was conducted to evaluate the effects of mitochondria-targeted antioxidants (MitoQ and Mito-TEMPO) and untargeted antioxidants (superoxide dismutase and CoQ10) on buck sperm quality during cooling process.

Materials and Methods: Semen samples were collected and diluted in an extender, and assigned into eight groups. First group served as the control, while other groups were supplemented with: superoxide dismutase (100 U/mL), CoQ10 (1 μM), MitoQ at two concentrations (50 and 100 nM), Mito-TEMPO at two concentrations (50 and 100 μM), and a combination of MitoQ and Mito-TEMPO (50 /50 μM). Samples were then cooled to 5°C for up to 50 hours, and sperm membrane integrity, lipid peroxidation, and mitochondrial activity potential were investigated at 0, 25, and 50 hours of cooling storage

Results: The results showed no significant differences ($P>0.05$) among the groups at time 0. However, after cooling, the group supplemented with MitoQ and Mito-TEMPO (MQ/MT) exhib-

ited the highest (P≤0.05) levels of membrane integrity, and mitochondrial activity, as well as the lowest (P≤0.05) level of lipid peroxidation.
Conclusion: In conclusion, supplementing buck sperm cooling extender with a combination of 50 nM MitoQ and 50 μM Mito-TEMPO, as powerful mitochondria-targeted antioxidants, is an effective strategy to preserve sperm quality during cooling storage
Keywords: Cooling, Mito-TEMPO, MitoQ, Goat, Spermatozoa

P-32: Effect of Hesperidin on Inhibition of Caspase-3-Dependent Apoptosis and DNA Damage In Frozen–Thawed Spermatozoa of Asthenoteratospermic Men

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Background: Cryopreservation and thawing of spermatozoa is considered one of the major fertility preservation strategies in assisted reproductive techniques. However, this process can induce oxidative stress, leading to impairment of vital sperm parameters including DNA integrity and activation of apoptosis in sperm cells. Hesperidin, a natural antioxidant flavonoid, possesses protective effects against oxidative damage induced by freezing and thawing procedures. Therefore, the present study aimed to investigate the protective effect of hesperidin on inhibition of caspase-3 dependent apoptosis and DNA damage in spermatozoa of asthenoteratozoospermic men during freezing and thawing.
Materials and Methods: Semen samples were obtained from 94 men diagnosed with asthenoteratozoospermia at the infertility center of Sina Hospital, Arak, Iran, and randomly divided into three experimental groups: control group, frozen-thawed group, and frozen–thawed supplemented with 5 μM hesperidin. DNA fragmentation was assessed using the sperm chromatin dispersion (SCD) assay kit according to the manufacturer's instructions. Acridine orange staining was performed to evaluate sperm DNA denaturation (single-stranded versus double-stranded DNA). In addition, cleaved caspase-3 immunoreactivity was examined using an antibody against cleaved caspase-3. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P<0.05 was considered statistically significant.
Results: Freeze–thaw process significantly increased sperm DNA fragmentation and DNA denaturation compared with the control group. In addition, strong cleaved caspase-3 immunoreactivity was observed in the cytoplasm of frozen–thawed spermatozoa. Hesperidin supplementation significantly inhibited DNA fragmentation and DNA denaturation compared with the frozen-thawed group. Furthermore, cleaved caspase-3 immunoreactivity was markedly reduced in hesperidin-treated spermatozoa compared with frozen–thawed spermatozoa.
Conclusion: Hesperidin, as a potent antioxdant, exerts protective effects during human sperm cryopreservation by protecting spermatozoa against DNA damage and suppressing caspase-3-dependent apoptotic pathways. Therefore, supplementation of freezing medium with hesperidin may improve post-thaw sperm quality in asthenoteratospermic men during freezing and thawing.

Keywords: Apoptosis, Caspase-3, DNA Denaturation, DNA Fragmentation, Hesperidin

P-33: Effect of rutin on catSper1 and catSper2 gene expression in NMRI mice following treatment with methotrexate

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Background: Methotrexate (MTX) is a widely used chemotherapy drug that disrupts folate metabolism and increases reactive oxygen species (ROS), leading to oxidative stress, DNA damage, and altered gene expression. The sperm specific ion channel genes CatSper1 and CatSper2 are essential for sperm motility and fertility and are vulnerable to oxidative damage. Rutin, a flavonoid with strong antioxidant properties, can inhibit ROS and protect against oxidative stress. This study investigated whether rutin can protect and improve the expression of *CatSper1* and *CatSper2* genes in male NMRI mice treated with methotrexate.
Materials and Methods: In this study, 24 adult male NMRI mice with an average weight of 31 ± 3 g were randomly assigned to four groups: control, methotrexate (20 mg/kg), rutin (100 mg/kg), and methotrexate + rutin. Treatments were administered for 35 days and at the end of the treatment period, total RNA was extracted from testicular tissue and, after quality assessment, converted to cDNA. The expression levels of *CatSper1* and *CatSper2* genes were measured using Real-Time PCR, with GAPDH serving as the internal control. Data were analyzed statistically using SPSS software, one-way analysis of variance (One-Way ANOVA), and the Tukey's test. Differences in means were considered significant at P<0.05.
Results: The mean expression levels of the catSper1 and *catSper2* genes in testicular tissue indicated a significant decrease in the methotrexate group when compared to the control group (P<0.001). However, in the methotrexate + rutin group, a significant increase in the expression levels of catSper1 (P<0.001) and *catSper2* (P<0.05) was seen compared to the methotrexate group and reached to the control group. No significant difference in the mean expression of the catSper1 and catSper2 genes in testicular tissue was detected between the rutin and control groups (P>0.05).
Conclusion: Our data showed that methotrexate significantly decreased *catSper1* and *catSper2* gene expression in testicular tissue, while rutin co treatment restored their expression to near control levels. These findings suggest a protective antioxidant effect of rutin against methotrexate induced reproductive toxicity.
Keywords: Methotrexate, Rutin, CatSper1, CatSper2

P-34: Referral Indications and Preimplantation Genetic Testing for Aneuploidy Findings in a Cohort of Infertile Patients with Pericentric Inversion of Chromosome 9

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Background: Pericentric inversion of chromosome 9 is among the most frequently reported structural chromosomal variants, with an estimated prevalence of approximately 1–3% in the general population. Although this inversion has traditionally been regarded as a benign variation, its possible contribution to reproductive failure remains controversial. Evidence regarding its association with infertility, recurrent pregnancy loss, implantation failure, stillbirth, and fetal anomalies is still limited and inconsistent. Therefore, further clinical observations are needed to clarify its potential reproductive significance.
Materials and Methods: In this report, we describe 24 individuals carrying pericentric inversion of chromosome 9 who presented with different reproductive complications, including infertility, recurrent pregnancy loss, implantation failure, stillbirth, and fetal malformations. In addition, we present preimplantation genetic testing for aneuploidy (PGT-A) findings from embryos obtained from a male carrier of chromosome 9 inversion. The carrier's wife had a history of pregnancy losses and implantation failure. Embryonic chromosomal status was evaluated to determine whether balanced or unbalanced chromosomal abnormalities were present and to identify embryos suitable for transfer.
Results: PGT-A analysis was performed on four embryos from the male carrier couple. The results demonstrated random aneuploidies in all examined embryos. Notably, three of the four embryos showed chromosome 9 imbalances, suggesting a possible relationship between the parental inversion and abnormal chromosomal segregation during gametogenesis or early embryonic development. Consequently, no chromosomally normal or transferable embryos were identified in this cycle.
Conclusion: These findings support the possibility that pericentric inversion of chromosome 9, although often classified as a benign chromosomal variant, may be associated with adverse reproductive outcomes in selected individuals. The observation of chromosome 9 imbalances in embryos from a male carrier adds further evidence to the ongoing debate regarding its clinical relevance. However, larger studies are required to determine its true reproductive risk and guide genetic counseling.
Keywords: Pericentric Inversion Chromosome 9, Recurrent Pregnancy Loss, Implantation Failure, Preimplantation Genetic Testing, Male Infertility

P-35: Curcumin nanomicelles enhances sperm quality in Oligoasthenoteratozoospermia by reducing oxidative stress and regulating the expression of apoptosis-related genes during cryopreservation

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Background: Sperm cryopreservation is a fundamental component of assisted reproductive technologies (ART). Despite its clinical utility, the freeze–thaw process induces substantial structural and functional damage to spermatozoa. The generation of excessive reactive oxygen species (ROS) during cryopreservation leads to lipid peroxidation, mitochondrial dysfunction, DNA fragmentation, and activation of apoptotic pathways, ultimately reducing post-thaw sperm quality. Curcumin, a natural polyphenolic compound with potent antioxidant and anti-apoptotic properties, has limited bioavailability in its native form; however, nanoformulation into micelles enhances its solubility and biological efficacy. This study evaluated the protective effects of nanomicelle curcumin supplementation in sperm freezing medium on oxidative stress markers and apoptosis-related gene expression in OAT samples.
Materials and Methods: semen samples were obtained from 25 men diagnosed with oligoasthenoteratozoospermia according to WHO criteria. Each sample was divided into two groups: control (standard cryopreservation medium) and treatment (medium supplemented with 1 µg/mL nanomicelle curcumin). Following cryopreservation and thawing, sperm parameters were assessed. Oxidative stress status was evaluated by measuring malondialdehyde (MDA) levels as an index of lipid peroxidation and total antioxidant capacity (TAC). The relative expression levels of apoptosis-related genes, BAX (pro-apoptotic) and BCL-2 (anti-apoptotic), were quantified using qRT-PCR. Statistical analysis was performed with significance set at $P<0.05$.
Results: Supplementation with 1 µg/mL nanomicelle curcumin significantly improved post-thaw sperm motility and the percentage of normal morphologically compared with the control group ($P<0.05$). A significant reduction in MDA levels and a concomitant increase in TAC were observed in the treatment group ($P<0.05$), indicating attenuation of oxidative stress. Gene expression analysis revealed downregulation of BAX and upregulation of BCL-2 in the curcumin-treated samples.
Conclusion: Nanomicelle curcumin effectively mitigates cryo-induced oxidative damage and apoptosis in sperm from men with oligoasthenoteratozoospermia, leading to improved post-thaw quality. These findings support its potential application as a protective additive in sperm cryopreservation protocols and warrant further investigation into optimized dosing strategies and synergistic antioxidant combinations.
Keywords: Assisted Reproductive Technology (ART), Cryopreservation, Nanomicelle Curcumin, Oxidative Stress, Sperm Quality

P-36: Effect of Pennyroyal (Mentha pulegium) Extract on Post-Thaw Quality of Rooster Sperm

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Background: The objective of this study was to evaluate the effect of different concentrations of pennyroyal (Mentha pulegium) extract on the post-thaw quality of rooster sperm.
Materials and Methods: Semen samples were collected from healthy adult roosters by abdominal massage method. Ejaculates were evaluated for volume, concentration, motility and morphology and only those with an adequate initial quality were selected. Pooled approved ejaculates were diluted with a standard poultry semen extender containing cryoprotectant to minimize individual variation. The diluted semen was divided into four experimental groups, a control group without extract and three treatment groups supplemented with 25, 50 and 100 µg/mL of pennyroyal extract. Samples were equilibrated at 4°C, frozen and stored in liquid nitrogen until post-thaw evaluation. After thawing, sperm quality parameters such as total motility, progressive motility, viability, plasma membrane integrity, acrosome integrity and abnormal morphology were assessed.
Results: The results revealed that the pennyroyal extract had a significant effect on post-thaw sperm quality (P<0.05). The best results were observed in the treatment group of 50 µg/mL. The total motility, progressive motility, viability, and membrane integrity in this group were 68.2%, 34.6%, 71.4%, and 63.8% respectively. These values were higher than those seen in the control, 25 µg/mL and 100 µg/mL groups. The 25 µg/mL concentration showed an increase in some parameters compared to the control group but was less effective than the treatment at 50 µg/mL. The highest concentration (100 µg/mL) did not show any further improvement and might have reduced the beneficial effect of the extract. The positive effect of pennyroyal extract could be attributed to the presence of antioxidant compounds especially phenolic and flavonoid compounds that can reduce oxidative stress during the process of freezing and thawing. Cryopreservation has been shown to increase the production of reactive oxygen species and can damage the plasma membrane, mitochondria and acrosome of the sperm cell. Hence, adequate antioxidant supplementation could improve the survival of sperm after thawing.
Conclusion: In conclusion, the post-thaw quality of rooster sperm was significantly improved by pennyroyal extract especially at 50 µg/mL. This concentration can be suggested as a natural antioxidant additive for cryopreservation of rooster semen.
Keywords: Semen, Extender, Cryopreservation, Antioxidant

P-37: Effect of Olive Leaf (Olea europaea) Extract on Post-Thaw Quality of Ram Epididymal Sperm

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Background: The present study was conducted to investigate the effect of different concentration of olive leaf (Olea europaea) extract on post-thaw quality of ram epididymal sperm.
Materials and Methods: The diluted sperm samples were split into 4 groups: a control group with no extract and 3 treatment groups with 25, 50 and 100 µg/mL of olive leaf extract. After equilibration at 4 °C, samples were placed in 0.25 mL straws, frozen in liquid nitrogen vapour and stored in liquid nitrogen until thawing. Parameters of sperm quality such as total motility, progressive motility, viability, plasma membrane integrity, acrosome integrity, abnormal morphology and lipid peroxidation were evaluated by standard laboratory procedures after thawing:
Results: The effect of olive leaf extract on post-thaw ram epididymal sperm quality was significant (P<0.05). The best results were obtained in the group treated with 50 µg/mL. The total motility, progressive motility, viability, and plasma membrane integrity in this group were 63.8, 29.6, 66.4, and 58.7 respectively. These values were higher compared to the values registered in control, 25 µg/mL and 100 µg/mL groups. The positive effect of the olive leaf extract can be related to phenolic compounds, particularly oleuropein, which are able to reduce the oxidative stress occurring during the freezing and thawing processes. In conclusion, the addition of olive leaf extract in the freezing extender improved the post-thaw quality of ram epididymal sperm
Conclusion: In the present study, among the tested concentrations, 50 µg/mL was the most effective concentration and it could be suggested as a suitable natural antioxidant additive for cryopreservation of ram epididymal sperm.
Keywords: Epididymal Sperm, Olive Leaf Extract, Viability

P-38: Hesperidin Confers Protection against Titanium Dioxide Nanoparticle Induced Testicular Damage through Suppression of Oxidative Stress and Inflammation in Mice

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Background: Titanium dioxide nanoparticles (TiO2 NPs) can cross biological barriers and accumulate in different organs, including the testes, which may disrupt testicular structure and impair spermatogenesis. This study evaluates whether hesperidin, as an antioxidant and anti-inflammatory agent, can mitigate these TiO2-induced adverse effects in mice.
Materials and Methods: In this experimental study, 24 male NMRI mice were randomly divided into four groups: control, TiO2 NPs treated, hesperidin treated, and TiO2 NPs plus hesperidin treated. TiO2 NPs and hesperidin were administered orally at a dose of 100 mg/kg/day for 35 days. At the end of the treatment period, testicular tissue was collected for stereological evaluation, and serum was obtained to measure oxidative stress factors. The expression levels of *IL-6* and *NLRP3* genes were also evaluated. Data were analyzed using one way ANOVA followed by Tukey's post hoc test, with P<0.05 considered statistically significant.
Results: TiO2 NP exposure led to severe testicular impairment, evidenced by significant reductions in testis and tubular volumes, germinal epithelium height, and all spermatogenesis indices (P<0.001). Concurrent increases in interstitial volume, MDA levels, and inflammatory gene expression (IL-6 and NLRP3) (P<0.001) confirmed the induction of oxidative stress and tissue damage. Co administration of hesperidin signifi-

cantly improved stereological parameters and Spermatogenesis indices and reduced inflammatory gene expression.
Conclusion: Hesperidin attenuated TiO2 NPs-induced testicular toxicity by improving testicular architecture, increasing germ cell numbers, restoring antioxidant balance (by elevating TAC and reducing MDA levels), and suppressing inflammatory gene expression.
Keywords: Titanium Dioxide Nanoparticles, Hesperidin, Testis, Inflammatory Gene Expression, Mice

P-39: Effect of Watercress (Nasturtium Officinale) Extract on Post-Thaw Quality of Ram Sperm

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Background: The objective of this study was to evaluate the effect of different concentrations of watercress (Nasturtium Officinale) extract on post-thaw quality of ram sperm.
Materials and Methods: Semen samples were collected from mature and healthy rams by artificial vagina. Ejaculates were immediately evaluated for volume, concentration, mass motility, individual motility and morphology after collection. Only ejaculates of acceptable initial quality were used for the experiment. To minimize individual variation, pooled samples were diluted in Tris-citric acid-fructose extender containing egg yolk and glycerol as cryoprotectants. The diluted semen was divided into four experimental groups, one control group without extract and three treatment groups supplemented with watercress extract at concentrations of 25, 50 and 100 µg/mL. After equilibration at 4°C, semen samples were packed in 0.25 mL straws, frozen in the vapor phase of liquid nitrogen and stored in liquid nitrogen until testing. The quality parameters of the sperm after thawing were assessed by standard laboratory methods which included total motility, progressive motility, viability, plasma membrane integrity, abnormal morphology and lipid peroxidation.
Results: The results revealed that the addition of watercress extract to the freezing extender at the concentrations used did not significantly alter post-thaw sperm quality compared with the control group (P>0.05). Total motility values were 58.6% in control, 59.8% in 25 µg/ml, 60.4% in 50 µg/ml and 57.9% in 100 µg/ml groups. Progressive motility was 23.2%, 24.1%, 24.7% and 22.5% respectively. Similarly, small numerical differences in viability, and membrane integrity were observed among the groups, but not statistically significant. Watercress contains antioxidant compounds such as phenolics, flavonoids and vitamin C, but the concentrations used in this work were not able to improve the resistance of ram spermatozoa to freezing and thawing damages. This may be due to lack of antioxidant activity, inappropriate dosage or possible interaction between the components of the extract and the extender medium.
Conclusion: In conclusions the addition of watercress extract at concentrations of 25, 50 and 100 µg/mL did not significantly improve the post-thaw quality of ram sperm. Therefore, watercress extract was not effective as a cryoprotective antioxidant supplement for ram semen freezing in the conditions of this study.

Keywords: Watercress, Ram, Cryoprotectant, Sperm

P-40: Effect of Selenium on Sperm Motility and Apoptosis Related Gene Expression During Cryopreservation in Asthenospermic Men

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Background: The sperm freeze–thaw process is widely used in assisted reproductive technologies (ART), but cryopreservation induced thermal shock and oxidative stress can impair sperm quality and motility. Caspase 3, a key apoptotic executor, promotes sperm cell death following oxidative damage, whereas HSPA2 protects sperm against cellular stress. Altered expression of these genes is linked to reduced sperm function. This study evaluated the effect of selenium as a Stronge antioxidant on sperm motility and *Caspase 3* and *HSPA2* expression in cryopreserved sperm from asthenozoospermic patients.
Materials and Methods: In this *in vitro* experimental study, semen samples from 30 asthenozoospermic patients were evaluated. To control for biological variation, each sample was divided into three distinct aliquots: 1) Control group (fresh sample), 2) Standard cryopreservation (Freeze group), and 3) Cryopreservation supplemented with selenium (at a concentration of 2 µg/ml). Following freeze-thaw procedures, sperm motility was assessed according to updated WHO guidelines. Transcriptome-level changes in *HSPA2* and *Caspase-3* gene expression were analyzed using quantitative Real-Time PCR (qRT-PCR). Statistical analysis involved repeated measures analysis followed by the Bonferroni post hoc test, with significance defined at P<0.05.
Results: A significant decrease in the mean total and progressive sperm motility was observed in the Freeze group compared with the control group (P<0.001). In addition, compared with the control group, the Freeze group showed a significant increase in the mean expression of the pro apoptotic gene Caspase 3 (P<0.001) and a significant decrease in *HSPA2* gene expression. In contrast, the Freeze + selenium group showed a significant increase in progressive sperm motility compared with the Freeze group. Moreover, a significant upregulation of the sperm maturation and function related gene *HSPA2* and a significant downregulation of the apoptotic marker gene *Caspase* 3 (P<0.001) were observed in this group compared with the Freeze group.
Conclusion: Selenium supplementation during cryopreservation improved progressive sperm motility and modulated apoptosis related gene expression in asthenozoospermic samples. Selenium decreased the expression of the pro apoptotic gene *Caspase 3* and increased *HSPA2* expression. These findings suggest that selenium may reduce cryopreservation induced oxidative stress and apoptotic damage, thereby improving post thaw sperm quality.
Keywords: Asthenozoospermia, Selenium, Caspase3, HSPA2, Sperm Cryopreservation

P-41: Theophylline Improves Sperm Motility During the Freeze–Thaw Process in Men with Asthenozoospermia by Modulating the Expression of Apoptosis- and Stress-Related genes (*Caspase* 3 and *HSPA2*)

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Background: Sperm cryopreservation is widely used in assisted reproductive technology (ART), but it may induce oxidative stress, leading to cellular damage, activation of apoptotic pathways, and reduced sperm quality. Caspase 3, an apoptosis mediator, and HSPA2, a heat shock protein involved in sperm maturation and cellular stress protection, are important markers associated with sperm function. This study aimed to evaluate the effect of theophylline supplementation on sperm motility and the expression of *Caspase 3* and *HSPA2* genes in asthenozoospermic semen samples during cryopreservation.
Materials and Methods: In this experimental study, 30 semen samples were collected from men with asthenozoospermia aged 25–40 years attending the Royan Infertility Treatment Center in Qom, Iran. each sample was divided into three equal aliquots and assigned to three experimental groups: 1) Control (fresh), 2) Freeze (standard cryopreservation without supplementation), and 3) Freeze + Theophylline (cryopreservation in the presence of 0.05 mM theophylline). Following thawing, sperm motility was evaluated according to the World Health Organization (WHO) guidelines. Additionally, the expression levels of *Caspase-3* and *HSPA2* genes were measured and analyzed using the qRT-PCR technique. Statistical analysis of the data was performed using Repeated Measures Analysis and the Bonferroni post-hoc test, and means difference was consider significant at $P<0.05$.
Results: Total and progressive sperm motility decreased significantly in the Freeze group versus control ($P<0.001$). The Freeze group exhibited increased pro-apoptotic *Caspase-3* expression and decreased *HSPA2* expression compared to controls. Conversely, the "Freeze + Theophylline" group showed significantly improved progressive motility compared to the Freeze group. Furthermore, compared to the Freeze group, the treatment group demonstrated significant upregulation of *HSPA2* and significant downregulation of *Caspase-3* ($P<0.001$). These findings suggest that theophylline treatment partially mitigates the adverse effects of freezing on sperm quality and gene expression.
Conclusion: The findings indicate that adding theophylline before sperm cryopreservation reduces cryo induced damage in asthenozoospermic samples. It improves progressive motility and regulates apoptosis and stress related genes. Reduced *Caspase 3* and increased *HSPA2* expression suggest a protective effect, supporting theophylline as a potential Supplement to enhance cryopreserved sperm quality in ART.
Keywords: Theophylline, Asthenozoospermia, *Caspase3, HSPA2*

P-42: Evaluation of the Effects of Different Doses of Dipyridamole on the Motility and Viability of Normal Human Sperm during the Freeze–Thaw Process

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Background: Sperm cryopreservation is widely used in assisted reproductive technologies (ART), but the freeze–thaw process increases reactive oxygen species (ROS) and oxidative stress, impairing sperm motility, viability, and overall quality. Dipyridamole, a phosphodiesterase inhibitor, can elevate intracellular cAMP and has reported antioxidant properties. Therefore, this study aimed to evaluate the effects of different dipyridamole concentrations and determine the optimal dose for improving motility and viability of normal human sperm during the freeze–thaw process.
Materials and Methods: Semen samples were collected from 8 normozoospermic men aged 25–40 years who attended the Infertility Treatment Center of Jahad Daneshgahi, Qom, Iran. Following initial evaluation based on the World Health Organization (WHO) criteria, each sample was divided into six groups: a control group (fresh semen), a freezing group (without treatment), and freezing groups supplemented with dipyridamole at concentrations of 25, 50, 75, and 100 µM. The treated groups were incubated with dipyridamole for 60 minutes prior to the freezing process. Sperm parameters, including total motility and progressive motility, were assessed using a Computer-Assisted Sperm Analysis (CASA) system, and sperm viability was evaluated using eosin–nigrosin staining. Statistical analysis was performed using repeated-measures analysis followed by the Bonferroni post hoc test. Differences between means were considered statistically significant at $P<0.05$.
Results: In the freezing group, the mean percentages of total motility, progressive motility, and sperm viability showed a significant decrease compared with the control group ($p \leq 0.001$). In contrast, supplementation with 50 µM dipyridamole during the freezing process resulted in a significant increase in total motility, progressive motility, and sperm viability compared with the freezing group ($p \leq 0.01$). However, the other tested concentrations did not show a significant effect on these parameters compared with the freezing group ($P>0.05$).
Conclusion: A concentration of 50 µM Dipyridamole appears to be the optimal dose for reducing freeze–thaw–induced cryodamage and improving human sperm quality. This effect may occur through reduced oxidative stress and protection of sperm structures, highlighting the importance of dose optimization and pretreatment strategies before cryopreservation in ART.
Keywords: Dipyridamole, Sperm Cryopreservation, Sperm Motility, Sperm Viability

P-43: Determination of the Optimal Ethanol Concentration for Improving the Motility and Viability of Normal Human Sperm during Cryopreservation

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Background: Sperm cryopreservation is an important method for preserving male fertility and a key component of assisted reproductive technologies. However, the freeze–thaw process can increase oxidative stress and cause damage to cellular structures, leading to reduced sperm motility and viability. In recent years, the induction of mild stress before freezing has been proposed as a novel approach to enhance cellular resistance to more severe stresses. Therefore, the study aimed to investigate the effects of ethanol concentrations and determine the dose on overall motility and viability of sperm in the freeze–thaw process.
Materials and Methods: In this experimental study, semen samples were obtained from eight normozoospermic men aged 25–40 years at the Infertility Treatment Center of Jahad Daneshgahi, Qom, Iran. Each sample was divided into seven groups: control (fresh), freezing with cryoprotectant only, and freezing groups supplemented with ethanol (0.01%, 0.03%, 0.05%, 0.07%, 0.09% v/v). Samples were incubated for 60 minutes and then cryopreserved and thawed. Sperm motility was analyzed using a Computer-Assisted Sperm Analysis (CASA) system, while viability was assessed by eosin–nigrosin staining. Statistical analysis was conducted using repeated-measures analysis with Bonferroni post hoc testing, with significance set at $P<0.05$.
Results: The freeze-thaw group showed a significant decrease in the mean percentage of total and progressive motility, as well as the mean percentage of sperm viability ($p \leq 0.001$) compared to the control group. In contrast, the freeze + ethanol group with a concentration of 0.05% showed a significant increase in the mean percentage of sperm motility and viability ($p \leq 0.05$) compared to the freeze group. Other ethanol concentrations did not have a significant effect on the mentioned parameters compared to the freeze group ($P>0.05$).
Conclusion: Freeze–thawing significantly reduced sperm motility and viability. However, pretreatment with 0.05% ethanol before cryopreservation significantly improved both parameters compared with the freezing-only group. Mild ethanol-induced stress may enhance sperm resistance to cryodamage. Thus, 0.05% ethanol could improve sperm quality during cryopreservation, though larger studies are required to confirm these findings.
Keywords: Human Sperm Cryopreservation, Ethanol Pretreatment, Oxidative Stress, Sperm Viability

P-44: Evaluation of the Effects of Sperm Zeta Potential and Short Abstinence Period on Sperm DNA Fragmentation and Protamination

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Background: Sperm DNA fragmentation (SDF), characterized by DNA strand breaks, is a key factor affecting male fertility and embryo quality. Proper protamination, involving histone replacement with protamines, is essential for maintaining sperm DNA integrity. The zeta potential–based sperm selection method is an advanced ART technique that isolates high-quality sperm based on surface charge properties. In addition, ejaculatory abstinence duration may influence sperm DNA quality.This study aimed to evaluate the combined effect of short (three-hour) ejaculatory abstinence and zeta potential–based sperm selection on sperm DNA fragmentation and chromatin protamination in men with persistently high DFI (>25%) despite previous one-day short abstinence and antioxidant use.
Materials and Methods: Semen samples were collected from 30 infertile men with previous IVF failure and persistently high DFI (>25%) despite previous one-day short abstinence and antioxidant use, at the Royan Research Institute, before and after a three-hour abstinence period. Sperm selection was performed using the zeta potential method. DNA fragmentation and protamination status were assessed using SCD and CMA3 assays, respectively. Data were analyzed using a paired t-test, with statistical significance set at $P<0.05$.
Results: Short abstinence alone did not significantly reduce DFI. However, zeta potential–based sperm selection significantly reduced DFI at both time points ($P<0.05$): from 34.37% ± 0.065 before selection to 23.75% ± 0.1098 (first sample) and 26.58% ± 0.081 (second sample after three-hour abstinence). Zeta selection also significantly decreased the proportion of CMA3-positive sperm.
Conclusion: Three-hour abstinence is not effective for all patients, whereas zeta selection effectively reduces DFI and improves chromatin quality regardless of abstinence duration, suggesting it enhances the selection of sperm with superior genetic integrity.
Keywords: Male Infertility, Short Abstinence, Sperm DNA Fragmentation, Sperm Selection, Chromatin Protamination

P-45: Assessment of Agreement between SCSA and γH2AX in Measuring Sperm DNA Damage among Infertile MAssessment of Agreement between SCSA and γH2AX in Measuring Sperm DNA Damage among Infertile Menen

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Background: Sperm DNA fragmentation (sDF) has emerged as an important functional biomarker in the evaluation of male infertility. However, currently available assays assess different aspects of sperm chromatin integrity and DNA damage, raising concerns about their interchangeability and clinical interpretation. This study compared the sperm chromatin structure assay (SCSA) with flow cytometric detection of γH2AX (Ser139), a sensitive marker of DNA double-strand break (DSB) signalling, to determine their level of agreement and analytical bias.
Materials and Methods: Semen samples obtained from infertile men (n=30) were analyzed simultaneously using both assays and categorized according to the SCSA-derived DNA

fragmentation index (DFI) into low (<20%) and high (>20%) fragmentation groups. To establish a controlled oxidative damage model, aliquots from 10 low-DFI samples were treated with 4 mM hydrogen peroxide (H_2O_2) for 15 minutes alongside matched untreated controls. Correlation, concordance, and agreement between methods were evaluated using Spearman's correlation, Lin's concordance correlation coefficient (CCC), and Bland–Altman analysis.

Results: Both SCSA and γH2AX assays detected significant increases in sDF following oxidative stress induction. In experimentally damaged samples, a strong correlation was observed between the two methods (Spearman's ρ=0.778, P=0.0001), although concordance remained only moderate (CCC=0.441), indicating proportional bias. Bland–Altman analysis demonstrated that SCSA consistently yielded higher damage estimates than γH2AX, with increasingly wider limits of agreement at higher sDF levels. In clinical infertility samples, the association between assays was weaker (Spearman's ρ=0.421, P=0.0206) and concordance was poor (CCC=0.087), suggesting that the assays are not clinically interchangeable.

Conclusion: SCSA and γH2AX evaluate overlapping but biologically distinct dimensions of sperm DNA damage. While SCSA reflects global chromatin susceptibility, γH2AX specifically identifies DSB-related signalling. Combining γH2AX with SCSA may therefore improve characterization of sperm DNA damage patterns and enhance the diagnostic assessment of male infertility.

Keywords: Sperm DNA Fragmentation, SCSA, γH2AX, Oxidative Stress, Male Infertility

P-46: Seminal Fructose and Sperm DNA Damage in Infertile Men

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Background: Male infertility is a multifactorial condition, and conventional semen parameters may not fully reflect sperm functional quality. Seminal fructose, mainly secreted by the seminal vesicles, is an important energy substrate for sperm motility. However, elevated fructose levels may also be associated with metabolic imbalance, oxidative stress, and impaired sperm DNA integrity. This study aimed to evaluate the association between seminal fructose levels, semen parameters, and sperm DNA fragmentation index (DFI) in infertile men.

Materials and Methods: This observational cross-sectional study was conducted on 100 infertile men referred to Pooyesh and Royesh Fertility Center, Isfahan, Iran. Semen analysis was performed according to the WHO 2021 guidelines. Semen volume, sperm concentration, and total motility were assessed using computer-assisted sperm analysis (CASA). Sperm morphology was evaluated by Diff-Quik staining. Seminal fructose concentration was measured using the indole colorimetric method, and sperm DFI was assessed by sperm chromatin structure assay (SCSA). Correlations were analyzed using Spearman's rank correlation test, with statistical significance set at P<0.05.

Results: Seminal fructose levels showed a significant negative correlation with total sperm motility (r=-0.27, P=0.005), indicating that higher seminal fructose concentrations were associated with reduced sperm motility. In addition, seminal fructose was significantly and positively correlated with sperm DNA fragmentation index (DFI) (r=0.315, P=0.0014), suggesting that elevated seminal fructose may be associated with increased sperm DNA damage. These findings may reflect a possible link between increased seminal fructose, oxidative stress, impaired sperm DNA integrity, and potentially reduced sperm functional competence. No significant correlations were observed between seminal fructose levels and semen volume, sperm concentration, or sperm morphology (P>0.05).

Conclusion: Higher seminal fructose levels were associated with decreased sperm motility and increased DNA fragmentation in infertile men. These findings suggest that elevated seminal fructose may reflect metabolic or seminal vesicle-related disturbances. Moreover, unhealthy dietary patterns, particularly excessive intake of fructose-rich foods and beverages, may contribute to oxidative stress, increased DFI, and reduced male fertility potential.

Keywords: Seminal Fructose, DNA Fragmentation Index, Sperm Motility, Male Infertility

P-47: Effect of Freezing Height Above Liquid Nitrogen on Post-Thaw Quality of Ram Epididymal Sperm

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Background: This study aimed to evaluate the effect of three different freezing heights on the surface of liquid nitrogen on the quality of ram epididymal sperm after thawing. Testes were collected from clinically healthy adult rams immediately after slaughter and were transported to laboratory under controlled temperature.

Materials and Methods: Spermatozoa were collected from the cauda epididymis by the technique of slicing and washing. Only samples with acceptable motility, concentration and morphology were used after initial evaluation. The recovered sperm samples were pooled to minimize individual variation and diluted with a Tris-based extender containing glycerol as cryoprotectant and loaded in to 0.25 mL straws. Straws were randomly assigned to 3 freezing treatments and exposed to nitrogen vapour at 2, 4 and 6 cm above the surface of liquid nitrogen for 10 minutes before plunging into liquid nitrogen for storage. Sperm quality parameters i.e. total motility, progressive motility, viability, plasma membrane integrity, acrosome integrity and abnormal morphology were measured after thawing.

Results: The results showed that freezing height significantly affected several post-thaw sperm parameters (P<0.05). The best results were achieved for the 4 cm treatment group. The values for total motility, progressive motility, viability, and membrane integrity in this group were 64.8%, 32.4%, 61.7%, and 56.8% respectively. These values were higher than those of the 2 cm and 6 cm groups. The lower sperm quality at 2 cm may be due to an overly rapid cooling rate, which could increase cold shock and membrane damage. On the other hand, the slower freezing in the 6 cm group could be caused by the lower quality, as slower freezing leads to more ice crystal formation and osmotic

stress.
Conclusion: In conclusion, the results showed that freezing height is an important factor in the cryopreservation of ram epididymal sperm. Among the treatments tested, exposure of straws to nitrogen vapor at 4 cm above the surface of liquid nitrogen for 10 min was the most suitable freezing condition and improved the post-thaw sperm quality.
Keywords: Sperm, Cryopreservation, Ram, Motility

P-48: Effects of L-Carnitine Supplementation on Mitochondrial Fatty Acid Transport Gene Expression in Testicular Tissue of High-Fat Diet-Induced Obese Mice

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Background: Obesity induced by a high-fat diet (HFD) is associated with impaired mitochondrial function and altered lipid metabolism in testicular tissue, potentially affecting male reproductive health. This study investigated the effects of L-carnitine supplementation on the mRNA expression of mitochondrial fatty acid transporters in the testes of HFD-fed mice.
Materials and Methods: Immature male mice were randomly assigned into three groups: control group fed a standard diet, HFD-O group fed a high-fat diet for 30 weeks, and HFD-O+LC group fed a high-fat diet for 30 weeks and treated with oral L-carnitine (2000 mg oral solution, 250 mg/kg) during the final 8 weeks. The mRNA expression levels of genes involved in mitochondrial fatty acid transport, including fatty acyl-CoA synthetase (FACS), carnitine palmitoyltransferase 1 (CPT1), carnitine-acylcarnitine translocase (CACT), carnitine palmitoyltransferase 2 (CPT2), and carnitine acetyltransferase (CRAT), were evaluated in testicular tissue.
Results: FACS expression was significantly increased in the HFD-O+LC group compared with controls ($P<0.05$), with no significant differences among other groups. CPT1 and CPT2 expression levels were significantly decreased in the HFD-O group compared with controls ($P<0.05$), while L-carnitine supplementation significantly increased their expression compared with the HFD-O group ($P<0.05$), restoring levels comparable to controls. CACT expression was significantly increased in the HFD-O+LC group compared with both control and HFD-O groups ($P<0.05$), while no difference was observed between HFD-O and controls. CRAT expression was significantly increased in the HFD-O group compared with controls ($P<0.05$) and significantly decreased following L-carnitine treatment compared with HFD-O ($P<0.05$), with no significant difference relative to controls.
Conclusion: L-carnitine supplementation may improve mitochondrial fatty acid transport and partially reverse obesity-induced alterations in testicular tissue, suggesting a protective role in metabolic and reproductive dysfunction.
Keywords: Obesity, L-carnitine, Testis, Mitochondrial Fatty Acid Transport

P-49: Rutin Enhances Human Sperm Quality during Freeze-Thaw Process

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Background: Sperm cryopreservation is a key method for fertility preservation in assisted reproductive technologies. However, the freezing and thawing process can damage sperm cellular components and increase the production of reactive oxygen species (ROS), which negatively affects sperm quality. The use of antioxidants has been proposed as a protective strategy to improve sperm survival during cryopreservation. Rutin, a flavonoid with strong antioxidant properties, can reduce oxidative stress and mitigate the harmful effects of ROS on sperm. Therefore, this study aimed to investigate the effect of rutin supplementation on the quality of human sperm during the freeze–thaw process.
Materials and Methods: After liquefaction, semen samples from 30 fertile men with normal parameters were divided into three groups: fresh, freeze (cryoprotectant only), and freeze + rutin (cryoprotectant supplemented with 10 μM rutin). Samples in the freezing groups were cryopreserved using a human sperm freezing medium and a rapid freezing method. After thawing, sperm motility was assessed according to WHO criteria, viability was evaluated using eosin–nigrosin staining, and morphology was examined using the Diff-Quick staining kit. Data were analyzed using SPSS software with repeated-measures analysis and the Bonferroni post hoc test. A P of less than 0.05 was considered statistically significant.
Results: The mean sperm motility, viability and normal morphology in the freeze group showed a significant decrease compared to the fresh group ($P<0.05$). Whereas, in the freeze + rutin group a significant increase was observed in the mentioned parameters compared to the freeze group ($P<0.05$).
Conclusion: Sperm cryopreservation significantly reduced motility, viability, and normal morphology. However, supplementation of the freezing medium with rutin (10 μM) significantly improved these parameters during thawing. These findings suggest that rutin, due to its antioxidant properties, may reduce oxidative stress–related damage during cryopreservation and enhance post thaw sperm quality in assisted reproductive techniques.
Keywords: Rutin, Human Sperm Cryopreservation, Sperm Morphology, Sperm Motility, Sperm Viability

P-50: Investigating the Correlation Between Seminal Plasma Exosomes and Oxidative Stress Factors in Azoospermic Men

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Background: Male-related factors contribute to nearly 50% of infertility cases worldwide, with azoospermia representing the most severe manifestation of spermatogenic failure. Azoospermia, defined as the complete absence of spermatozoa in the ejaculate. Emerging evidence highlights the crucial role of seminal plasma exosomes—nano-sized extracellular vesicles secreted by epididymal and accessory gland epithelial cells—in regulating spermatogenesis, sperm maturation, and intercellular communication within the male reproductive tract. These vesicles carry proteins, lipids, and nucleic acids that may reflect testicular microenvironment status. Concurrently, oxidative stress has been recognized as a key contributor to male infertility, primarily through excessive reactive oxygen species (ROS) production and insufficient antioxidant defense. However, the potential relationship between seminal exosomal characteristics and oxidative stress biomarkers, such as malondialdehyde (MDA) and total antioxidant capacity (TAC), remains insufficiently elucidated.
Materials and Methods: In this study, semen samples were obtained from 20 men diagnosed with azoospermia and 20 age-matched fertile men. Seminal plasma exosomes were isolated using differential centrifugation techniques and characterized by dynamic light scattering (DLS) for size distribution and bead-based flow cytometry for surface marker validation (CD9 and CD63). Total exosomal protein concentration was quantified using the Bradford assay. Oxidative stress parameters were assessed by measuring MDA levels and TAC using standardized ELISA kits.
Results: Our findings revealed significantly elevated MDA levels and markedly reduced TAC levels in azoospermic men compared to healthy controls ($P<0.05$), indicating enhanced oxidative stress. Exosomes were successfully validated by the expression of CD9 and CD63 markers in both groups. Although no significant difference was observed in exosome size distribution, total exosomal protein concentration was significantly lower in the azoospermic group ($P<0.05$).
Conclusion: The present study demonstrates a significant association between increased oxidative stress and reduced seminal exosomal protein content in azoospermic men. These findings suggest that oxidative imbalance may adversely affect exosome biogenesis or cargo composition, contributing to impaired intercellular communication within the testicular microenvironment. This multifactorial interaction may represent an important mechanistic pathway in azoospermia and highlights the potential of seminal exosomes as diagnostic biomarkers in male infertility.
Keywords: Azoospermia, Seminal Plasma Exosomes, Oxidative Stress, Male Infertility

P-51: Effects of Hydrogen Peroxide–Induced Mild Oxidative Stress on Sperm Parameters in Cryopreserved Normozoospermic Samples

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Background: Sperm cryopreservation is an effective method for preserving male fertility. However, the cryopreservation process exposes sperm to physical and biochemical stresses that may impair sperm parameters. Preconditioning sperm with sublethal oxidative stress prior to cryopreservation has emerged as a potential strategy to enhance their resistance to cryo induced damage. This study aimed to evaluate the effects of mild oxidative stress induced by hydrogen peroxide on post thaw human normal sperm parameters.
Materials and Methods: Semen samples from 30 normozoospermic men aged 25–45 years who attended Rastak Infertility Treatment Center, Arak, Iran, were divided into three groups: fresh (not cryopreserved), freeze (cryopreserved without treatment), and freeze + hydrogen peroxide (treated with cryoprotectant containing 1 μM hydrogen peroxide). Cryopreservation was performed using a rapid freezing protocol. Sperm motility was assessed by light microscopy according to World Health Organization (WHO) criteria. Sperm viability was evaluated using eosin–nigrosin staining, and sperm morphology was assessed using the Diff Quick staining kit. Data were analyzed using repeated measures analysis followed by the Bonferroni post hoc test. The significant level was considered at $P<0.05$.
Results: In the freeze group, mean sperm motility, viability, and normal morphology were significantly reduced compared with the fresh group ($P<0.05$). In contrast, the freeze + hydrogen peroxide group showed a significant increase in the mentioned parameters compared with the freeze group ($P<0.05$).
Conclusion: Mild oxidative preconditioning with hydrogen peroxide may reduce cryo induced damage and improve post thaw sperm motility, viability, and morphology in normozoospermic samples. Controlled exposure to low oxidative stress before cryopreservation may enhance sperm cryosurvival.
Keywords: Mild Oxidative Stress, Hydrogen Peroxide, Sperm Cryopreservation, Sperm Parameters

P-52: Measurement of Mitochondrial Membrane Potential of Sperm and Its Relationship with Sperm ROS Production and Sperm DNA Fragmentation in Individuals with Normozoospermia and Asthenozoospermia: A Case-Control Study

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Background: Infertility is an increasing concern globally, affecting approximately 15% of couples Around the worldwide. This project aims to delve deeper into these aspects and exam-

ine the relationship between the role of Mitochondrial Membran Potential (MMP), reactive oxidation species (ROS), DNA Fragmentation Index (DFI) in fertility and infertility status.
Materials and Methods: A case-control study collecting semen samples from 84 individuals, such as 45 normozoospermia and 39 asthenozoospermia, Following the sperm analysis, the extent of DNA fragmentation was evaluated. MMP and ROS levels were measured using flow cytometry.
Results: The results of the nonparametric Mann-Whitney test showed that the MMP and ROS levels in the normozoospermia group were significantly higher than those in the asthenozoospermia group (P=0.001 and P=0.008, respectively). While the results of the parametric T-test showed that the mean DFI level in the asthenozoospermia group (19.43 ± 6.24) was not significantly different from that in the normozoospermia group (16.98 ± 7.56) (P=0.111).
Conclusion: Our results show that higher MMP levels are associated with better sperm motility and function, reinforcing the idea that mitochondrial health is critical for optimal sperm function. High levels of reactive oxygen species (ROS) can be beneficial under certain conditions, implying a delicate balance between oxidative stress and sperm function. Further research is necessary to clarify the complex relationships between these parameters and their implications for male fertility.
Keywords: Infertility, Sperm Motility, Mitochondria, DNA Fragmentation, Ros

P-53: Gonadoprotective Efficacy of Aloe vera Gel in Streptozotocin-Induced Diabetic Rats via Modulation of Oxidative Stress, Testosterone Levels, and Sperm Quality

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Background: Diabetes mellitus compromises male fertility through hyperglycemia, oxidative stress, and endocrine dysregulation, impairing spermatogenesis, sperm quality, testicular histoarchitecture, and testosterone levels. Consequently, antioxidant and cytoprotective natural compounds are gaining attention to attenuate diabetic gonadal injury. Although Aloe vera exhibits diverse tissue-protective effects, its mechanistic efficacy against diabetes-induced reproductive impairment remains poorly elucidated. Therefore, this study evaluated the gonadoprotective efficacy of Aloe vera gel in streptozotocin-induced diabetic rats.
Materials and Methods: 48 adult male Wistar rats were randomly assigned into six experimental groups :control, Aloe vera gel–treated healthy groups (50 and 100 mg/kg), diabetic, and diabetic rats treated with Aloe vera gel (50 and 100 mg/kg). Diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg). Aloe vera gel was orally administered for 56 consecutive days.At the end of the experimental period, animals were euthanized, and blood, testicular tissue, and epididymal sperm samples were collected for hormonal, histopathological, biochemical, and reproductive assessments. Serum testosterone levels and oxidative stress biomarkers, including malondialdehyde (MDA), ferric reducing antioxidant power (FRAP), and catalase activity, were evaluated. Histomorphometric analyses were performed to quantify seminiferous tubule diameter.In addition, sperm count, motility, viability, chromatin/DNA integrity, and nuclear maturity were assessed using epididymal sperm samples. Fertility potential was further evaluated through *in vitro* fertility analysis.
Results: Histomorphometric and biochemical analyses demonstrated that diabetes markedly impaired testicular structure, antioxidant status, and sperm quality, as evidenced by reduced seminiferous tubule diameter, Leydig cell density, FRAP levels, catalase activity, sperm motility, and viability, alongside increased lipid peroxidation, sperm DNA damage, and chromatin immaturity ($P<0.05$). Treatment with Aloe vera gel significantly ameliorated these alterations and improved IVF outcomes, with the 100 mg/kg dose showing the greatest protective efficacy ($P<0.05$).
Conclusion: Aloe vera gel exerts potent gonadoprotective and antioxidant effects against diabetes-induced reproductive dysfunction. By mitigating oxidative stress, preserving testicular histoarchitecture, and ameliorating sperm quality and chromatin integrity, Aloe vera gel substantially enhances *in vitro* fertilization potential, representing a promising therapeutic agent for managing diabetes-associated male infertility.
Keywords: Diabetes Mellitus, Aloe Vera Gel, *In Vitro* Fertilization, *In Vitro* Fertilization,Male Infertility

P-54: Optimal Leydig Cell Conditioned Medium Concentration Enhances Spermatogonia Differentiation in Agarose Cultured Immature Mouse Testis

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Background: Leydig cells regulate spermatogenesis via androgens and paracrine signals; replicating these effects *in vitro* may improve spermatogonia differentiation. Background: To identify the optimal Leydig cell conditioned medium (LC CM) concentration for supporting spermatogenesis in immature NMRI mouse testicular tissue cultured on agarose.
Materials and Methods: Leydig cell lines (Pasteur Institute; passages 1-3) were cultured and medium was collected after 72 hours to produce LC CM. Testes from 3-5 day old NMRI mice were fragmented and placed on 1.5% soft agar. Tissue explants were cultured 28 days with LC CM at 0% (control), 10%, 25%, or 50%. Endpoints included qPCR for Sycp3, 3βHsd, Acrosin,

Vasa, Sox9, Bcl2, and Bax; histology staining (H&E) for spermatogenesis; ELISA for testosterone; and immunohistochemistry for 3βHSD, ACROSIN, and SYCP3 in the 10% group
Results: Histological evaluation revealed the presence of round spermatids in the control, 10% and 25% LC-CM groups, with less tissue damage and better structural integrity observed in the 10% LC-CM group. On day 21, testosterone levels were elevated in both the control and 10% groups; however, by day 28, the 25% group had the highest levels ($P<0.05$). Acrosin showed a significant expression in the 10% group, while Vasa and Sycp3 were highest in the 25% group; 3βHsd expression was significantly increased at 50% LC CM group ($P<0.05$). Moreover, Sox9 was upregulated in all LC CM groups versus control. Immunohistochemistry confirmed presence of spermatogenic markers in 10% and 25% groups.
Conclusion: A 10% LC-CM supplement in agarose-based culture optimally balanced tissue preservation, steroidogenic activity, and the progression of spermatogonia differentiation to round spermatids in immature mouse testicular culture, indicating its suitability for *in vitro* support of early spermatogenesis.
Keywords: Immature Mouse Testicular Tissue, Leydig Cells, Conditioned Medium, *In Vitro* Spermatogenesis

P-55: Liposomal Kinetin Nanoparticles as a Novel Cryoprotective Strategy to Improve Post Thaw Human Sperm Quality

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Background: Cryopreservation of sperm is one of the major parts of ART (Assisted Reproductive Technology); however, the freezing and thawing procedures cause significant damage because of oxidative stress, osmotic stress, and ice crystal formation that lead to dysfunction and damage of genetic material. Kinetin, being an antioxidant cytokinin, was shown to have a protective effect during cryopreservation experiments; however, there were not sufficient studies concerning the usage of kinetin in a nano-formulated form for cryopreservation of human spermatozoa. The objective of this study was to examine the protective effect of liposomal nanoparticles loaded with kinetin on post-thaw sperm parameters.
Materials and Methods: In the present study, 20 samples of normozoospermic semen were studied. Nanoparticles based on liposomes with encapsulated kinetin were prepared and analyzed via dynamic light scattering (DLS) and Fourier transform infrared spectroscopy (FTIR). Samples were divided into six groups: control, freezing media, empty liposomes control, and three kinetin nanoparticle groups (0.01, 0.1, and 1 mg/mL). Samples were subjected to rapid freezing using liquid nitrogen vapor phase and direct contact with liquid nitrogen. Sperm analysis for parameters including motility, viability, morphology, DNA fragmentation, chromatin integrity, and intracellular ROS was conducted based on WHO 2021.
Results: Nanoparticle characterization indicated the successful preparation of nanoparticles with the appropriate size and encapsulation efficiency. Among all the different concentrations used, 0.1 mg/mL kinetin nanoparticles exhibited the highest protective effects. Compared to the freezing control group, 0.1 mg/mL kinetin nanoparticles showed a remarkable improvement in progressive motility and viability of sperm. DNA fragmentation and chromatin immaturity were also remarkably lower in the kinetin nanoparticle-treated groups than in the freezing controls. Besides, the intracellular ROS content was significantly lower in the kinetin nanoparticle-treated groups than in the freezing controls. However, higher concentrations at 1 mg/mL did not show any protective effect.
Conclusion: The cryodamage in human spermatozoa was successfully inhibited using kinetin-loaded liposomal nanoparticles due to improvement in post-thaw functional properties and maintenance of genome integrity via reduction in oxidative stress. Kinetin nano formulation shows promise as a sperm cryopreservation technique.
Keywords: Kinetin, Liposomal Nanoparticles, Sperm Cryopreservation, DNA Fragmentation, Oxidative Stress

P-56: Evaluation of Ferroptosis-Associated Biochemical Markers in Seminal Plasma of Normozoospermic and Asthenozoospermic Men

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Background: Ferroptosis is a type of programmed cell death driven by lipid peroxidation that has been implicated in various pathological conditions, including male infertility. In asthenozoospermia (AZS), oxidative stress and disrupted iron homeostasis may promote ferroptotic damage to spermatozoa. Malondialdehyde (MDA) has been reported to be elevated in the seminal plasma of AZS samples, reflecting the activation of ferroptosis. Ferritin protein and total antioxidant capacity (TAC) are other nodes in the ferroptosis pathway. This study aimed to compare seminal plasma levels of MDA, ferritin, and TAC between normozoospermic and asthenozoospermic men and to examine their correlations with sperm progressive motil-

ity (PR%).
Materials and Methods: Seminal samples were obtained from 34 normozoospermic controls and 40 men diagnosed with AZS according to WHO 2021 criteria. Seminal plasma was isolated by centrifugation at 10,000 × g (rcf) and the supernatant was stored at −80°C until analysis. Sperm PR% was assessed under a light microscope. MDA was measured using a colorimetric thiobarbituric acid (TBA) assay kit, and TAC was determined by a colorimetric ferric reducing antioxidant power (FRAP) kit. Ferritin concentration was quantified using a chemiluminescence immunoassay.
Results: MDA levels were significantly higher in the AZS group compared with controls (P<0.05) and showed a negative correlation with PR% (r=-0.302, P<0.01). Seminal TAC was paradoxically elevated in the AZS group (P<0.05) and also correlated negatively with PR% (r=-0.236, P<0.05). Ferritin concentration was markedly higher in AZS samples (P<0.001) and displayed the strongest negative association with PR% (r=-0.355, p=0.001).
Conclusion: This is the first study to concurrently quantify seminal plasma MDA, ferritin, and TAC as a ferroptosis-oriented biochemical panel in AZS. Our findings confirm prior reports of elevated MDA and ferritin in AZS and, notably, reveal a counterintuitive increase in TAC. The negative correlations between all three markers and progressive motility suggest that ferroptosis-associated molecular alterations may contribute to impaired sperm motility. This biomarker triad warrants further investigation as a diagnostic and therapeutic target in male infertility.
Keywords: Ferroptosis, Male Infertility, MDA, Ferritin, TAC

P-57: Comparison of Fresh and Frozen-Thawed Embryo Transfer Obtained from TESE-ICSI Treatment Cycles in Men with Non-Obstructive Azoospermia

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Background: Non-obstructive azoospermia (NOA) is a severe form of male infertility. Testicular Sperm Extraction (TESE) combined with Intracytoplasmic Sperm Injection (ICSI) offers affected men the possibility of biological parenthood; however, treatment outcomes remain variable. This study evaluated clinical outcomes and identified predictors of reproductive success in NOA patients undergoing TESE-ICSI, with a comparison of fresh and frozen embryo transfer cycles.
Materials and Methods: A retrospective analysis was conducted on 170 TESE-ICSI treatment cycles performed at a tertiary fertility center. All cycles involved embryos derived from testicular sperm obtained from men diagnosed with NOA and subsequently cryopreserved by vitrification. Patients were categorized according to the use of fresh or frozen-thawed testicular sperm. Embryological parameters, including fertilization rate and embryo morphology, were assessed alongside clinical outcomes, including implantation, clinical pregnancy, live birth, and miscarriage rates. Multivariable logistic regression analysis was used to identify independent predictors of treatment success and pregnancy loss.
Results: Fresh embryo transfer cycles achieved significantly better outcomes than frozen cycles. A higher proportion of good-quality embryos (Grade A/AB) independently predicted clinical success (OR=1.016, 95% CI: 1.006–1.027, p=0.002). Each additional embryo increased the likelihood of success by 20.7% (OR=1.207, p=0.002), whereas increasing paternal age reduced the odds of a favorable outcome (OR=0.945, p=0.041). Fertilization rate was not an independent predictor after adjustment (p=0.090). While embryo quality was not associated with miscarriage risk, a greater number of embryos transferred was linked to higher odds of pregnancy loss (p=0.016).
Conclusion: Embryo quantity and morphological quality are the strongest independent predictors of reproductive success in NOA patients undergoing TESE-ICSI, whereas advancing paternal age adversely influences outcomes. Fresh embryo transfer cycles achieve superior results compared with frozen cycles. These findings underscore the importance of comprehensive embryo assessment and optimized embryo transfer strategies to maximize live birth rates while minimizing miscarriage risk in couples affected by NOA.
Keywords: Non-Obstructive Azoospermia, Testicular Sperm Extraction, Intracytoplasmic Sperm Injection, Frozen Embryo Transfer

P-58: Microvesicles derived from Seminal plasma Modulate Human Sperm Quality After Vitrification: Functional and molecular Parameters Based Assessment

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Background: The advancement of assisted reproductive technologies (ART) has been crucial for the treatment of infertility. Vitrification represents an innovative technique for sperm cryopreservation. Evidence suggests that seminal plasma (SP) and microvesicles derived from Seminal plasma (MVs-SP) modulate sperm function, specifically motility and viability. The present study aimed to evaluate the effect of supplementing the vitrification medium with MVs-SP and SP
Materials and Methods: The experimental procedure involved the isolation of MVs from SP, followed by the determination of SP and MVs concentrations and the characterization of extracted MVs in terms of size and morphology. Subsequently, these components were incorporated into the vitrification medium at concentrations of 50 mM and 100 mM for both SP and MVs-SP. Following thawing, assessments were conducted on sperm total and progressive motility, viability, plasma membrane integrity, DNA fragmentation, and the expression levels of apoptosis-related genes (*BAX,BCL2,CASPAE3,* and *CASPASE9*).
Results: The results indicated that the lower concentration (50

mM) of MVs-SP led to significant improvements in sperm total and progressive motility, viability, and plasma membrane integrity compared to the control group (P<0.05), and also regulated the expression of genes associated with apoptosis. In contrast, only the higher concentration (100 mM) of SP demonstrated a significant effect.
Conclusion: These observations suggest that MVs derived from SP are more effective at enhancing post-thaw sperm quality at a lower concentration compared to SP alone.
Keywords: Microvesicles, Q-PCR, Seminal Plasma, Vitrifictaion

P-59: Taurine Improves Sperm Membrane, Acrosomal and DNA Integrity During Swim-Up Preparation

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Background: Sperm preparation is a critical step in assisted reproductive technology (ART); however, the process often induces oxidative stress, which can compromise sperm motility, viability, and overall fertilizing potential. Antioxidant compounds, such as taurine, are being investigated for their ability to mitigate these detrimental effects. The present study was done to evaluate the impact of adding varying concentrations of taurine to the preparation medium on the functional parameters of human sperm during the swim-up process.
Materials and Methods: Semen samples from 10 normozoospermic individuals were divided into five equal aliquots. Each aliquot was processed using the swim-up method in media (Ham's F10) supplemented with different concentrations of taurine (0, 1, 3, 5, and 7 mM). Post-preparation, the sperm samples were analyzed for motility, morphology, viability, plasma membrane integrity, acrosome integrity, and DNA integrity.
Results: The addition of 5 mM taurine to the preparation medium significantly improved sperm motility, viability, plasma membrane integrity, and acrosome integrity (P<0.05) compared to the control group. Additionally, 3 mM taurine significantly enhanced viability and plasma membrane integrity. No significant differences were observed in sperm morphology or DNA integrity across the different taurine concentrations compared to the control.
Conclusion: Incorporating taurine into the sperm preparation medium, specifically at an optimal concentration (5 mM), can effectively enhance the quality and functional integrity of human sperm prepared via the swim-up method, potentially improving outcomes in ART procedures.
Keywords: Taurine, Sperm Preparation, Swim-up Method, Antioxidant, Assisted Reproductive Technology (ART)

P-60: Reduced Expression of ARRDC5 in Spermatozoa of Teratozoospermic Men: A Potential Molecular Marker for Sperm Morphology Defects

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Background: Teratozoospermia, a condition characterized by abnormal sperm morphology, is a major contributor to male infertility. Animal studies suggest that ARRDC5, an alpha-arrestin family gene, is crucial for normal spermatogenesis and sperm structure. However, its role in human teratozoospermia remains unclear. This study aimed to compare ARRDC5 expression in spermatozoa of normozoospermic versus teratozoospermic men.
Materials and Methods: Semen samples were collected from 26 normozoospermic (control) and 26 teratozoospermic (case) men attending Royan Institute after informed consent. Specimens were obtained by masturbation following 2–7 days of abstinence. *ARRDC5* expression was measured by real-time PCR, with β-actin as the housekeeping gene. Relative expression was calculated using the ΔΔCt method. An independent t-test was performed using SPSS version 21, with $p \leq 0.05$ considered significant.
Results: Mean relative expression of *ARRDC5* was significantly lower in the teratozoospermic group (0.72 ± 0.49) compared to controls (1.14 ± 0.60). The difference between groups was statistically significant (p=0.009).
Conclusion: Our findings show a marked reduction in *ARRDC5* expression in spermatozoa of men with teratozoospermia. This suggests that ARRDC5 dysregulation may be involved in abnormal sperm morphology in humans and could serve as a potential molecular biomarker for teratozoospermia.
Keywords: *ARRDC5*, Teratozoospermia, Sperm Morphology, Male Infertility, Gene Expression

P-61: Effects of cumulus cells-derived extracellular vesicles on sperm quality parameters following chemotaxis-based sperm selection

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Background: In the female reproductive tract, several physical and chemical barriers prevent low-quality spermatozoa from reaching and fertilizing the oocyte. However, in assisted reproductive technologies (ART), these natural selection barriers are bypassed. Therefore, precise selection of spermatozoa is essential for improving ART outcomes. In the nature, oocyte and its

surrounding cumulus cells secrete chemoattractants that direct spermatozoa toward the site of fertilization. Previous studies have shown that chemotaxis-based sperm selection enhances both sperm quality and fertilization potential. In this study, we aimed to select spermatozoa based on their chemotactic response to extracellular vesicles secreted by cumulus cells, using a simple microfluidic system.

Materials and Methods: Cumulus cells were collected from 10 women undergoing ICSI procedures for infertility treatment. The inclusion criteria were: women under the age of 40, follicle count above 10, and no history of endometriosis, polycystic ovary syndrome, or malignancy. Cumulus-oocyte complexes were dissected, and the cells were dispersed using hyaluronidase enzyme. The cell suspensions were cultured for 24 hours in FBS-free medium. The culture supernatants were then collected, and extracellular vesicles were isolated using a commercial exosome isolation kit. Sperm parameters including concentration, total motility, progressive motility, viability and DNA integrity were evaluated before and after sperm exposure to 5, 25 and 50 µg/ml concentration of extracellular vesicles.

Results: Sperm selection disks containing 25 µg/ml cumulus cells extracellular vesicles showed the significantly higher sperm concentration, total motility and progressive motility compared to control group. There were no statistically significant differences between the study group regarding sperm viability and membrane integrity. Moreover, percentage of sperm cells with large, medium halo which indicate sperm DNA intactness were not significantly differed between the control and study groups.

Conclusion: Our findings suggest that cumulus cell derived extracellular vesicles particularly at a concentration of 25 µg/ml, possess significant chemoattractant properties that can selectively enrich sperm populations with improved concentration, total motility and progressive motility. These results highlight the potential of cumulus cell derived extracellular vesicles as a novel tool for selecting high-quality spermatozoa for ICSI procedure.

Keywords: Cumulus Cells, Extracellular Vesicles, Chemotaxis, Sperm Selection

P-62: Selenium Attenuates Human Sperm Cryoinjury Through Antioxidant and Anti-Autophagic Mechanisms

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Background: Sperm cryopreservation is a common and important procedure in assisted reproductive technology (ART) laboratories. The freezing-thawing process causes serious detrimental changes in sperm function. The aim of the present study was to investigate the beneficial effect of sodium selenite (Se) antioxidant on sperm parameters, DNA damage, autophagy, and total antioxidant capacity in freeze-thawed sperm.

Materials and Methods: In this study, sperm samples from 20 healthy individuals were collected according to ethical criteria in the infertility treatment department of Firouzabadi Hospital and divided into two groups: semen samples without selenium and those with sodium selenite (5 µg/ml). Subsequently, semen analysis was performed before and after the freezing process using Computer-Assisted Sperm Analysis (CASA) software. After thawing, DNA damage was evaluated using the Sperm DNA Fragmentation Assay (SDFA) kit, and Total Antioxidant Capacity (TAC) was evaluated via colorimetric assay using an ELISA reader. Additionally, the expression level of LC3 protein was assessed by Western blotting.

Results: The results showed that selenium antioxidant improved sperm parameters, including sperm progressive motility, total motility, and viability, compared to the group without selenium. Moreover, selenium was associated with higher values of total antioxidant capacity. DNA integrity was significantly affected by selenium, as in Se-treated samples, DNA fragmentation was decreased compared to the group without selenium. Western blot results showed a significant decrease in LC3 protein expression in the selenium-frozen group.

Conclusion: In conclusion, this work provides evidence to better understand the impact of cryopreservation on sperm quality and identifies three consequences of cryopreservation injury to human sperm: impaired motility, DNA damage, and autophagy. The addition of antioxidants to sperm cryopreservation media can improve sperm cryopreservation protocols for use in ART laboratories and help overcome various adverse situations resulting from chemotherapy, radiotherapy, and surgery.

Keywords: Sperm, Sodium Selenite, Freeze, Autophagy

P-63: Investigating the of Effect of Rosmarinic Acid on Human Sperm Parameters Following Vitrification

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Background: Cryopreservation plays an important role in fertility preservation and assisted reproductive technologies. Among available methods, vitrification has gained attention due to its rapid cooling rate and ability to prevent intracellular ice crystal formation. Despite these advantages, sperm vitrification is still associated with cellular injuries, mainly caused by excessive production of reactive oxygen species (ROS) during the freezing and warming processes. Elevated oxidative stress can adversely affect sperm motility, viability, membrane integrity, and DNA quality. Rosmarinic acid is a naturally occurring phenolic compound with well-documented antioxidant properties. Owing to its ability to neutralize free radicals and reduce oxidative damage, rosmarinic acid may represent a promising additive for improving sperm quality following vitrification.

Materials and Methods: In this experimental study, semen samples were collected from normozoospermic men aged 20–35 years following 2–5 days of sexual abstinence. After initial

semen processing and sperm selection using the Swim-Up technique, the samples were divided into four groups: one control group without rosmarinic acid and three experimental groups supplemented with 50, 100, and 200 µM rosmarinic acid, respectively. Samples were vitrified in liquid nitrogen and evaluated one week later after warming. Sperm quality parameters, including motility, viability, membrane integrity, DNA integrity, antioxidant capacity, and lipid peroxidation, were assessed using routine laboratory methods.

Results: Rosmarinic acid at a concentration of 50 µM demonstrated a tendency to preserve sperm quality against the adverse effects of Among the tested concentrations, 50 µM RA appeared to produce the most favorable outcomes; however, these improvements were not statistically significant compared with the control group. RA treatment was associated with improved sperm viability and membrane integrity, although the observed differences did not reach statistical significance. Similarly, the proportion of sperm with intact DNA was higher in the 50 µM group, but this increase was not statistically significant.

Conclusion: Overall, these findings suggest that rosmarinic acid may partially protect sperm against oxidative stress and cryopreservation-induced damage.

Keywords: Rosmarinic Acid, Cryopreservation, Verification, Sperm Quality

P-64: Evaluation of Rosmarinic Acid on Total Antioxidant Capacity and Lipid Peroxidation in Vitrified Human Sperm

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Background: Oxidative stress is one of the major factors contributing to sperm damage during cryopreservation procedures. Although vitrification minimizes ice crystal formation, excessive generation of reactive oxygen species (ROS) during cooling and warming can disrupt the oxidant-antioxidant balance, leading to lipid peroxidation and impaired sperm function. Rosmarinic acid (RA), a naturally occurring polyphenolic compound, has attracted attention due to its potent antioxidant properties and its potential role in protecting cells against oxidative damage. This study aimed to evaluate the effects of RA supplementation during sperm vitrification on total antioxidant capacity (TAC) and malondialdehyde (MDA) levels.

Materials and Methods: In this experimental study, semen samples were collected from normozoospermic men aged 20–35 years following 2–5 days of sexual abstinence. After sperm selection using the Swim-Up technique, samples were allocated into four groups: a control group and three treatment groups supplemented with 50, 100, and 200 µM RA. Following vitrification and storage in liquid nitrogen, samples were warmed after one week. Oxidative stress-related parameters, including TAC and MDA levels, were assessed using standard laboratory assays.

Results: Supplementation of vitrification media with RA resulted in slight alterations in oxidative stress markers compared with the control group. The 50 µM concentration showed a tendency toward increased TAC and reduced MDA levels following warming. However, no statistically significant differences were observed among the experimental and control groups regarding either TAC or MDA values ($P>0.05$).

Conclusion: The findings of this study suggest that although rosmarinic acid may exert modest antioxidant effects during sperm vitrification, its supplementation at the tested concentrations did not significantly modify total antioxidant capacity or lipid peroxidation levels. Further studies with larger sample sizes and different treatment protocols are warranted to clarify the potential role of RA in reducing cryopreservation-induced oxidative stress.

Keywords: Verification, Rosmarinic Acid, Malondialdehyde, Total Antioxidant Capacity

P-65: Serum Total Antioxidant Capacity and Sperm Morphology in Aluminum Chloride-Exposed Male NMRI Mice Following Silymarin Co-administration

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Background: Aluminum chloride exposure has been linked to oxidative imbalance and male reproductive toxicity. Silymarin is a plant-derived flavonolignan complex with antioxidant and membrane-stabilizing properties; however, its protective effects on sperm structural quality following aluminum exposure require further experimental evaluation. Among sperm structural defects, head abnormalities are particularly relevant because the sperm head contains the nucleus and acrosome, both of which are essential for fertilization. This focused analysis aimed to evaluate serum total antioxidant capacity and sperm morphology in aluminum chloride-exposed male NMRI mice following silymarin co-administration.

Materials and Methods: Twenty-four adult male NMRI mice were divided into four groups (n=6/group): control, aluminum chloride ($AlCl_3$), silymarin, and $AlCl_3$ + silymarin. Treatments were administered orally by gavage for four weeks. The $AlCl_3$ group received aluminum chloride at 100 mg/kg body weight, the silymarin group received silymarin at 50 mg/kg body weight, and the combined group received $AlCl_3$ followed three hours later by silymarin. At the end of the treatment period, serum total antioxidant capacity (TAC) was measured, and epididymal sperm morphology was assessed. Morphological evaluation included normal morphology, abnormal morphology, and head, neck, and tail abnormalities. Data distribution and homogeneity of variances were assessed, and normally distributed data were analyzed using one-way ANOVA followed by Tukey's post hoc test, and $P<0.05$ was considered statistically significant.

Results: Serum TAC differed significantly among groups ($p=0.00004$). The $AlCl_3$ group showed the lowest TAC value (0.0610 ± 0.0056 µmol/mL), while TAC was higher in the silymarin-only group (0.1210 ± 0.0154 µmol/mL) and in the $AlCl_3$ + silymarin group (0.1165 ± 0.0078 µmol/mL). In sperm mor-

phology analysis, the $AlCl_3$ group showed a higher percentage of sperm head abnormalities compared with the $AlCl_3$ + silymarin group (25.66 ± 4.95% vs. 13.88 ± 3.99%, respectively; P<0.05). Normal sperm morphology was numerically higher in the $AlCl_3$ + silymarin group compared with the $AlCl_3$ group, whereas neck and tail abnormalities did not show significant differences among groups.

Conclusion: Silymarin co-administration was associated with improved serum antioxidant status and reduced sperm head abnormalities in aluminum chloride-exposed male NMRI mice. These findings suggest a partial protective effect of silymarin on oxidative and structural reproductive alterations associated with aluminum chloride exposure.

Keywords: Aluminum Chloride, Silymarin, Total antioxidant Capacity, Sperm Morphology, Sperm Head Abnormality

P-66: Relationship between ARSA Gene Expression in Sperm and IUI Outcome: A Preliminary Analysis

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Background: Intrauterine insemination (IUI) is a first line assisted reproductive technique (ART) with 10-15% success rate. Arylsulfatase A (ARSA) is a sperm protein that translocates to the sperm surface during capacitation and facilitates sperm–oocyte binding by interacting with sulfated glycans on the zona pellucida. This study evaluates the relationship between sperm ARSA gene expression and IUI outcome.

Materials and Methods: This ongoing cohort study initially planned to enroll 60 infertile men (age <49 years, BMI ≤30 kg/m²) whose partners underwent IUI. For this preliminary analysis, we included 15 men (8 with successful IUI, 7 with failed IUI based on serum beta hCG at 14 days post IUI). Exclusion criteria were leukocytospermia, varicocele, or reproductive tract infections. Ethics approval was obtained from the Royan Institute. Excess semen samples on the day of IUI were obtained from the Embryology lab of Royan Institute. Sperm RNA was extracted with TRIzol, cDNA synthesized using SMOBio kit. Gene expression was quantified by real-time PCR in three technical replicates and analyzed using the $2^{-\Delta\Delta Ct}$ method, employing β-actin as the housekeeping gene. Groups were compared using independent t-test (P<0.05 significant).

Results: Sperm ARSA expression was significantly higher in the IUI success group (1.09 ± 0.42, n=8) than in the IUI failure group (0.15 ± 0.14, n=7); mean difference=0.94 [95% CI: 0.48–1.40], p = 0.001 (two tailed).

Conclusion: These preliminary findings suggest a strong association between increased sperm ARSA expression and IUI success, supporting the role of ARSA in sperm–oocyte interaction. Larger sample sizes are required to confirm this biomarker potential.

Keywords: IUI, ARSA, Sperm, Gene Expression

P-67: Interleukin-10 (IL-10) Levels in Seminal Plasma in Relation to IUI Outcome

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Background: Successful fertilization and embryo development require a balanced immune environment in the male reproductive tract. Seminal plasma contains various cytokines that help regulate the immune system, support sperm function and maintain reproductive health. One of these cytokines, interleukin-10 (IL-10), is an anti-inflammatory and immune-modulating factor that may protect sperm against inflammatory and oxidative damage. Intrauterine insemination (IUI) is a widely used assisted reproductive technique that several immunological factors have been suggested to influence its outcome. This study aimed to evaluate the association between seminal plasma IL-10 levels and IUI outcomes.

Materials and Methods: This cohort study involved 46 infertile men (BMI ≤30 kg/m², age ≤45 years old) whose partners were undergoing IUI at the Royan Institute. The study was approved by the Royan Institute Ethics Committee. Excess of semen samples were obtained on the day of IUI from embryology laboratory, seminal plasma separated and stored at −20 °C until evaluation. Success was defined as a positive serum beta hCG two weeks after IUI. Concentrations of IL-10 in seminal plasma was measured by enzyme-linked immunosorbent assay (ELISA) method in duplicate. Data were analyzed with SPSS using independent t-tests and results are reported as mean ± standard deviation (SD). A P less than 0.05 is considered statistically significant.

Results: Seminal plasma IL-10 levels were 85.5 ± 90.7 pg/mL in the IUI success group (n=16) and 95.3 ± 73.86 pg/mL in the IUI failure group (n=30), with no significant difference between two groups (P=0.6). Statistical evaluation revealed that power of this sample size was 99%. Regression analysis confirmed no significant association between IL-10 concentration and IUI outcome.

Conclusion: The findings suggest that seminal plasma levels

of IL-10 may not directly affect IUI outcome nor serve as a reliable predictive biomarker for IUI success. Given the complex nature of the reproductive immune environment, a thorough evaluation of multiple cytokines and immune mediators is needed to clarify the relationship between immune regulation and IUI success.

Keywords: Male Infertility, IUI, IL-10, Seminal Plasma

P-68: The Relationship Between Seminal TNF-α Concentration and IUI Outcome

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Background: Intrauterine insemination (IUI) is widely used as a first-line treatment for infertile couples; however, predicting its outcome remains challenging. One of the factors that may influence fertilization outcome is seminal plasma, which contains various bioactive components, particularly cytokines. In recent years, inflammatory cytokines present in seminal plasma have attracted attention due to their possible effects on sperm function and fertility. Tumor necrosis factor-alpha (TNF-α) is one of the major inflammatory cytokines involved in oxidative stress-related sperm dysfunction. The present study aimed to investigate the relationship between seminal TNF-α concentration and IUI outcome.

Materials and Methods: In this cohort study, semen samples were collected from infertile men (BMI ≤30 kg/m², age ≤45 years) whose partners underwent IUI cycles at Royan Institute. We included 46 men (16 with successful IUI, 30 with failed IUI based on serum beta hCG at 14 days post IUI). Following sample collection on the day of IUI, seminal plasma was separated and samples were stored at −20°C until evaluation. TNF-α concentration was measured using enzyme-linked immunosorbent assay (ELISA) with two technical replicates. Statistical analysis was performed using independent T-test in SPSS software, and P <0.05 was considered statistically significant.

Results: Seminal plasma TNF α concentration was higher in the IUI success group (165.74 ± 135.51 pg/mL, n=16) compared to the IUI failure group (130.67 ± 38.84 pg/mL, n=30). This difference was not statistically significant (p = 0.3; 95% CI for the difference: −65.2 to +135.3 pg/mL).

Conclusion: According to these preliminary data, no significant association was observed between seminal TNF-α concentration and IUI outcome in the studied population. For definite conclusion, completion of sample size is needed.

Keywords: TNF-α, Semen, IUI, Cytokine, Infertility

P-69: The Effect of Cryopreservation on miR-34b-5p Expression in Spermatozoa from Normozoospermic and Teratozoospermic Men

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Background: Teratozoospermia is a prevalent cause of male infertility and is frequently associated with impaired sperm function and reduced fertilization potential. Although sperm cryopreservation is an essential component of assisted reproductive technologies (ART), the freeze–thaw process induces membrane disruption and molecular alterations that may be particularly detrimental in spermatozoa with pre-existing morphological abnormalities. MicroRNA-34b-5p (miR-34b-5p), a key post-transcriptional regulator involved in spermatogenesis, apoptosis, and sperm functional competence, has been implicated in male reproductive health. However, its expression profile and potential role in the cryopreservation response of teratozoospermic men have not yet been elucidated.

Materials and Methods: Normozoospermic and teratozoospermic men were enrolled according to the WHO 2021 criteria. Each semen sample was divided into fresh and cryopreserved aliquots. Sperm motility (CASA), morphology (Papanicolaou), viability (eosin–nigrosin), protamine deficiency (CMA3), and nuclear maturity (aniline blue) were assessed. miR-34b-5p expression was quantified in a representative subset using qRT-PCR. Data were analyzed using one-way ANOVA with Tukey's post hoc test or the Kruskal–Wallis test, with significance set at P<0.05.

Results: Cryopreservation significantly reduced sperm progressive motility, viability, and normal morphology, while increasing protamine deficiency and nuclear immaturity in both groups (P<0.001), with greater deterioration observed in teratozoospermic samples. miR-34b-5p expression differed significantly among the four groups (P<0.05). Expression levels were lower in the teratozoospermic group than in the normozoospermic group, regardless of cryopreservation status. However, cryopreservation did not significantly affect miR-34b-5p expression within either group (P>0.05), suggesting that miR-34b-5p abundance is associated with sperm morphology rather than cryopreservation-induced damage.

Conclusion: The teratozoospermic group exhibited consistently lower miR-34b-5p expression than the normozoospermic group in both fresh and cryopreserved samples. While cryopreservation significantly compromised conventional sperm parameters and chromatin integrity, it did not significantly affect miR-34b-5p expression. These findings suggest that reduced miR-34b-5p expression is associated with sperm morphological abnormalities rather than cryopreservation-induced damage, highlighting

its potential as a molecular marker of sperm quality in ART settings.

Keywords: Cryopreservation, MicroRNA, Sperm, Normozoospermia, Teratozoospermia

P-70: Effect of ROCK Inhibitor Y-27632 on Acrosome and Membrane Integrity of Human Sperm Following Vitrification

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Background: Sperm vitrification is an effective method for fertility preservation; however, cryopreservation-induced damage can impair sperm function by disrupting plasma membrane and acrosome integrity. The Rho-associated coiled-coil containing protein kinase (ROCK) signaling pathway is involved in cellular stress responses and cytoskeletal regulation. Inhibition of ROCK by Y-27632 has been shown to exert cytoprotective effects in various cell types. This study aimed to investigate whether supplementation with Y-27632 during vitrification could improve post-warming sperm membrane and acrosome integrity

Materials and Methods: Semen samples were collected from 20 normozoospermic men following 3–5 days of sexual abstinence. After routine semen analysis, samples were divided into four groups: control, and Y-27632-treated groups at concentrations of 5, 10, and 20 µg/mL. Samples were vitrified, stored for one week, and subsequently warmed. Sperm membrane integrity was assessed using the hypo-osmotic swelling test (HOST), while acrosome integrity was evaluated by FITC-PNA staining. Post-warming parameters were compared among groups.

Results: No significant differences were observed in sperm membrane integrity among the control and Y-27632-treated groups following warming ($P>0.05$). In contrast, Y-27632 supplementation improved acrosome preservation, with the 20 µg/mL concentration exhibiting the highest percentage of acrosome-intact spermatozoa compared with the other treatment groups. Although the protective effect was concentration-dependent, the improvement was more pronounced for acrosomal integrity than for membrane integrity

Conclusion: Y-27632 supplementation during sperm vitrification may contribute to the preservation of acrosome integrity, particularly at a concentration of 20 µg/mL. ROCK inhibition appears to have limited effects on plasma membrane integrity but may represent a promising strategy for reducing acrosomal damage during sperm cryopreservation.

Keywords: Vitrification, ROCK Inhibitor, Membrane Integrity, Acrosome Integrity

Animal

P-71: Investigating the Expression of Interferon-Stimulated Genes in Endometrium Mediated by IVF and SCNT Derived Blastocysts in *EX Vivo* Condition in Bovine Species

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Background: In bovine, embryos reach the blastocyst stage approximately on day 7 post-fertilization. While pre-blastocyst development is largely autonomous, subsequent development and survival rely critically on uterine secretions. Simultaneously, blastocyst-derived factors, notably interferon-tau (IFNT) modulate endometrial gene expression to facilitate maternal recognition of pregnancy. Embryos derived via Somatic cell nuclear transfer (SCNT) - frequently suffer from implantation failure, which is often attributed to defective embryo-maternal cross talk. This study aimed to compare the expression profiles of interferon-stimulated genes (ISGs) in bovine endometrial explants co-cultured with either *in vitro* fertilized (IVF) or SCNT-derived blastocysts, as well as their respective conditioned media (CM).

Materials and Methods: Bovine embryos were produced via IVF and SCNT. Blastocysts (n=20 per group and in 4 independent replicates) were co-cultured for 24 hours with endometrial explants obtained from cyclic heifers during the early-to-mid luteal phase (days 5–10 of the estrous cycle). Following co-culture, the CM from each group was collected and incubated with fresh endometrial explants. The relative mRNA expression levels of key ISGs (*MX1, MX2, OAS1Y, ISG15,* and *RSAD2*) in the endometrial tissues were quantified using real-time PCR.

Results: Direct co-culture with IVF blastocysts significantly upregulated the expression of all evaluated ISGs in the endometrial explants compared to the control group. Conversely, SCNT blastocysts elicited significantly lower expression levels of MX1, ISG15, and RSAD2. Although MX2 and OAS1Y were significantly upregulated by SCNT blastocysts, their expression remained markedly lower than that observed in the IVF group. Furthermore, treatment with IVF-derived CM significantly increased ISG expression, albeit at a lesser extent than direct co-culture. In contrast, CM derived from SCNT co-cultures induced negligible or non-significant alterations in ISG expression relative to the blastocysts resulted in minimal or non-significant changes in gene expression compared to control.

Conclusion: IVF-derived blastocysts demonstrate a superior capacity to induce endometrial ISG expression compared to their SCNT counterparts. Blastocyst-secreted factors, presumably including IFNT, retain their biological activity in the conditioned medium, emphasizing their vital role in embryo-maternal communication. The diminished ability of SCNT blastocysts to adequately stimulate endometrial ISG expression reflects a compromised maternal recognition of pregnancy, which likely contributes to their higher rates of implantation failure.

Keywords: SCNT, IVF, Endometrium, Bovine Blastocyst, Interferon-Stimulated Genes

P-72: The Comparison Between the Amino Acid Profiles of Spent Embryo Culture Medium (SECM) of SCNT Male

and Female Goat

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Background: Amino acid patterns in culture medium are a reflection of the metabolic activity of the embryo and can be used as a measure to investigate embryo metabolism. Non-invasive approaches using SECM (Spent Embryo Culture Media) are promising. The objective was to investigate the metabolism of amino acids in SECM of SCNT (Somatic Cell Nuclear Transfer) goat embryos in terms of differences in their uptake and release.
Materials and Methods: A total of 18 SCNT goat embryos (good grade), including 9 male goat embryos and 9 female goat embryos, were examined. They were first cultured in groups until day 5, and then individually from day 5 to 7. After the embryo was removed from the culture medium, a sample of the remaining SECM was collected for each embryo. Also, 3 samples of Blank culture medium (only incubated culture medium without embryos) and 3 samples of Fresh culture medium were collected. HPLC was used to determine the amino acid profile of SECM, and amino acid uptake and release were measured.
Results: In total, 20 amino acids were identified in SECM by HPLC. The amino acids included Aspartate, Asparagine, Arginine, Tyrosine, Tryptophan, Valine, and Isoleucine were significantly ($P<0.05$) decreased (uptake) compared to the average incubator culture medium. The amino acids, including Glutamine, Alanine, and Lysine, were significantly ($P<0.05$) increased (released) compared to the average incubator culture medium. The data also showed that other amino acids, including Glutamate, Histidine, Serine, Glycine, Threonine, Methionine, Phenylalanine, and Leucine, did not differ significantly in the SECM compared with the average incubator culture medium. Due to the very low concentrations of citrulline and ornithine in SECM, these two amino acids were omitted. Concentration in SECM was not significantly affected by embryo sex.
Conclusion: The uptake and release of amino acids by the embryo in SECM shed more light on embryonic metabolism, which is independent of sex. Our primary data pave the way for investigating the metabolism of SCNT embryos and warrant further study.
Keywords: Embryo Metabolism, SECM (Spent Embryo Culture Medium), SCNT (Somatic Cell Nuclear Transfer), Amino Acid Profile, Non-invasive Method

Embryology

P-73: Relationship between Sperm DNA Fragmentation and ICSI (Fertilization Until Day 2 and Day 3 of Cell Division)

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Background: Sperm DNA fragmentation (SDF) occurs during spermatogenesis due to defective chromatin condensation, aberrant apoptosis, and oxidative stress. Although male factor infertility accounts for nearly 50% of cases globally, standard semen analysis fails to identify about 15% of infertile men with normozoospermia. Evidence on the impact of high DNA fragmentation index (DFI) on ICSI outcomes remains conflicting. This study investigated the correlation between SCSA-measured SDF and early ICSI outcomes in couples with isolated male factor infertility, ≥2 failed IUIs (mean: 2.4 ± 0.7), normal WHO semen, female age <38 years, no severe endometriosis, and ≥5 mature oocytes.
Materials and Methods: This prospective study was conducted at Royan Institute on 50 patients undergoing a single ICSI cycle. On the day of ICSI, DFI was assessed using SCSA by flow cytometry. Patients were stratified into three groups: Group 1 (DFI<15%, n=15), Group 2 (DFI 15-25%, n=16), and Group 3 (DFI>25%, n=19). After exclusion criteria, 28 patients were included in embryo quality analysis. Embryo quality was assessed on day 3 using Royan grading. Statistical analysis used one-way ANOVA and chi-square test ($P<0.05$).
Results: Mean fertilization rate showed no significant difference among groups ($P>0.05$). Notably, one patient with DFI of 61.8% achieved 15 normal zygotes, confirming high DFI does not prevent fertilization. Regarding embryo quality, patients with good-quality embryos (Grade A/B) were 67% in Group 1, 56% in Group 2, and 60% in Group 3. Patients with DFI>25% produced lower-grade embryos (Grade C/D) significantly more often than those with DFI<15% ($P<0.05$), while the intermediate group showed no significant difference from either.
Conclusion: High DFI does not significantly impair fertilization in ICSI ($P>0.05$), but DFI>25% is significantly associated with reduced embryo quality ($P<0.05$). However, DFI assessment alone is insufficient to definitively predict embryo quality, as high-quality embryos can still be obtained even with very high DFI. Thus, DFI may serve as a useful but not absolute predictor of embryonic development in couples with prior IUI failure and normal semen analysis. Larger prospective studies are warranted.
Keywords: Sperm DNA Fragmentation, SCSA, ICSI, Embryo Quality

P-74: Mitigating Cryoinjury in Vitrified Mouse Ovaries Via Luteolin Supplementation and Alginate Encapsulation:

Early Post-Warming Functional Dynamics

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Background: Intact cryopreservation of ovarian tissue represents an essential approach in fertility preservation. However, this process may result in cryo-injury, causing follicle depletion, cell death through apoptosis, and impaired tissue functioning. The objective of this research was to examine the early post-thaw dynamics of cryoinjury in intact mouse ovaries and investigate the potential effectiveness of the developed approach based on a combination of biochemical and biomechanical treatment including supplementation with luteolin and alginate encapsulation.
Materials and Methods: Ovarian tissues from KM female mice (4–6 weeks old) were vitrified according to the two-stage protocol for cryoprotectant addition (7.5% and 15% EG + DMSO + 0.5 M sucrose). Intact samples of ovaries were maintained *in vitro* in culture for 0.5, 2, and 4 hours after thawing to examine the initial stage of injury in ovarian tissue dynamics. Eight groups were formed: untreated control, vitrified control, vitrified with different concentration of luteolin, and vitrified with alginate + luteolin. Evaluation of the degree of injury included histological examination (hematoxylin-eosin staining) and follicles counts, LDH activity determination, cleaved caspase-3.
Results: Vitrification resulted in notable disorganization and damage to the follicles and stromal cells immediately following warming, and subsequent degeneration continued throughout the four hours of culture. The increase in LDH production and caspase-3 activity was found to be progressive over time in the vitrified control group, proving that cryo-injury is a continuing event that extends past the warming phase. Supplementation with luteolin was able to maintain primordial and growing follicles to some degree, depending on the dosage, with 25 μM being the most effective concentration.
Conclusion: The results show that the early post-thawing stage can be considered an essential time point for intervention and indicate that using a combination of a flavonoid-derived antioxidant along with a biomaterial-based structural support can significantly minimize the effects of vitrification on ovarian tissues. These promising results may suggest that the dual-strategy method might be a promising clinical approach to enhancing outcomes of ovarian tissue cryopreservation.
Keywords: Ovarian Tissue Cryopreservation, Luteolin, Alginate Encapsulation, Vitrification, Apoptosis

P-75: Automated Embryo Quality and Implantation Prediction Using Ensemble Deep Learning on Time-Lapse Imaging

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Background: Despite significant advances in assisted reproductive technologies, the success rate of *in vitro* fertilization (IVF) remains suboptimal. Accurate embryo selection is a critical determinant of treatment outcome; however, conventional morphology- based assessment is subjective and may not adequately reflect dynamic embryonic development. Furthermore, manual interpretation of time-lapse imaging is time consuming and prone to inter-observer variability.
Materials and Methods: We developed an ensemble deep learning framework for automated embryo quality assessment and pregnancy outcome prediction using time-lapse embryo imaging. A total of 6,300 annotated blastocyst images obtained from 243 patients undergoing 527 embryo transfer cycles between 2022 and 2025 were analyzed. Four deep learning architectures, including ResNet-50, EfficientNet-B4, Vision Transformer (ViT-B16), and DenseNet-161, were trained to predict Gardner grading parameters, namely blastocyst expansion, inner cell mass (ICM), and trophectoderm (TE) quality. To enhance model robustness and mitigate class imbalance, transfer learning, focal loss, data augmentation, and self-supervised pretraining using SimCLR on over 100,000 unlabeled embryo images were employed. Morphokinetic features were further integrated through a decision-tree-based predictive model.
Results: The stacking ensemble achieved AUC values of 0.731 for blastocyst expansion grading, 0.734 for ICM classification, and 0.693 for TE assessment. For pregnancy outcome prediction, the ensemble model achieved an AUC of 0.610, outperforming all individual deep learning architectures. Prospective validation on independent clinical samples demonstrated consistent and stable predictive performance.
Conclusion: The proposed framework demonstrates the potential of combining ensemble deep learning with morphokinetic modeling to support automated embryo evaluation in IVF. By integrating morphological grading, developmental kinetics, and pregnancy prediction into a unified decision-support system, this approach may improve embryo prioritization and contribute to more objective and efficient embryo selection workflows.
Keywords: Embryo Selection, IVF, Deep Learning, Ensemble Learning

P-76: Human Follicular Fluid-Derived Extracellular Vesicles Improve *In Vitro* Maturation in a Mouse Model of Polycystic Ovary Syndrome

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Background: This study investigated the effects of enriching *in vitro* maturation (IVM) medium with human follicular fluid-derived extracellular vesicles (hFFEVs) on the maturation of immature oocytes in normal and polycystic ovary syndrome (PCOS) mouse models.
Materials and Methods: PCOS was induced in adult female NMRI mice using a single intramuscular injection of estradiol valerate. hFFEVs were isolated via differential ultracentrifugation and characterized. First, germinal vesicle (GV) oocytes

from normal mice were cultured with various hFFEV concentrations (0, 10, 20, and 40 µg/mL) to determine the optimal dose. Subsequently, GV oocytes from control and PCOS mice were allocated into four groups: Control (C), C+EV, PCOS (P), and P+EV. Nuclear maturation was assessed after 18 hours of culture.

Results: Supplementation with 20 and 40 µg/mL of hFFEVs significantly increased maturation rates compared to lower doses ($P<0.05$), with 20 µg/mL yielding the optimal outcome. The 40 µg/mL dose significantly increased degeneration and parthenogenetic activation ($P<0.05$). In the main experiment, hFFEV supplementation significantly enhanced nuclear maturation in the C+EV group compared to the C group (64.7% vs. 51.9%, $P<0.05$). The maturation rate in the PCOS group was significantly lower than that in the control group (39.0% vs. 51.9%, $P<0.05$), whereas EV treatment significantly improved maturation in the P+EV group compared to the P group (47.9% vs. 39.0%, $P<0.05$). Strikingly, no significant difference was observed between the P+EV and control groups

Conclusion: Supplementing IVM medium with hFFEVs (specifically at 20 µg/m) effectively enhances oocyte maturation. This approach represents a promising strategy to improve IVM outcomes and clinical efficiency in both normal and PCOS conditions.

Keywords: *In Vitro* Maturation, Polycystic Ovary Syndrome, Extracellular Vesicles, Follicular Fluid

P-77: A Magnetic cffDNA Kit for Non-invasive Embryo Assessment

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Background: Cell-free fetal DNA (cffDNA) serves as a powerful biomarker for non-invasive prenatal testing (NIPT) and the early diagnosis of fetal sex, genetic syndromes during the initial weeks of pregnancy. For analysis, cffDNA must first be extracted from the mother's blood plasma, but its low concentration poses challenges for its efficient extraction.

Materials and Methods: Considering the ease, rapidity, and high efficiency of magnetic extraction, the present project aims to develop kits containing Fe3O4-coated magnetic nanoparticles for the separation of cffDNA from the blood plasma of pregnant mothers that can reduce the aforementioned problems. Nanoparticles were synthesized using a hydrothermal method. All the steps required for extracting cffDNA from blood plasma using magnetic nanoparticles, including the addition of proteinase K, lysis buffer, binding buffer, washing buffer, and release buffer, were considered in the form of a magnetic cffDNA separation kit. The extraction and analysis of plasma were repeated 3 times. The performance of this kit was investigated using the gender detection kit and real-time PCR method, and compared with the commercial clinical kit used in NIPT tests. For this purpose, blood plasma samples of mothers with male fetuses were used, and DYS14 was assessed for its Ct. Also, HBB was used as an internal control gene to assess the presence of sufficient DNA in the extraction.

Results: Real-time PCR results showed that the developed kit provided a lower Ct than the commercial kit with the same amount of plasma. Mean Ct for the developed kit was 23.2 ± 0.17 compared to 24.58 ± 0.33 for the commercial kit. Also, this magnetic kit does not require advanced equipment or many centrifugation steps. It also requires less time and fewer steps, saving time and cost.

Conclusion: Due to its non-invasive nature, low cost, and rapid response, the developed kit can be used for NIPT, such as fetal gender detection, and has potential for aneuploidy screening like Down syndrome, in the first weeks of pregnancy (week 9 or 10).

Keywords: cffDNA, Magnetic Nanoparticles, NIPT, Liquid Biopsy

P-78: Human Follicular Fluid Enhances Cell Survival and Reduces Apoptosis in Vitrified–Warmed Human Ovarian Tissue *In Vitro* Culture

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Background: A large number of young women of reproductive age have been diagnosed with cancer, however recent advances in cancer diagnosis and the introduction of new chemoradiotherapy protocols have significantly increased the survival rates in both children and young women with cancer. These new advances had let scientists to find new methods to preserve female fertility. Among these methods, ovarian cryopreservation has proven to be practical, but there is still some challenges. Human follicular fluid presents itself as a biomolecule-rich reservoir which is predominantly composed of proteins, steroid hormones, and it also contains cytokines, enzymes, reactive oxygen species, growth factors, and metabolites like amino acids and lipids.

Materials and Methods: Human ovaries were provided from transsexual patiants with consent. Vitrified-warmed human ovarian tissue ($2\times2\times0.5$ mm^3) were devided into three groups: Control (the non-cultured fragments), Culture (fragments were cultured without follicular fluid), Culture+FF (fragments were cultured with 10% human follicular fluid). In Culture and Culture+FF, fragments were cultured for one week in Alpha-MEM, 1% ITS, 0.5 IU/ml FSH and 10% HSA. Every other day half of the media was changed and stored in -20 ◦C for further assessments. Concentration of Lactate dehydrogenase (LDH) on day 6, and the gene expression level for *BCL2* and *BAX*

genes were measured 5 times for each group.
Results: The expression level of *BCL2* was significantly increased in Culture+FF compared to the Control and Culture groups. The expression level of *BAX* was decreased in the Culture+FF group when compared to the Control and Culture groups, this difference was not significant. The anti-apoptotic *BCL2/BAX* ratio was 5.62 ± 4.56, 33.09 ± 20.47 and 149.7 ± 34.82 in Control, Culture and Culture+FF groups respectively and the difference between Control and Culture+FF and also Control and Culture groups were significante (P<0.05). The mean of lactate dehydrogenase on day 6 was 112 ± 62.5 IU/ml and 35.66 ± 61.8 IU/ml in Culture and Culture+FF groups, respectively, this difference was not significant.
Conclusion: Based on the results shown in this study, it can be concluded that human follicular fluid supports the survival of follicles and ovarian cells in human ovarian vitrified-warmed fragments.
Keywords: Human Follicular Fluid, Human Ovarian Tissue, Vitrification, *In Vitro* Culture, BCL2 And BAX

P-79: Individual or Group Culture of Human Embryo: Quantify Analysis of Spent Culture Medium and its Relationship to Embryo Growth

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Background: Individual and group culture of human embryo are designed for optimization preimplantation human embryo culture systems. Quantifying the spent culture medium (SCM) may provide insights into how individual or group culture conditions influence the biochemical landscape surrounding embryos and help determine which approach offers the greatest developmental benefit.
Materials and Methods: This cross-sectional study, analyzed SCM collected from embryos cultured for 72 and 96 hours. Controlled ovarian stimulation followed the GnRH antagonist protocol. After ovum pick-up, Intra Cytoplasmic Sperm Injection used by fresh sperm. Fertilization confirmation occurred by presence of two pronuclei. Then embryos were placed in droplets following: individual embryo culture; two embryos co-culture (2 e. co-culture); more than two embryos co-culture (> 2 e. co-culture). Following embryo transfer or freezing, SCM collected of embryos with 3 quality subgroups (Good, Fair, Poor) for miR-191-5p, total antioxidant capacity (TAC), and reactive oxygen species (ROS) measurements.
Results: Based on this study, after 72 and 96 hours of embryos culture, significant differences were observed in miR-191-5p, TAC, and ROS levels across embryo quality subgroups (expect in > 2 e. co-culture) within each culture group (P<0.05). Comparisons of Good-subgroups across culture groups (vs. control) showed significantly higher miR-191-5p and TAC, and lower ROS in co-culture conditions (P<0.05), the 2 e. co-culture exhibiting the highest miR-191-5p and lowest ROS.Pearson's correlation analysis revealed direct correlations between miR-191-5p and TAC (P<0.001). So, inverse correlations were observed between miR-191-5p and ROS (P<0.001), and between TAC and ROS (P<0.001).
Conclusion: These findings indicate that group culture, especially of 2 e. co-culture, yields superior miR-191-5p, and TAC levels in SCM compared to individual culture or larger groups (> 2 e. co-culture).
Keywords: Assisted Reproductive Technology (ART), Embryo Culture, Spent Culture Medium (SCM), Group Culture, Individual Culture

P-80: Wharton's Jelly Derived Mesenchymal Stem Cells Exosome Improve *In Vitro* Bovine Embryo Production

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Background: Since demanding for milk and meat production is increasing, development and application of IVF in bovine is progressing too. Despite this improvements, IVF technology still requires some modifications. In this study, we explored whether using exosomes extracted from mesenchymal stem cells (MSCs) could improve the quality of bovine embryos produced in a lab setting. They isolated exosomes from the Wharton's jelly MSCs of a human fetal umbilical cord and collected the conditioned media for exosome isolation. Using DLS, western blotting, TEM, and SEM, they confirmed that these MSCs secrete exosomes.
Materials and Methods: For the experiment, cumulus-oocyte complexes derived from a slaughterhouse were matured and fertilized *in vitro*. The resulting zygotes on day 3 were divided into three culture groups: Group I (conventional medium containing 5% FBS), Group II (conventional medium containing 2.5% FBS and 2.5% Wharton's jelly exosomes), and Group III (conventional medium containing 5% Wharton's jelly exosomes).
Results: The results showed no significant difference in cleavage rates across the groups. However, the blastocyst rate was significantly higher in Group II (23.24 ± 1.35) compared to Groups I, and III [17.07 ± 1.64 (P<0.05), and 11.38 ± 0.23 (P<0.05), respectively]. Additionally, the blastocyst cell number (ICM /TE %) was significantly higher in Groups II (28.13 ± 3.39) compared to the groups I and III [23.1 ± 3.23 (P<0.05) and 15.15 ± 2.37(P<0.05)].
Conclusion: The study concludes that MSCs secrete various beneficial growth factors, and incorporating MSC derived exosomes—which carry these factors—into the culture medium can effectively enhance the development and quality of bovine

embryos produced *in vitro*.
Keywords: Wharton's Jelly, Mesenchymal Stem Cells, Exosomes, *In Vitro* Embryo Production, Bovine

P-81: Trajectory-Guided Network Mining Reveals Early Developmental Control Points for Zebrafish Gonadal Repair

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Background: Reproductive repair requires coordinated restoration of germline identity, gonadal architecture, and immune remodeling. This study aimed to integrate network enrichment and developmental trajectory perturbation to identify regulatory checkpoints linking early embryonic patterning with zebrafish reproductive competence.
Materials and Methods: Literature-curated zebrafish genes were assigned to germline, gonadal, and immune-remodeling modules. Protein–protein interaction (PPI) networks were expanded in STRING and analyzed in Cytoscape. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment, false discovery rate (FDR)-based filtering, and topological centrality were combined to prioritize non-redundant hubs. Phenotype relevance was evaluated with FishEnrichr. To test whether early developmental regulators could redirect later reproductive programs, ZebraHub/CellOracle in silico knockout simulations were examined at 10, 19, and 24 hours post-fertilization (hpf).
Results: The analysis identified 17 hub genes distributed across three biologically connected modules. The germline module was dominated by RNA-binding, germ-granule, and piRNA-associated regulators, including nanos3, dazl, dnd1, piwil1/2, tdrd1, and tdrd7a. The gonadal module prioritized sex-determination and steroidogenic regulators, including dmrt1, wt1a, cyp19a1a, amh, and bmp15. The immune-remodeling module highlighted cytokine and repair mediators, including il1b, tnfa, tgfb1a, cxcl8a, and stat3. FishEnrichr supported strong enrichment for oocyte, ovary, and gonad-related phenotypes, indicating reproductive coherence of the hub set. CellOracle simulations suggested stage-dependent vulnerability of mesodermal trajectories: wt1a loss mainly disrupted early mesodermal fate stabilization, whereas tbx16 loss produced a more persistent impairment of somite/mesodermal differentiation. These effects link embryonic patterning with the somatic scaffold required for later germ-cell colonization and gonadal organization.
Conclusion: A trajectory-guided network strategy nominated a compact 17-gene regulatory signature that integrates germline maintenance, gonadal differentiation, and immune remodeling in zebrafish reproductive repair. The findings suggest that early mesodermal control points may condition later gonadal architecture and regenerative capacity, offering a computational framework for prioritizing fertility-related regulators in vertebrate models.
Keywords: Zebrafish, Reproductive Regeneration, Gene Regulatory Networks, Celloracle, Gonadal Development

P-82: Biocompatibility Study of Different Doses of Zinc Oxide Nanoparticles on Sheep Stromal Cells for Use in The Cryopreservation Process

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Background: Zinc oxide nanoparticles act as an effective material in improving heat transfer, controlling the freezing microstructure, and increasing the stability of the system in freezing processes. However, their dose-dependent cytotoxicity makes it necessary to determine safe and biocompatible concentrations for therapeutic applications, especially in endangered veterinary species where data are limited. This study evaluated the cytotoxicity of chemical zinc oxide nanoparticles to identify the appropriate biologically safe dose in the ovarian tissue cryopreservation process for future applications.
Materials and Methods: Primary stromal cells isolated from sheep ovarian tissue were cultured in T25 flasks. After passage 3, cells were exposed to zinc oxide nanoparticles (1-6 µg/mL) for 24, 48, and 72 hours. Viability and cytotoxicity were measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at 570 nm and Hemotoxin and Eosin. Antioxidant capacity was assessed via the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay at similar concentrations of nanoparticles. All experiments were performed in triplicate.
Results: The MTT assay showed a dose-dependent decrease in cell viability, with concentrations of 1–2 µg/mL maintaining high metabolic activity and minimal cytotoxicity compared to the control group. Concentrations above 2 µg/mL showed a significant decrease in viability, indicating the onset of nanoparticle-induced stress. Examination of ovarian tissue using a concentration of 1 µg/mL of zinc oxide in the freezing solution showed improvement in the freezing process of ovarian tissue.
Conclusion: Zinc oxide nanoparticles demonstrate a clear dose-dependent therapeutic window for ovarian stromal cells cryopreservation. Cytotoxicity becomes significant at higher doses (≥2 µg/mL), while concentrations within the 1–2 µg/mL range maintain high cell viability and metabolic activity. Specifically, a concentration of 1 µg/mL was identified as optimal, successfully combining minimal cytotoxicity with a demonstrable improvement in the histological preservation of ovarian tissue during freezing. This balance highlights its potential for enhancing bio-protection in cryopreservation protocols. The sharp decline in biocompatibility at elevated doses underscores the critical importance of precise concentration selection in nanobiotechnology applications. Therefore, zinc oxide nanoparticles at 1 µg/mL present a promising strategy for biomedical applications in fertility preservation, warranting further investigation to elucidate the underlying protective mechanisms *in vivo*.
Keywords: Zinc Oxide Nanoparticles, MTT Assay, Sheep, Stromal Cells

P-83: Optimization of Culture Conditions for Ovarian Follicular Fluid-Derived Stem Cells

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Background: Human ovarian follicular fluid-derived stem cells represent a promising resource for regenerative medicine; however, their *in vitro* expansion is hindered by limited viability and low culture efficiency. Optimizing culture conditions is therefore critical to enhance their proliferation and functional properties. The aim of the present study was to optimize the culture conditions of these cells to enhance their functional efficiency and application potential.
Materials and Methods: In this study, follicular fluid samples were collected from 10 patients attending the infertility center of Amir al-Momenin Hospital, Semnan, Iran. Patients with a history of endometriosis, Polycystic Ovary Syndrome (PCOS), or diabetes were excluded from the study. In order to optimize culture conditions, several parameters were evaluated, including different concentrations of FBS (10%, 15%, and 20%), types of culture vessels (flasks and plates), and centrifugation conditions, such as the number of centrifugation cycles (single vs. double centrifugation) and temperature (25°C and 17–19°C). Cells were cultured under these conditions, and cell number and adhesion were subsequently assessed.
Results: The optimal culture conditions were determined to be the use of 20% FBS, culture in plates, and a single centrifugation step at 17–19°C. Under these conditions, the highest cell viability and adhesion were observed. It should be noted that, due to simultaneous variation of parameters, the independent effect of each factor was not assessed separately.
Conclusion: The results of this study led to the identification of optimal culture conditions. However, further studies with a larger sample size are required to confirm these findings and to investigate the contribution of each parameter in greater detail.
Keywords: Ovarian Follicular Fluid-Derived Stem Cells, Follicular Fluid, Optimal Culture

P-84: Effect of Increasing hCG Dose on Oocyte Maturation in Mouse Preantral Follicle Culture

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Background: Human chorionic gonadotropin (hCG) is commonly used as a LH like trigger for final oocyte maturation in follicle culture systems. However, *In vitro* oocyte maturation remains suboptimal due to impaired cumulus-oocyte communication, inadequate gonadotropic signaling, and intrinsic oocyte limitations. Previous findings demonstrated improved maturation outcomes with 5 IU hCG, suggesting that optimized gonadotropin exposure may partially overcome these barriers. Therefore, we investigated whether increasing the hCG dose to 7.5 IU could further enhance meiotic maturation in an *in vitro* preantral follicle culture model.
Materials and Methods: Preantral follicles (120–130 μm) were mechanically isolated from prepubertal mouse ovaries and individually cultured for 14 days to allow follicular growth and antrum formation. On day 13, follicles were randomized to receive either 5 IU or 7.5 IU hCG in maturation medium. After 20–22 hours, cumulus-oocyte complexes were retrieved and oocytes were categorized as GV, GVBD, MII, degenerated, or total matured (GVBD+MII). Data were expressed as mean percentage ± SEM, and analyzed statistically using t-test ($P \leq 0.05$).
Results: No significant differences were observed between the 5 IU and 7.5 IU groups in GV, GVBD, MII, total matured oocytes, or degeneration rates. Although minor variations in stage distribution were detected. Oocytes exposed to 5 IU hCG exhibited superior morphological quality compared with the 7.5 IU group.
Conclusion: Increasing hCG from 5 IU to 7.5 IU did not improve meiotic maturation outcomes. Importantly, the better morphological quality observed at 5 IU suggests that optimal hormonal balance, rather than increased stimulation intensity, is critical for achieving competent meiotic maturation *in vitro*.
Keywords: Meiotic Maturation, Preantral Follicle, *In Vitro* Follicle Culture, Human Chorionic Gonadotropin

P-85: Improving Early Embryonic Development and Zygotic Genome Activation in Vitrified Mouse GV Oocytes Using Fe_3O_4/Honey Nanocomposite Supplementation

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Background: Oocyte vitrification can impair subsequent embryonic developmental competence and alter gene expression profiles, particularly during zygotic genome activation (ZGA). This study aimed to investigate whether supplementation of the vitrification medium with Fe_3O_4/honey nanocomposite (FHNC) could mitigate cryodamage and improve cleavage rates, blastocyst formation, and expression of key genes involved in ZGA in immature mouse germinal vesicle (GV) oocytes.
Materials and Methods: GV-stage oocytes were collected from adult NMRI mice and randomly divided into three groups: non-vitrified control (nVit), vitrified (Vit), and vitrified supple-

mented with 0.004% Fe_3O_4/honey nanocomposite (FHNC). After 7 days of storage in liquid nitrogen, oocytes were warmed and subjected to *in vitro* maturation (IVM) followed by *in vitro* fertilization (IVF). Two-pronuclear (2PN) zygotes were cultured in SAGE medium up to the blastocyst stage. Expression levels of ZGA-related genes (*Krt8*, *Zscan4*, *Gata2*) at the 2-cell stage and Cadherin-1 (Cdh1) at the blastocyst stage were evaluated using real-time PCR.

Results: The 2-cell formation rate was significantly higher in the FHNC group (97.77 ± 2.22%) compared to the Vit group (82.27 ± 3.63%, P<0.01). The 4-cell formation rate in the Vit group (59.36 ± 6.25%) was significantly lower than both nVit (75.5 ± 6.60%) and FHNC (76.55 ± 1.81%) groups (P<0.01). Blastocyst formation rate was markedly improved in the FHNC group (40.35 ± 7.85%) compared to the Vit group (17.08 ± 2.49%, P<0.0001) and approached the nVit control level (44.93 ± 8.56%). At the 2-cell stage, Zscan4 expression was significantly higher in FHNC than Vit (P<0.05), and Gata2 was upregulated in FHNC compared to both Vit (P<0.01) and nVit (P<0.05). *Krt8* and *Cdh1* expressions were significantly downregulated in the Vit group (P<0.001 and P<0.05, respectively).

Conclusion: Supplementation of the vitrification medium with Fe_3O_4/honey nanocomposite significantly enhanced embryonic cleavage, blastocyst development, and the proper expression of key zygotic genome activation and compaction-related genes in vitrified immature mouse GV oocytes. These findings suggest that FHNC is a promising additive for improving cryotolerance and developmental competence of cryopreserved oocytes.

Keywords: Vitrification, Fe3o4 /Honey Nanocomposite, Embryonic Development, Zygotic Genome Activation, GV Oocytes

P-86: Comparison of Amino Acid Profiles in One Commonly Used Culture Medium in Clinical Embryology Laboratories Under Different Condition

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Background: In assisted reproductive technology (ART), metabolomics and biochemical assessment of embryo culture media play an important role in optimizing laboratory conditions and improving clinical outcomes. However, limited information is available regarding the stability and changes in amino acid (AA) profiles in commonly used embryo culture media, particularly in fresh media as well as media close to their expiration date, under different incubation conditions. In addition, the effects of dry and humid incubators on AA composition have not yet been fully clarified. This study aimed: a) Evaluation of the effect of incubator conditions (dry and humid) on changes in AA profiles in the culture medium. b) Assessment of the effect of incubator door opening and closing (compared with continuous close) on the stability of AA profiles. c) Investigation of the effect of culture medium status (fresh versus near expiration date) on alteration of AA profiles.

Materials and Methods: Samples from a one step embryo culture medium used in infertility clinics were incubated in dry and humid incubator, different expiration statuses, and varying incubator opening/closing door conditions. Droplets were incubated for 3 days, collected, and then analyzed for detection of 21 amino acids (AAs) using High Performance Liquid Chromatography (HPLC).

Results: Among 21 amino acids which HPLC could detect, 18 amnio acids were detected. In culture medium near expiration compared to fresh medium, 15 of 18 amino acids (≈83%) increased significantly (P<0.05). Three amino acids (Glutamic acid, Asparagine, and Histidine) were not significantly affected by fresh and expiration date. The greatest increases in AA concentration were observed in branched-chain amino acids (BCAAs) family, Glutamine and Arginine. No significant differences in AA profiles were observed under incubator door opening/closing conditions or between dry and humid incubator.

Conclusion: In culture medium near its expiration date, the increased AA concentrations may be attributed to time-dependent changes in the protein components of the medium, particularly human serum albumin (HSA) inclusion. These alterations may lead to structural modifications or partial degradation of proteins, resulting in the release of free amino acids into the culture medium which warrants further studies.

Keywords: Culture Media, Amino Acid Profiles, Dry and Humid Incubator, Storage Time

P-87: Human Follicular Fluid Reduces Cell Death in Cultured Human Isolated Ovarian Cells

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Background: Reproductive regenerative medicine has created great hope for patients with premature ovarian insufficiency (POI). Creating artificial ovary and benefiting from natural scaffolds to restore the ovarian function in this context, is a new approach with commercial and therapeutic features that, in addition to creating the possibility of having children from one's own cells, does not require mandatory costs for other treatments such as egg donation, embryos and surrogate uterus and can play a special role in improving the quality of life of these patients. Therefore, in this study, we aimed to optimize human ovarian cell isolation and culture to support cell survival and

proliferation to produce an artificial ovary.
Materials and Methods: Cortical fragments of human ovarian tissue were obtained from 3 transsexual (T) and 3 POF (P) patients for scaffold preparation and cell isolation, respectively. Cortical ovarian cells were extracted by mechanical and enzymatic digestion following filter process. After passage 2, these cells were injected into the scaffolds, producing the artificial ovaries which were distributed into four groups; (1) base medium (BM), (2) BM supplemented with 10% follicular fluid (FF), (3) BM supplemented with 50% cumulus-conditioned medium (CC) and (4) BM supplemented with 10% knockout serum replacement (KSR). The morphology of the cells was assessed in the presence of different culture media after one week of culture. Cell death was also assessed by measuring Lactate Dehydrogenase Enzyme (LDH) secreted in the culture media.
Results: The extracted cells were able to survive, grow, and proliferate. They usually had a high proliferation capacity and reached about 1200000 cells in passage 2. The cells injected in the scaffolds indicated three different morphological characteristics after one week; spindle-shaped and granular cells in the presence of BM and KSR, multi-faceted and granular cells in the presence of CC, and multi-faceted, granular cells with round oocyte-like cells (20-30 micrometer diameter) in the FF media. A significant reduction in the cell death was also observed in the FF group.
Conclusion: We modified the cell substrate using ovarian cortical tissue from transsexual patients as a scaffold and investigated the optimal culture conditions, showing that the FF medium can help support an artificial ovary suitable for use in POF patients. The possibility of isolating and using ovarian cortical cells from patients with ovarian failure is a big step towards achieving artificial ovaries and supporting these patients, and FF with complex compounds can be suggested as a vital supplement to support ovarian cells.
Keywords: Ovarian Cortical Cell Isolation, Ovarian Scaffold, POI, Human Follicular Fluid, Artificial Ovary

P-88: Transzonal Projection Associated Gene Expression in Human *In Vitro* Derived Follicle Like Structures

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Background: Transzonal projections (TZPs) are actin-based filopodia-like structures that facilitate bidirectional communication between oocytes and surrounding granulosa cells during folliculogenesis. Proper TZP formation is essential for oocyte growth and maturation. However, establishing functional oocyte–somatic cell communication remains challenging in in-vitro derived follicle-like structures (FLSs). This study aimed to evaluate TZP associated gene expression (MYO10, DAAM1, and FSCN) in a 3D human FLS model.
Materials and Methods: In this study, FLSs were generated using a three-dimensional (3D) culture system composed of three cell lineages: human theca stem cells (hTSCs), human cumulus cells (hCCs), and germ cell-like cells (hGCLCs) differentiated from hTSCs. TZP formation was indirectly assessed by analyzing the expression of TZP associated genes (MYO10, DAAM1, and FSCN1) using qRT-PCR. The experimental group consisted of in-vitro derived FLSs, while hGCLCs served as the control group. Analyses were performed in four biological replicates (n=4). Each experimental replicate contained approximately 30 FLSs (≈1000 cells), and controls contained ≈1000 hGCLCs. Statistical comparisons were performed using an independent t-test.
Results: MYO10 expression was significantly increased in FLSs compared with controls ($P<0.0001$), suggesting enhanced TZP elongation and activity. FSCN1 expression was also significantly upregulated ($P<0.001$), indicating increased actin filament bundling and structural stabilization. In contrast, DAAM1 expression was significantly decreased ($P<0.01$), which may reflect stage-specific regulation of actin nucleation during TZP formation.
Conclusion: These findings suggest activation of TZP associated molecular pathways in the 3D FLS model, potentially supporting communication between oocyte-like cells and surrounding somatic cells. The 3D culture system may therefore provide a favorable microenvironment for promoting TZP formation and improving the developmental competence of *in vitro* derived oocyte like cells.
Keywords: Transzonal Projections (TZPs), Follicle-like Structures (FLSs), 3D Culture, MYO10, FSCN1, DAAM1

P-89: Metformin Co-Treatment Improves the Embryonic Developmental Competence of MII Oocytes in Cyclophosphamide-Treated Immature Mice

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Background: Cyclophosphamide (CP), a widely used chemotherapeutic agent in childhood cancers, exerts gonadotoxic effects on the ovary and may lead to fertility loss through direct damage to oocytes. Metformin has been reported to possess antioxidant and cytoprotective properties. This study aimed to evaluate the protective effect of metformin on oocyte maturation and embryonic developmental competence in cyclophosphamide-treated immature mice.
Materials and Methods: Two-week-old female NMRI mice were randomly assigned into four groups: control, metformin-only (MET), cyclophosphamide-only (CP), and cyclophosphamide plus metformin (CP+MET). Metformin (150 mg/kg) was administered intraperitoneally for 11 consecutive days, while cyclophosphamide (60 mg/kg) was administered intraperitoneally on days 3, 6, and 9. Four weeks after the final injection, immature germinal vesicle (GV) oocytes were collected

from the ovaries and assessed for nuclear maturation rate, *in vitro* fertilization (IVF) rate, cleavage rate, and embryonic development. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test in SPSS version 26.
Results: CP treatment significantly reduced the nuclear maturation rate to 54%, whereas co-treatment with metformin restored this parameter to 72% (P<0.0001). Compared with the CP group, the CP+MET group showed a significantly improved IVF rate (P< 0.0001). Although no significant difference was observed in cleavage rate between the CP and CP+MET groups, all 2-cell embryos in the CP group were morphologically abnormal or developmentally arrested at this stage. In contrast, metformin co-treatment promoted embryonic progression to the early blastocyst stage, whereas no embryos in the CP group reached the blastocyst stage (P<0.0007).
Conclusion: Metformin co-treatment improves oocyte nuclear maturation and embryonic developmental competence following cyclophosphamide exposure. These findings suggest that metformin may serve as a potential adjunctive strategy for fertility preservation in prepubertal females undergoing cyclophosphamide treatment.
Keywords: Cyclophosphamide, Metformin, Oocyte Maturation, Embryonic Development, Ovary

P-90: Machine Learning-Based Prediction of *In Vitro* Fertilization-Embryo Transfer Outcomes Using Clinical and Embryological Parameters

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Background: *In vitro* fertilization (IVF), a cornerstone of ART, offers a vital treatment option for infertility stemming from conditions such as endometriosis, genetic disorders, or compromised oocyte and sperm quality. Nevertheless, predicting *in vitro* fertilization and embryo transfer (IVF-ET) outcomes remains challenging due to complex interactions among clinical, hormonal, and embryological factors. Although machine learning (ML) approaches have shown promise, many studies have reported limited predictive performance and insufficient validation. This single-center study evaluated the real-world predictive ability of multiple ML models using routinely collected parameters.
Materials and Methods: This cross-sectional study included 694 patients who underwent first-cycle IVF-ET at the IKHC Infertility Clinic between March 2022 and March 2025. Eighteen variables were analyzed, including female age, body mass index (BMI), infertility duration, anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), endometrial thickness, stimulation protocol, gamete source, and embryo quality. Clinical pregnancy was the primary endpoint. Data were preprocessed and randomly divided into training (70%) and testing (30%) sets using stratified sampling. Logistic Regression (LR), Random Forest (RF), and Extreme Gradient Boosting (XGBoost) models were developed. Model performance was evaluated using area under the receiver operating characteristic curve (AUROC) and area under the precision-recall curve (AUPRC). Feature importance was assessed using permutation importance and SHAP analyses.
Results: The overall clinical pregnancy rate was 29.7%. Female age, AMH level, endometrial thickness, embryo quality, and gamete source demonstrated the strongest associations with IVF-ET outcome. LR achieved the highest predictive performance (AUROC: 0.638; AUPRC: 0.433), followed by RF (AUROC: 0.599) and XGBoost (AUROC: 0.578). Overall, all models demonstrated modest discriminatory performance. Feature importance analyses consistently identified female age, ovarian reserve markers, endometrial characteristics, and embryo quality as the principal prognostic factors.
Conclusion: Female age, ovarian reserve, and endometrial thickness emerged as the most critical prognostic factors. Machine learning models using routine clinical and embryological variables achieved only modest predictive performance in this single-center study. These results highlight the limited incremental value of current ML approaches with standard parameters and underscore the need for larger multicenter datasets incorporating time-lapse imaging, endometrial receptivity markers, or multi-omics data. External validation and prospective evaluation are essential before clinical implementation.
Keywords: *In Vitro* Fertilization, Embryo Transfer, Artificial Intelligence, Machine Learning, Prediction Model

P-91: Hydrogen Sulfide Donor (NaHS) Improves Preimplantation Mouse Embryo Development via Regulation of Redox Homeostasis and Implantation-Associated Molecules

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Background: Hydrogen sulfide (H2S) is an endogenous gaseous signaling molecule with important regulatory roles in cell survival, oxidative stress, and tissue development. Sodium hydrosulfide (NaHS), as an exogenous H2S donor, may influence preimplantation embryo development by modulating redox balance and implantation-related pathways. This study aimed to evaluate the effects of NaHS supplementation during *in vitro* culture of mouse embryos on blastocyst development, hatching ability, oxidative status, and expression of implantation-associated factors.
Materials and Methods: In this experimental study, mouse oocytes were collected from hormonally stimulated mice and subjected to *in vitro* maturation, fertilization, and embryo culture. In the first stage, embryos (2PN stage) were cultured with different concentrations of NaHS, and their developmental outcomes were assessed, including blastocyst formation rate, hatching rate, and total cell number. Inner cell mass (ICM)

and trophectoderm (TE) cell allocation were also evaluated. In addition, intracellular reactive oxygen species (ROS) and glutathione (GSH) levels were measured to assess oxidative balance. Based on the optimal concentration identified in the first stage, the second stage examined zona pellucida dissolution time using acid Tyrode's solution, as well as IL-1α protein expression and the levels of MMP2 and MMP9 in developed blastocysts.

Results: NaHS supplementation improved embryo developmental competence *in vitro*. Compared with the control group, NaHS-treated embryos showed enhanced blastocyst development and hatching performance. Moreover, NaHS increased the total number of blastocyst cells and improved the allocation of ICM and TE cells. The treatment also reduced intracellular ROS levels while increasing GSH content, indicating better redox homeostasis. In the mechanistic analysis, NaHS accelerated zona pellucida dissolution, increased IL-1α protein expression, and altered MMP2 and MMP9 protein levels in blastocysts, suggesting a positive role in promoting implantation-related processes.

Conclusion: NaHS supplementation during *in vitro* embryo culture enhances mouse blastocyst development and hatching, at least in part through improvement of oxidative balance and regulation of implantation-associated molecules. These findings suggest that NaHS, as an H2S donor, may have potential applications in improving embryo quality and preimplantation developmental outcomes in assisted reproductive technologies.

Keywords: Hydrogen Sulfide (H2S), Sodium Hydrosulfide (NaHS), Blastocysts, Embryo Development, Mouse

P-92: Investigation the Effect of Adding One-Carbon Cycle Cofactors and Substrates in CAPA_IVM System on Cytoplasmic Lipid Droplet Accumulation in Goat Oocytes

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Background: Lipid metabolism and cytoplasmic lipid droplets are crucial for the energy supply and developmental competence of mammalian oocytes. Optimizing culture media by targeting metabolic pathways can enhance oocyte quality. This study aimed to investigate the impact of adding cofactors and substrates (zinc, VitB2, VitB3, VitB6, VitB12, Methylenetetrahydrofolate, Betaein, L-cystine,) of the One-Carbon Cycle (1CC) during a capacitated *in vitro* maturation (CAPA-IVM) system on the polar body extrusion rate and accumulation of cytoplasmic lipid droplets in goat oocytes.

Materials and Methods: In this experimental study, immature goat oocytes were cultured in CAPA-IVM system. The experimental groups included: 1) control group (CNP-MM) it means no supplementation was added neither in CNP (pre-IVM phase) nor in maturation medium (MM), 2) CNP+1CC-MM group that only CNP medium was supplemented 1CC cofactors and substrates, 3) CNP-MM+1CC group that only MM was supplemented with cofactors and substrates, 4) CNP+1CC-MM+1CC group that both CNP and MM were supplemented with cofactors and substrates. Following maturation period, polar body extrusion rate and cytoplasmic lipid droplet content was evaluated using Nile Red staining. Data were analyzed using one-way ANOVA followed by Tukey HSD post-hoc test.

Results: The polar body extrusion rate was similar among various treatment group ($P > 0.05$). The Nile Red staining revealed that the presence or absence of 1CC cofactors and metabolites either in CNP or in MM did not affect lipid droplet accumulation in matured oocytes among various treatment groups ($P > 0.05$).

Conclusion: In conclusion, modulating one-carbon metabolism by supplementing 1CC cofactors and substrates during the CAPA-IVM system does not significantly affect the polar body extrusion rate or the dynamics of cytoplasmic lipid droplets in goat oocytes. Therefore, under the current experimental conditions, 1CC supplementation does not alter the oocyte's lipid profile.

Keywords: Goat Oocyte, CAPA-IVM, One-Carbon Cycle, Lipid Droplet, Polar Body.

P-93: The Impact of Sodium Hydrosulfide (NaHS) as A Hydrogen Sulfide Donor on *In Vitro* Oocyte Maturation and Developmental Competence in Mice

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Background: Hydrogen sulfide (H_2S) is the third endogenous gasotransmitter, playing critical roles in redox homeostasis and cellular stress responses. This study evaluated the effects of sodium hydrosulfide (NaHS), a rapid-releasing H_2S donor, on mouse oocyte maturation and developmental competence when applied exclusively during the IVM phase.

Materials and Methods: Immature GV oocytes from NMRI mice were matured in media supplemented with 0, 0.5, 1, 2, or 4 mM NaHS. Matured oocytes were assessed for nuclear maturation, ROS, and GSH levels. Following IVF, developmental rates to 2-cell, morula, blastocyst, and hatching stages were recorded. Blastocyst quality was evaluated by TCN and ICM/TE ratio.

Results: NaHS did not affect polar body extrusion ($P>0.05$). All NaHS concentrations significantly reduced ROS vs. control ($P<0.05$), but only 2 mM NaHS significantly increased GSH ($P<0.05$). Fertilization and 2-cell rates were significantly higher at 2 and 4 mM NaHS vs. control ($P<0.05$). Morula and blastocyst formation were significantly improved at 2 and 4 mM NaHS vs. control and 0.5 mM NaHS ($P<0.05$). Hatching rate did not differ among groups. The 4 mM NaHS group showed the highest ICM, TE, TCN, and ICM/TE ratio ($P<0.05$), followed by 2 mM NaHS.

Conclusion: The lack of effect on polar body extrusion confirms that NaHS does not disrupt nuclear maturation. The 2 mM NaHS uniquely reduced ROS while increasing GSH, creating

an optimal redox environment for cytoplasmic maturation. Improved fertilization, cleavage, morula, and blastocyst rates at 2 and 4 mM NaHS indicate that positive effects during IVM are sustained through preimplantation development. Enhanced ICM, TE, TCN, and ICM/TE ratio at 2 and 4 mM NaHS reflect better embryo quality and implantation potential. Stage-specific application of NaHS during IVM is crucial for maximizing its benefits in ART.

Keywords: NaHS, Developmental Competence, IVM

P-94: The Effect of Platelet-Rich Fibrin and PRF-Loaded Chitosan Nanoparticles on the *In Vitro* Development Competence of Ovine Embryos

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Background: As the livestock, industry seeks to optimize production and flock sizes, improving assisted reproductive technologies (ARTs) is of paramount importance. Enhancing *in vitro* culture (IVC) media is essential for increasing the efficiency of *in vitro* embryo development following *in vitro* fertilization (IVF). Platelet-rich fibrin (PRF) is a unique, biocompatible compound rich in growth factors and peptides known to promote embryonic growth. While polymeric nanocarrier, such as chitosan, are widely utilized for the controlled delivery of biomolecules, their efficacy in reproductive applications requires investigation. This study aimed to evaluate the impact of PRF-loaded chitosan nanoparticles versus unencapsulated PRF on the developmental competence of ovine IVF embryos.

Materials and Methods: PRF-loaded chitosan nanoparticles were synthesized utilizing the ionic gelation method. Ovine zygotes derived from IVF were cultured for 7 days in IVC media supplemented with these nanoparticles at varying concentrations: 0, 21, 42, 83, 166, 332, 664, 1329, and 2657 µg/ml. Concurrently, a separate experiment was conducted to evaluate the direct application of unencapsulated PRF in IVC media at concentrations of 0, 2.5, 5, 10, 25, and 50 mg/ml. Cleavage rates, blastocyst formation rates, and the total number of cells per blastocyst were evaluated across all experimental groups.

Results: The supplementation of IVC medium with PRF-loaded chitosan nanoparticles yielded no positive impact on either the cleavage or the blastocyst rates of the embryos. Notably, exposure to a nanoparticle concentration of 664 µg/ml resulted in a significant reduction in the blastocyst rate compared to the control group. Conversely, culturing potential zygotes in media enriched with unencapsulated PRF demonstrated a significant increase in the blastocyst rate at an optimal concentration of 2.5 mg/ml compared to the controls.

Conclusion: While unencapsulated PRF acts as a potent source of growth factors and cytokines that significantly enhance ovine embryo development *in vitro*, utilizing chitosan as a nanocarrier for PRF fails to improve its efficacy and may even exert detrimental effects. Therefore, chitosan nanoparticles are not a suitable delivery vehicle for PRF in embryonic culture media.

Keywords: Ovine, Blastocyst Development, Chitosan Nanoparticles, *In Vitro* Fertilization, Platelet-Rich Fibrin

P-95: Impact of N-acetylcysteine Supplementation on the Developmental Potential of Cultured Preantral Mouse Follicles

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Background: Although ovarian follicle culture is a pivotal technique for fertility preservation, enhancing its functional efficiency remains a significant challenge. This study aimed to investigate the impact of N-acetylcysteine (NAC) on the developmental outcomes of cultured preantral follicles.

Materials and Methods: Fourteen-day-old female NMRI mice were euthanized via cervical dislocation. Following ovary isolation, preantral follicles with diameters ranging from 110 to 130 µm were mechanically extracted. The follicles were randomly assigned to various groups within a scaffold-free 3D culture system and treated with different concentrations of NAC (0.1, 0.3, 0.6, and 1 mM).

Results: Supplementation with 1 mM NAC in the base culture medium (containing 2 mIU FSH) significantly enhanced follicular development. Compared with the control group, NAC-treated follicles exhibited larger diameters ($p \leq 0.05$), higher survival rates ($p \leq 0.05$), and superior morphological integrity.

Conclusion: Targeted supplementation with 1 mM NAC significantly enhances the developmental competence of preantral follicles by promoting growth, survival, and morphological integrity. These findings highlight that NAC acts as an effective antioxidant, offering a promising strategy to optimize *in vitro* follicle culture protocols and improve the overall efficiency of fertility preservation techniques.

Keywords: Mice Preantral Follicle Culture, N-Acetylcysteine, Scaffold-Free 3D Culture System, Antioxidant

P-96: Enhancing Zebrafish Embryo Viability Through Optimized Cryoprotectant Exposure at Different Developmental Stages

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Background: The depletion of genetic resources in aquatic species due to overexploitation and environmental changes has highlighted the urgent need for effective cryopreservation strategies to preserve biodiversity and support biomedical research. Despite the widespread use of zebrafish (Danio rerio) as an important vertebrate model organism in biomedical studies, successful embryo cryopreservation remains a major challenge because of the low survival rates of cryopreserved embryos. This limitation is primarily attributed to the embryos' sensitivity to cryoprotective agents (CPAs) and their susceptibility to osmotic and toxic injuries. This study aimed to optimize the exposure conditions to cryoprotective agents (CPAs) by identifying suitable CPA concentrations and exposure durations to maximize the viability of zebrafish (Danio rerio) embryos prior to cryopreservation.
Materials and Methods: TU strain zebrafish embryos at the cleavage and blastula stages were collected and allocated into two groups: A) cleavage and B) blastula stages (20 repeats and 600 embryos in each group). The embryos were sequentially exposed to an Equilibration Solution (ES) containing 7.5% dimethyl sulfoxide (DMSO) and 7.5% ethylene glycol (EG), followed by exposure to a Vitrification Solution (VS) containing 15% DMSO + 15% EG and 0.5 M sucrose. Subsequently, the embryos were washed in three successive solution (W1, W2 and W3) using decreasing concentrations of sucrose (1 M, 0.5 M and 0.25 M) and finally rinsed in E3 medium. The control group consisted of embryos that were not exposed to cryoprotectant agents (CPAs). Embryo viability and hatching rates were assessed at 24, 48, and 72 hours after treatment.
Results: The results showed that the embryo stage was affective on the toxicity test outcome. Compared with the group A, the optimized protocol (group B) resulted in a marked reduction in early embryonic mortality and improved hatching rates over 90% survival rate.
Conclusion: The identification of appropriate embryo stage contributed to maintaining the viability of zebrafish (Danio rerio) embryos and may serve as a foundation for the development of successful embryo cryopreservation protocol in aquatic species and experimental model systems.
Keywords: Cryopreservation, Zebrafish (Danio Rerio), Embryo Viability, Cryoprotective Agents (CPAs)

P-97: Stepwise Increase in Cryoprotectant Agents Concentration in the Equilibration Solution for Mature Mouse Oocyte Vitrification

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Background: Oocyte vitrification is a pivotal assisted reproductive technique for women seeking to postpone fertility due to various social, economic, medical, or personal factors. However, osmotic shock remains one of the major challenges in this process, making its mitigation essential. Therefore, this study aimed to investigate the effects of a stepwise increase in cryoprotectant agent (CPA) concentrations using a manual method.
Materials and Methods: Mature oocytes obtained from NMRI mice via *in vitro* maturation (IVM) were randomly assigned to control and experimental groups. In the control group, oocytes were equilibrated in an equilibration solution (ES) containing 7.5% dimethyl sulfoxide (DMSO) and 7.5% ethylene glycol (EG) for 12 minutes, followed by 1 minute in a vitrification solution (VS) composed of 15% DMSO, 15% EG, and 0.5 M sucrose. In the experimental group, a stepwise equilibration was performed: oocytes were initially exposed to 2.5% DMSO and EG for 1 minute, then to 5% DMSO and EG for 1 minute, followed by 10 minutes in the standard ES. Finally, they were transferred to the VS for 1 minute. Following at least 24 hours of vitrification, oocytes from both groups were warmed using a standardized protocol. Statistical significance was set at $P<0.05$.
Results: The survival rate in the control group (88.50%) was significantly lower than the experimental group (93.58%) ($p=0.023$). Furthermore, the mitochondrial membrane potential in the experimental group was significantly increased by 29.33% compared with the control group ($p=0.003$). Additionally, oxidative stress levels in the experimental group were decreased significantly by 56.55% compared with the control group ($P<0.001$).
Conclusion: This study demonstrates that a stepwise increase in CPA concentration significantly enhances the quality of vitrified-warmed oocytes by increasing mitochondrial membrane potential and reducing oxidative stress. By effectively mitigating osmotic shock, this stepwise approach serves as a superior, less toxic alternative to conventional equilibration protocols, offering a practical and more efficient strategy for optimizing clinical oocyte vitrification outcomes.
Keywords: Oocyte Vitrification, Osmotic Shock, CPA Concentration

P-98: Protective Effect of Menadione Against Cyclophosphamide-Induced Ovarian Toxicity in Mice: A Histological Study

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Background: Cyclophosphamide (CP), a widely used chemotherapeutic agent, is known to induce ovarian damage, reduce the ovarian reserve, and ultimately lead to infertility. Fertility preservation in patients undergoing chemotherapy remains a major challenge in reproductive medicine. Previous studies suggest that menadione (Vitamin K3) may modulate cellular

signaling pathways involved in tissue injury. Therefore, the present study aimed to histologically evaluate the protective effect of menadione against cyclophosphamide-induced ovarian toxicity in an NMRI mice model.

Materials and Methods: In this experimental study, 6–8-week-old female NMRI mice were randomly allocated into five groups: normal saline (control), DMSO (vehicle control), CP-only, menadione-only, and co-treatment (menadione + CP). Menadione (5 and 10 mg/kg) was administered intraperitoneally (IP) for 11 consecutive days, while CP (120 mg/kg, IP) was injected on days 3, 6, and 9. All animals were euthanized on day 12. Following the treatments, ovarian tissues were collected, fixed, and processed for histological analysis. Hematoxylin and eosin (H&E) staining was performed, and the numbers of primordial, primary, preantral, antral, and atretic follicles were quantified and compared across the experimental groups.

Results: Histological evaluation demonstrated that CP administration caused marked ovarian damage, significantly reducing the number of primordial follicles and increasing the number of atretic follicles compared to the control groups ($P<0.05$). In contrast, co-administration of menadione with CP partially preserved the ovarian histological architecture and increased the surviving proportion of primordial follicles ($P<0.05$) Furthermore, the co-treatment groups exhibited fewer atretic follicles compared to the CP-only group, although this reduction did not reach statistical significance, possibly due to the limited sample size($P>0.05$). Notably, the protective efficacy of menadione appeared to be dose-dependent, being more pronounced at the 10 mg/kg dose compared to the 5 mg/kg dose.

Conclusion: The findings of this study suggest that menadione can partially protect ovarian tissue against CP-induced structural damage and contribute to the preservation of the ovarian follicular reserve. However, further molecular investigations are warranted to elucidate the exact underlying mechanisms of this protective effect.

Keywords: Cyclophosphamide, Menadione, Ovarian Toxicity, Ovarian Reserve

P-99: Comparison of Oocyte and Embryo Quality and Quantity in Women with Low Anti-Müllerian Hormone Level in Two Groups of Age, Under and Over 40 Years Old

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Background: Anti-Müllerian hormone (AMH), produced by the granulosa cells of ovarian follicles can reflect the biological age of women. throughout the menstrual cycle, AMH levels do not change significantly, but tend to decrease with advancing age. Previous studies revealed a positive correlation between the AMH level and the oocyte quality and quantity regardless of age. However, some clinical studies reported that in an IVF cycle, AMH level could not predict the oocyte quality in women below the age of 40 years. Therefore, as a predictor of oocyte quality, the role of AMH in ART remains controversial. In this regard, this study compared the quality and quantity of oocytes and embryos in women with low AMH level in two different age groups, under and over 40 years old.

Materials and Methods: The data were collected retrospectively from the medical records of 50 women between 25-43 years old refer to Rastak Fertility Center in resent month for IVF treatment. The included patients were divided into two groups: the low AMH group (>1.1 ng/ml) under 40 years old and the low AMH (=<1.1 ng/ml) group over 40 years old. Using statistical analysis, the oocyte quality and quantity, fertilization Rate and embryo quality were compared between two groups.

Results: the oocyte quality and quantity, fertilization Rate and embryo quality were not significantly differed in women with low AMH group (>1.1 ng/ml) under 40 years old and in women with low AMH (=<1.1 ng/ml) group over 40 years old ($P<0.05$).

Conclusion: Although the number of high-quality eggs was higher in women with low AMH (=<1.1 ng/ml) group over 40 years old, statistical analysis of the data did not show a significant difference. more accurate results could be obtained by increasing the number of women studied in future research.

Keywords: Anti-Müllerian Hormone, Embryo Quality, Oocyte Quality

P-100: Human Follicular Fluid Extracellular Vesicles Improve Preimplantation Embryo Development in a Chemotherapy-Induced DOR Mouse Model

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Background: Diminished ovarian reserve (DOR), particularly when induced by gonadotoxic chemotherapy, severely impairs oocyte quality and *in vitro* fertilization (IVF) outcomes. Extracellular vehicles (EVs) derived from human follicular fluid (FF) have emerged as potential modulators of oocyte competence and early embryonic development. This study investigated the therapeutic potential of FF-EVs in improving preimplantation embryogenesis and blastocyst formation in a chemotherapy-induced mouse model of DOR.

Materials and Methods: FF-EVs were isolated and characterized using transmission electron microscopy, dynamic light scattering (mean diameter: 103.9 nm), flow cytometry (CD9+/CD63+), and protein quantification. DOR was induced in 8-week-old female mice using a single injection of busulfan (1.2 mg/kg) and cyclophosphamide (12 mg/kg). Model validation was performed 21 days later by assessing ovarian-to-body weight ratio, follicle counts, and serum FSH levels. Following dose optimization (10–80 µg/mL), 20 µg/mL FF-EVs was selected for IVF experiments. Oocytes were divided into four groups: control, control+EV, DOR, and DOR+EV.

Results: Chemotherapy successfully induced DOR, confirmed by reduced ovarian-to-body weight ratio, follicle depletion, and elevated serum FSH levels ($P<0.05$). *In vitro*, untreated DOR oocytes showed significantly reduced fertilization and increased developmental arrest at the 2-cell stage ($P<0.05$). Supplementation with 20 µg/mL FF-EVs significantly improved fertilization and cleavage rates and reduced embryonic arrest,

leading to increased blastocyst formation compared with untreated DOR oocytes (P<0.05). Additionally, FF-EVs enhanced embryonic development parameters in control oocytes without detectable adverse effects. Higher concentrations showed cytotoxic effects and developmental impairment.
Conclusion: Human follicular fluid-derived EVs at an optimized dose improve fertilization efficiency and early embryonic development in a chemotherapy-induced DOR model. These findings suggest that FF-EVs may represent a promising cell-free strategy for improving preimplantation developmental competence in compromised ovarian environments.
Keywords: Extracellular Vesicles, Follicular Fluid, Ovarian Reserve, Fertilization

P-101: Computer Vision in Digital Histology for Assessing Hyaluronic Acid Preservation in Decellularized Wharton's Jelly

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Background: Hyaluronic acid (HA) is a critical glycosaminoglycan (GAG) in Wharton's jelly extracellular matrix, which plays an important role in ovarian tissue engineering and artificial ovary construction. In tissue engineering, biological tissue-derived scaffolds require decellularization; however, this process may compromise extracellular matrix (ECM) integrity, particularly the preservation of HA. Therefore, identifying decellularization protocols that minimize ECM damage is essential. In addition, conventional methods for HA quantification are often costly, labor-intensive, and require specialized equipment. This study compared two decellularization strategies for Wharton's jelly to develop a computational image-based approach for indirect semi-quantitative assay of HA.
Materials and Methods: Human umbilical cord samples were sectioned into 5×5×2 mm^3 fragments and allocated to two decellularization groups: SDS 1% (SDS) and SDS 0.03%+DNase (S-DNase). Following decellularization, tissues were fixed, paraffin-embedded, and stained with Alcian Blue at pH 2.5 to detect total GAGs (sulfated GAGs (sGAGs) and non-sulfated GAG (HA)) and pH 1.0 to detect only sGAGs. Images were acquired under light microscopy in negative mode (At least 23 fields per sample were analyzed). A custom Python/OpenCV workflow was designed to segment stained regions and quantify color characteristics. Quantitative evaluation was performed using the CIEDE2000 (ΔE) color-difference metric.
Results: At pH 1.0, the difference between SDS + DNase treatment (S-DNase group) and SDS-treated tissues was (ΔE ≈ 1.26), indicating damage to sGAGs following SDS treatment. Also, at pH 2.5, the difference in total GAGs increased markedly (ΔE≈8.48), demonstrating greater preservation of total GAGs content in the S-DNase group. Notably, the increase in ΔE observed at pH 2.5 compared with pH 1.0 was substantially greater in the S-DNase group. Since the difference between pH 2.5 and pH 1.0 primarily reflects non-sulfated GAGs (HA), these findings indicate that HA preservation was approximately seven-fold higher in S-DNase group decellularized tissues than in SDS 1% treated samples.
Conclusion: SDS + DNase-based decellularization more effectively preserves ECM components, particularly HA, than only SDS treatment. Furthermore, the proposed ΔE-based digital histological analysis represents an innovative and low-cost strategy for objective ECM assessment and indirect HA semi-quantification. By integrating routine histological staining with computational image analysis, this approach offers a promising platform for tissue engineering applications and quantitative histopathological evaluation.
Keywords: Decellularization, Wharton's Jelly, Glycosaminoglycan, Hyaluronic Acid, Digital Histology

P-102: The Impact of Ambient Air Pollution on Assisted Reproductive Technology Outcomes: A Systematic Review of Cohort Studies

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Background: Ambient air pollution has emerged as a potentially modifiable environmental risk factor for reproductive failure, yet its impact on outcomes of assisted reproductive technology (ART) remains incompletely synthesised. This systematic review quantifies the association between exposure to criteria air pollutants and clinical outcomes after ART using a uniform cohort design.
Materials and Methods: The review (March 2026) involved five systematic stages: question formulation, comprehensive search, data extraction, study selection, and structured reporting. PubMed, Scopus, Web of Science, Magiran, IranDoc, ScienceDirect, and Google Scholar were searched using predefined terms. From 742 initial records, 17 original population-based retrospective cohort studies published between 2010 and 2023 were included after PRISMA 2020 screening. All studies linked ART cycle data with ambient pollutant concentrations ($PM_{2.5}$, PM_{10}, NO_2, O_3, SO_2) during follicular, implantation, or pregnancy windows. Two reviewers independently extracted data and assessed quality with the Newcastle-Ottawa Scale (cohort version): five scored 8, seven scored 7, and five scored 6. Findings were synthesised by pollutant, exposure window, and outcome.
Results: Three consistent patterns emerged. 1) Live birth and clinical pregnancy: The majority of studies reported significant inverse associations between $PM_{2.5}$, NO_2, and live birth, with adjusted odds ratios typically ranging from 0.85 to 0.95 per interquartile range increase. O_3 and SO_2 showed less consistent effects. 2) Exposure window sensitivity: The follicular and implantation phases were identified as critical windows; adverse effects were most pronounced when elevated pollution occurred during ovarian stimulation and early embryonic development. 3) Embryo and gamete quality: Several studies observed reduced fertilization rates and poorer embryo qual-

ity with higher pollutant exposure, though these outcomes were less frequently reported. The direction of effect was remarkably consistent across diverse geographic settings (USA, China, Europe, South Korea, Brazil).
Conclusion: Convergent evidence from 17 cohort studies indicates that short-term exposure to traffic-related air pollutants, particularly $PM_{2.5}$ and NO_2, significantly reduces the probability of a live birth following ART. These findings strengthen the rationale for integrating air quality counselling into fertility care and underscore the need for regulatory measures to protect reproductive health. Future research should incorporate personal exposure monitoring and multi-pollutant models.
Keywords: Air Pollution, Assisted Reproductive Technology, Particulate Matter, Pregnancy Outcome

Ethics

P-103: Choice of Delivery Method in Surrogacy: A Right of the Surrogate Mother or the Applicant Couple?

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Background: A surrogacy contract is concluded to prevent future disputes between the surrogate mother and the applicant couple. One of the clauses of this contract deals with the method of delivery (cesarean section or natural birth) and undoubtedly raises the question of which of the two parties has the right to decide on the delivery method?
Materials and Methods: This research has been conducted with a descriptive-normative and analytical method.
Results: Field studies on surrogate mothers show that applicant couples prefer cesarean section to natural birth for various reasons. The possibility of planning and determining the time of delivery in cesarean section (unlike the natural birth), performing a cesarean section in a hospital, which allows the applicant couple to control the situation, and preventing the formation of an emotional bond between mother and baby, more likely to happen in a natural birth, are among the reasons for the aforementioned preference.
Conclusion: Cesarean section can be lifesaving for both mother and baby in case of medical emergency, but performing such a surgery without medical necessity can sometimes result in irremediable complications, especially for the mother. Infection, bleeding, blood clotting, and problems in subsequent pregnancies are among the main complications of cesarean section. Accordingly, prescribing cesarean section as the method of delivery to a surrogate mother, in advance and without a medical necessity, highly likely leads to inflicting avoidable harm on her. Full and comprehensive support for the surrogate mother, who is often the weaker party to the surrogacy contract, is a moral duty. Therefore, the delivery method should be chosen at the time of delivery, after taking into account all medical considerations and risks and based on the fully informed consent of the surrogate mother. On the other hand, legitimate and reasonable expectations and concerns of the applicant couple in this regard have to be given close attention in line with the aforesaid ethical interests of the surrogate mother and the baby.
Keywords: Delivery Method, Surrogate Mother, Cesarean Section, Vaginal Delivery

Female

P-104: Preliminary Evaluation of Extracellular Vesicles from Adipose Derived Mesenchymal Stem Cells in A Chemotherapy Induced Mouse Model of Primary Ovarian Insufficiency

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Background: Primary ovarian insufficiency (POI) is characterized by premature ovarian follicle depletion by the age of 40. Guided by a preliminary bioinformatic analysis that highlighted a protein-protein interaction network, we investigated the effect of using human adipose-derived mesenchymal stem cell-derived extracellular vesicles (hADSC EVs) to ameliorate chemotherapy-induced POI in a mouse model.
Materials and Methods: In the bioinformatics section, differentially expressed genes from the GSE39501 dataset were analyzed, and an interaction network including hub regulators was constructed. In the experimental scetion, hADSCs were isolated, expanded, and characterized. EVs were collected from conditioned medium using differential ultracentrifugation and confirmed using transmission electron microscopy, dynamic light scattering, protein quantification, and Western blotting. Female mice were randomly assigned to control, sham (normal saline), POI, and EV-treated POI groups. GFP-labeled hADSC EVs were injected via the tail vein. Two weeks after treatment, serum estradiol (E2), FSH, and LH levels were measured, and ovarian morphology and follicle number were assessed. Expression patterns of selected hub genes and their protein expression are currently being evaluated by qRT PCR and Western blot.
Results: The obtained hub genes were the main regulators of folliculogenesis (*MAPK3, GDF9, FOXO3, BMP15*). Chemotherapy-induced POI significantly changed the hormonal profile characterized by increased FSH levels and decreased E2 and LH concentrations compared to the control group. Systemic administration of hADSC extracellular vesicles partially restored this hormonal imbalance, indicating enhanced ovarian endocrine function. Histological examination showed that ovaries treated with extracellular vesicles not only had more growing follicles (primary, secondary and antral), but also showed fewer morphological features consistent with granulosa cell apoptosis.
Conclusion: These findings provide proof of concept for a cell-free, extracellular vesicle-based strategy aimed at preserving ovarian function and fertility, while more comprehensive molecular analyses are underway to inform future translational applications.

Keywords: Extracellular Vesicles, Primary Ovarian Insufficiency, Bioinformatics, Folliculogenesis,

P-105: Clinical Pregnancy and Reproductive Outcomes Following Frozen Embryo Transfer in Natural Versus Artificial Endometrial Preparation Cycles

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Background: The optimal endometrial preparation strategy for frozen embryo transfer (FET) in recurrent implantation failure (RIF) remains uncertain. We compared outcomes between natural and artificial cycles and developed pre transfer prediction models for clinical pregnancy.
Materials and Methods: In this single center cohort study conducted in 2023 at Erfan Hospital in Karaj, Iran, women aged 18 to 40 years with RIF undergoing FET were included and received either natural or artificial hormone replacement therapy cycles. Clinical pregnancy was the primary outcome, with biochemical pregnancy, ongoing pregnancy, and live birth as secondary outcomes. Associations were evaluated using logistic regression with LASSO based adjustment, and pre transfer prediction models were developed and validated using nested cross validation across multiple classifiers.
Results: A total of 190 women with RIF underwent FET (96 natural, 94 artificial). Mean age was about 32 years and body mass index (BMI) about 27 kg/m^2, with no between-group differences. Nulliparity, polycystic ovary syndrome, and metformin use were more frequent in artificial cycles. Male factor differences were observed, with lower sperm count in the artificial group. Pregnancy outcomes, including clinical pregnancy and live birth, did not differ between protocols. In adjusted analyses, the preparation protocol was not independently associated with clinical pregnancy; however, higher right uterine artery resistance index and abnormal prolactin were associated with increased odds, while higher sperm count and a greater number of blastocysts transferred were associated with reduced odds. In predictive analyses, pre transfer variables showed good discrimination, with random forest performing best.
Conclusion: Among women with RIF undergoing FET, natural and artificial endometrial preparation protocols showed similar pregnancy outcomes, indicating no clear advantage of either approach. A concise set of routinely available pre transfer clinical, Doppler, embryologic, and male factor variables provided useful prediction of clinical pregnancy, supporting individualized counseling and targeted optimization of modifiable factors before embryo transfer. External validation in independent cohorts is needed.
Keywords: Frozen Embryo Transfer, Recurrent Implantation Failure, Endometrial Preparation, Clinical Pregnancy, Predictive Modeling

P-106: High Prevalence of Simultaneous HMGA2 Overexpression and MED12 Mutation in Iranian Uterine Leiomyomas

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Background: Uterine leiomyomas (UL) are the most common benign tumors of the female reproductive system. They contribute to symptoms, including abnormal uterine bleeding, pelvic pain, and infertility. HMGA2 overexpression and MED12 mutations are two of the most important genetic alterations in UL. This study aims to assess HMGA2 gene expression and MED12 mutation in UL, and to investigate their association with solitary versus multiple tumor presentation.
Materials and Methods: In this cross-sectional study, 35 uterine leiomyoma tissue samples and 8 adjacent normal myometrial tissues were collected from Iranian women undergoing surgery for UL. Total RNA and DNA were extracted and analyzed for HMGA2 mRNA expression by quantitative real-time PCR and MED12 mutations using Sanger sequencing. Based on patients' ultrasonography record, tumors were classified as solitary or multiple.
Results: Solitary leiomyomas (n=13) showed significantly higher HMGA2 expression than multiple leiomyomas (n=22) (.0.014). Overall, exclusive overexpression of HMGA2 (Samples carrying only HMGA2 overexpression) was detected in 15 of 35 tumors (42%), while exclusive MED12 mutations were identified in 3 cases (8%). Coexistence of both HMGA2 overexpression and MED12 mutation was observed in 9 tumors (26%).
Conclusion: Our results support the presence of distinct molecular subtypes in solitary and multiple ULs, but the detection of concurrent HMGA2 overexpression and MED12 mutation in a considerable proportion of tumors suggests that these molecular pathways are not fully contradictory. As HMGA2 is more associated with solitary large tumors, it may serve as a useful biomarker for molecular subclassification of leiomyomas and a target for therapeutic approaches.
Keywords: Uterine leiomyoma, HMGA2 overexpression, MED12 mutation, Solitary UL, multiple UL

P-107: Comparing Sequential Frozen Embryo Transfer to Standard Blastocyst Transfer for Patients with Repeated Implantation Failure- A Randomized Controlled Trial

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Background: This study investigated the comparative efficacy of sequential embryo transfer (ET) versus blastocyst-stage transfer in patients experiencing repeated implantation failure (RIF) during frozen-thawed embryo transfer (FET) cycles.
Materials and Methods: A prospective randomized clinical trial was conducted at the IVF Center of Mehr Medical Institute in Rasht, Iran, between December 2021 and February 2024. Two hundred patients diagnosed with RIF were enrolled and divided into two groups. The sequential ET group underwent the transfer of two day-3 embryos, with one subsequently cultured to the blastocyst stage. The control group received only day-5 blastocyst transfers.
Results: Primary outcome measures, including biochemical pregnancy, clinical pregnancy, implantation, and multiple pregnancy rates, showed no statistically significant differences between the sequential ET and blastocyst transfer groups (P=0.63, 0.90, 0.99, and 0.11, respectively). Likewise, ongoing pregnancy, live birth, and early miscarriage rates were comparable across both groups (P 0.87, 1.00, and 0.48, respectively).
Conclusion: The findings suggest that sequential embryo transfer does not offer a significant advantage over blastocyst-stage transfer in enhancing pregnancy outcomes for patients with repeated implantation failure.
Keywords: Assisted Reproductive Techniques, Blastocyst Transfer, Implantation, Intracytoplasmic Sperm Injections, Pregnancy outcomes

P-108: To Evaluate the Relationship Between Endometrial Compaction and Reproductive Outcome Following Transfer of Frozen-Thawed Embryos in Stimulated Natural Cycles

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Background: Research indicates that endometrial compaction occurs in a specific group of patients, characterized by the progesterone-induced reduction in endometrial thickness observed between the end of the estrogenic phase and the day of embryo transfer. This study was performed to examine the relationship between endometrial compaction and reproductive outcome following frozen-thawed embryo transfers (FET) in stimulated natural cycles.
Materials and Methods: This observational cohort study included two hundred two women who underwent their FET cycles from 2021 to 2025. Endometrial thickness was measured using transvaginal ultrasound on the LH Peak day and again on the day of embryo transfer. The changes in endometrial thickness were recorded. In this study, women with age ≥ 40 years, body mass index ≥ 35, uterine anomalies, untreated hydrosalpinx, severe endometriosis, severe male factor infertility, recurrent miscarriage, and recurrent implantation failures did not include the study. Patients were divided into two groups: those with endometrial compaction and those without endometrial compaction. The primary outcome measured was the clinical pregnancy rate, while the secondary outcomes included the implantation rate, miscarriage rate, and ongoing pregnancy rate.
Results: Demographic characteristics were comparable between the two groups. Endometrial compaction was observed in about 56% of the FET cycles. There was no significant difference between the two groups in terms of clinical pregnancy rate, ongoing pregnancy rate, and miscarriage rate. Furthermore, subgroup analysis based on endometrial compaction rates indicated a similar reproductive outcome in cycles showing endometrial compaction or not. The degree of endometrial compaction was unrelated to the progesterone levels measured on the ET day.
Conclusion: The results of the present study showed that regardless of the type of endometrial thickness change in stimulated natural cycles, there was no association between endometrial compaction and reproductive outcome. Further prospective studies on endometrial compaction in different protocols of transfer cycles are recommended.
Keywords: Endometrial Thickness, Endometrial Compaction, Reproductive Outcome, Frozen-Thawed Embryo Transfer.

P-109: The Evidence-Practice Gap in Immunotherapy for Recurrent Miscarriage: A Systematic Review

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Background: Corticosteroids, IVIG, lymphocyte immunotherapy (LIT), intralipid, and TNF-α antagonists are widely prescribed for recurrent miscarriage (RM) despite weak evidence and recommendations against routine use by ESHRE (2023) and ASRI (2025). We critically appraised the evidence-practice gap in RM immunotherapy.
Materials and Methods: PubMed, Scopus, Web of Science, and EMBASE were searched from inception to February 2026 using MeSH terms for RM and immunotherapeutic agents. Inclusion criteria comprised RCTs and cohort/case–control studies comparing immunotherapy with placebo or standard care, reporting live birth or miscarriage rate. Animal studies, case reports, reviews, uncontrolled studies, and conference abstracts without full text were excluded. Two reviewers independently screened, extracted data, and assessed risk of bias (Cochrane RoB-2; Newcastle-Ottawa Scale). PRISMA 2020 guidance was followed.
Results: From 1,742 records, 32 studies (14 RCTs, 18 obser-

vational; n=6,340) were included. A narrative synthesis was conducted across four intervention categories. Corticosteroids: Four RCTs reported live birth rates of 38–42% versus 33–37% for placebo; a definitive large trial is ongoing. IVIG: Six RCTs and seven cohort studies yielded conflicting results observational data reported up to 86% live birth, whereas prior meta-analyses detected no significant benefit. Positive studies showed selection bias and absent immunological stratification. Intralipid and LIT: Available evidence was restricted to small, underpowered studies; no adequately powered RCT supports either intervention. TNF-α antagonists: Only one retrospective study met eligibility criteria, reporting improved live birth rates (71.2% vs 50.9%) with TNF-α inhibitors; however, the evidence remains limited to non-randomized designs. Across all included studies, 18/32 lacked predefined immunological entry criteria, and findings from 22/32 had not been replicated in independent cohorts. Only five studies demonstrated low risk of bias across all domains.

Conclusion: A substantial evidence-practice gap exists: clinical use of immunotherapies for RM far exceeds supporting evidence, with TNF-α antagonists exemplifying interventions whose uptake has outpaced rigorous evaluation. Most treatments are given without validated immunological criteria. Heterogeneity precludes reliable meta-analysis. Current practice frequently contradicts guidelines, exposing women to unproven and potentially harmful interventions. Large, immunologically stratified RCTs with standardized core outcomes are urgently needed.

Keywords: Recurrent Miscarriage, Immunotherapy, Evidence-Practice Gap, Systematic Review.

P-110: To Compare the Clinical Outcomes Following Mixed Double-Embryo Transfer and Double Transfer of Blastocysts in Frozen-Thawed Embryo Transfer Cycles

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Background: The human endometrium is receptive to the embryo during a specific time known as the window of implantation (WOI). A possible reason for the implantation failures in patients may be the displacement of their WOI. Selecting the optimal stage of embryo development for transfer and deciding on the ideal number of embryos are both crucial factors for ensuring successful implantation. In this study, an attempt was made to compare pregnancy rate following simultaneous double-embryo transfer of cleavage and blastocyst stages and transfer of blastocyst double-embryo transfer in frozen-thawed embryo transfer (FET) cycles.

Materials and Methods: This randomized controlled trial study was conducted between 2019 and 2024 on 40 women undergoing an FET cycle. Women with a history of at least one failure in implantation in a cycle of ART in which at least two good quality embryos were transferred in each cycle, aged less than 40 years old, with a body mass index (BMI) less than 30 kg/m2, and with at least four good quality frozen embryos were included in the study. Women with severe male factor infertility, poor ovarian response, previous uterine surgery, uterine factor infertility, recurrent abortion, known genetic disorders, and a history of more than 3 *in vitro* fertilization failures were not included in the study. Women were randomly assigned to one of the two groups of double-embryo transfer of cleavage and blastocyst stages, or blastocyst double-embryo transfer. The primary outcome measure was clinical pregnancy, and secondary outcome measures were implantation rate, twin rate, miscarriage rate, and live birth rate. For data analysis, SPSS software was used. In all tests, the significance level was considered less than 0.05.

Results: Both groups were comparable in BMI, infertility duration, cause of infertility, and endometrial thickness on progesterone injection day. There were no statistically significant differences with respect to rates of clinical pregnancy, implantation, miscarriage, and live birth in the two groups. However, the rate of twin pregnancy was significantly higher in blastocyst double-embryo transfer compared to double-embryo transfer of cleavage and blastocyst stages.

Conclusion: These data suggest that in women with at least one or two failures in *in vitro* fertilization/intracytoplasmic sperm injection cycles, double-embryo transfer at the cleavage and blastocyst stages has the advantage of double-blastocyst transfer without increasing the twin rate. Further clinical trials with a larger sample size are recommended to confirm these findings.

Keywords: Blastocyst, Cleavage embryo, Frozen-Thawed, Embryo Transfer, Clinical Pregnancy Outcome.

P-111: To Evaluate the Effect of Endometrial Compaction on Reproductive Outcomes Following Frozen-Thawed Embryo Transfers in Endometrial Preparation Cycles

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Background: Endometrial receptivity is considered a crucial element affecting IVF success. Initial research suggests that assessing endometrial thickness via ultrasound may be an alternative to invasive procedures such as endometrial biopsy for determining a receptive endometrium during frozen-thawed embryo transfers. This study was conducted to investigate the relationship between endometrial compaction (EC) and reproductive outcome following frozen-thawed embryo transfers (FET) in endometrial preparation cycles with HRT.

Materials and Methods: In this prospective cohort study, women who were candidates for an FET cycle and had been referred to Royan Research Institute between 2021 and 2024 were included. EC was defined as a decrease in endometrial

thickness measured by transvaginal sonography on the day of embryo transfer, compared to the day the progesterone injection began. The patients were divided into a group of women with EC and those without EC. The primary outcome was clinical pregnancy rate, and the secondary outcomes were implantation rate, miscarriage rate, and live birth rate. For data analysis, SPSS software was used. In all tests, the significance level was considered less than 0.05.

Results: One hundred forty-five women were included. The majority of cycles demonstrated EC. There were no significant differences in terms of female age, body mass index, infertility duration, and infertility diagnosis. Baseline clinical characteristics were comparable between groups. Clinical pregnancy rate did not differ between groups with EC and no EC. Implantation, miscarriage, and live birth rates were also similar between groups. Additionally, subgroup analysis based on EC rates revealed a similar implantation rate, clinical pregnancy rate, and live birth rate in cycles showing EC or not.

Conclusion: Endometrial compaction does not appear to be associated with clinical pregnancy rate, miscarriage rate, or live birth rate in FET cycles in this cohort. Further multicenter prospective studies could help to demonstrate whether EC may be important for specific patient groups.

Keywords: Endometrial Thickness, Endometrial Compaction, Reproductive Outcome, Frozen-Thawed Embryo Transfer.

P-112: One Patient, Two Panels, Two Fates: A Systematic Review of Diagnostic Discordance in Commercial Gene Panels for Female Infertility

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Background: NGS gene panels for female infertility vary widely in gene content, potentially yielding conflicting diagnoses (genetic infertility vs. normal) depending on the commercial panel used. This review systematically quantified diagnostic discordance and evaluated adherence to evidence-based reporting standards.

Materials and Methods: PubMed, Scopus, Web of Science, and EMBASE were searched (January 2015–February 2026) using MeSH and free-text terms for female infertility, gene panel, and NGS. Included were studies describing/validating commercial multigene panels with reported gene lists and diagnostic outcomes; excluded were single-gene studies, WES/WGS without a panel component, and non-commercial custom panels. Two reviewers independently screened records, extracted gene lists from each panel, and assessed quality using a modified ACCE framework (Analytic validity, Clinical validity, Clinical utility, Ethical implications). PRISMA 2020 was followed.

Results: Of 1,023 records, 18 studies (12 distinct commercial panels) met criteria. Narrative synthesis found: (1) Gene content overlap – Only 26 of 384 unique genes (6.8%) were shared by all 12 panels; key infertility genes (*FSHR*, *BMP15*, *GDF9, NOBOX*) were absent from at least one panel. (2) Reporting standards – Just 5 panels provided public evidence for gene-disease validity; 3 included genes with "limited" or "no known" association per ClinGen expert curation. No panel had undergone external clinical validation in an independent cohort. Across studies, risk of bias was moderate to high, mainly due to retrospective design and lack of blinding.

Conclusion: Profound gene-content discordance creates a diagnostic roulette: a woman may be labelled genetically infertile by one panel yet normal by another. This evidence-practice gap undermines precision medicine in reproductive health. Standardized gene selection, mandatory clinical validity evidence, and external validation are urgently needed before these panels can be considered reliable diagnostic instruments.

Keywords: Female Infertility, Gene Panel, Diagnostic Discordance, Systematic Review

P-113: Investigating Oxidative Stress and Inflammatory Markers in the Follicular Fluid of Women with Endometriosis

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Background: Endometriosis is associated with significant alterations in the biochemical environment of follicular fluid, which can affect oocyte quality and its fertilization potential. The aim of this study was to evaluate oxidative stress and inflammatory markers in the follicular fluid of women with endometriosis compared with those of healthy individuals.

Materials and Methods: This study included 60 women aged 25–40 years who were referred to the Infertility Treatment Centre of Qom Jahan Daneshgahi. Participants were divided into two groups: 30 women diagnosed with endometriosis based on the presence of endometrioma cysts, and 30 healthy women without endometriosis or any underlying disease, whose infertility was attributed to male factors, serving as the control group. Follicular fluid samples were collected during oocyte retrieval. Levels of malondialdehyde (MDA), total antioxidant capacity (TAC), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) in the follicular fluid were measured using ELISA kits according to the manufacturer's instructions. Statistical analyses were performed using the independent t-test and Mann–Whitney U test. A P of less than 0.05 was considered statistically significant.

Results: The mean levels of MDA and TNF-α in the follicular fluid of the endometriosis group were significantly increased compared to the control group ($P<0.001$). In addition, IL-6 levels in the follicular fluid of the endometriosis group showed a significant increase ($P<0.01$). In contrast, the mean TAC level in these patients was significantly decreased compared to the control group.

Conclusion: Women with endometriosis showed increased oxidative stress and inflammatory responses in follicular fluid, indicated by elevated MDA, TNF-α, and IL-6 levels and reduced TAC. These changes may impair the follicular microenvironment, negatively affecting oocyte quality and fertility potential. The findings suggest that oxidative stress and inflammation

contribute to endometriosis-associated infertility and may serve as potential targets for therapeutic intervention.
Keywords: Endometriosis, Follicular Fluid, Malondialdehyde Total Antioxidant Capacity, Tumor Necrosis Factor-Alpha

P-114: Vitamin D Supplementation Mitigates Ovarian Oxidative Stress in Letrozole-Induced Polycystic Ovary Syndrome Rats

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Background: Polycystic ovary syndrome (PCOS) causes anovulatory infertility which is accompanied by sustained redox imbalance that disrupts oocyte maturation, triggers granulosa apoptosis, and arrests follicular development. Although vitamin D boosts antioxidant enzymes, its exact effect on ovarian oxidative markers remains unclear. This study evaluated whether vitamin D modulates ovarian malondialdehyde (MDA) and total antioxidant capacity (TAC).
Materials and Methods: Forty Sprague Dawley rats (180–230 g, in proestrus phase) were randomized into (n=10) G1: control, G2: sham (ethanol 0.01%), G3: PCOS, and G4: PCOS + vitamin D. PCOS was induced/maintained by daily oral letrozole gavage (1 mg/kg/day) in G3–G4 for 9 weeks, confirmed by vaginal smear, testosterone level, and ovarian histology. G4 received vitamin D (2500 IU/kg/once a week, i.p.) from week 3 to week 9. On day 60, the left ovaries were dissected to weigh and assay MDA and TAC levels (ZellBio GmbH, Germany). Statistical analyzes were performed using one-way ANOVA with Tukey post hoc testing. Results are reported as mean ± SEM and $P \leq 0.05$ is considered significant.
Results: Ovary weight was statistically elevated in G3 (31.1 ± 2.7 mg) compared to G1, G2, and G4 (16.4 ± 1.3 mg, 21.2 ± 1.6 mg, 23.3 ± 2.3 mg, respectively). MDA was significantly lower in G4 (3.5 ± 0.4 µM/mg tissue) relative to both G1 (12.2 ± 1.6 µM/mg tissue; $P<0.001$) and G2 (8.9 ± 1.1 µM/mg tissue; $P \leq 0.05$). TAC was significantly reduced in G3 (1.2 ± 0.1 mM/mg tissue) and G4 (1.3 ± 0.1 mM/mg tissue) compared to G1 (2.2 ± 0.2 mM/mg tissue; $P<0.001$ and $P \leq 0.003$, respectively). Consequently, the MDA/TAC ratio was markedly decreased in G4 compared to G1 and G2.
Conclusion: Although G4 received letrozole for 60 days, vitamin D attenuated ovarian enlargement, lowered MDA, and reduced the MDA/TAC ratio, indicating a substantial improvement in ovarian redox equilibrium. By mitigating oxidative stress, it is likely that vitamin D could protect follicular apoptosis and arrest. These findings support vitamin D as a redox-modulating adjunct for oxidative stress-mediated infertility in PCOS. Further researches are needed weather to confirm the exact effects of vitamin D on preserving fertility through modulating oxidative stress.
Keywords: Polycystic Ovary Syndrome, Letrozole, Oxidative Stress, Malondialdehyde, Vitamin D

P-115: Drug Discovery of Stable Phytochemical CYP24A1 Inhibitors from Iranian Traditional Medicine to Rescue Endometrial Dysfunction in Recurrent Implantation Failure

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Background: Recurrent implantation failure (RIF) affects 8–12% of couples undergoing assisted reproduction. It is associated with impaired endometrial receptivity, chronic inflammation, and abnormal vitamin D signaling. CYP24A1, as one of the key enzymes responsible for vitamin D catabolism, may contribute to this dysfunctional state. Endometrial transcriptomics was Integrated with structure-based phytochemical screening to discover Iranian traditional medicine–derived CYP24A1 inhibitors as potential new therapeutic candidates for RIF.
Materials and Methods: Four human endometrial RNA-seq datasets (three bulk and one single-cell-derived pseudo-bulk; n=122; RIF=56, controls=66) were harmonized using ComBat-seq and RUVSeq. Differential expression analysis was performed with DESeq2 (FDR<0.05). Six fertility-related medicinal plants in Iranian traditional medicine—Foeniculum vulgare, Nigella sativa, Fraxinus excelsior, Marrubium vulgare, Myristica fragrans, and Peganum harmala—were selected, and their phytochemicals extracted from databases. CYP24A1 was modeled by AlphaFold and validated by Ramachandran analysis (91.9% favored residues), ERRAT (quality factor 97.1), and ProSA-web. A PHASE pharmacophore model (survival score 5.72) was generated by using Cyp24A1 Binding DB inhibitors, yielding 77 compounds for downstream screening. Hits with calcitriol and 25-hydroxyvitamin D3 as controls were evaluated by molecular docking, QSAR modeling (PLS; R^2=0.84), ADMET profiling, and 100 ns molecular dynamics (MD) simulations.
Results: Integrated transcriptomic analysis identified 59 differentially expressed genes which CYP24A1 consistently upregulated alongside inflammatory and epithelial remodeling markers. Pharmacophore-screened compounds revealed docking energies ranging from −4.3 to −9.6 kcal/mol. QSAR and ADMET prioritization identified CID131623 (derived from F. vulgare) and 10467 (derived from N. sativa) as lead phytochemicals. In MD simulations, both candidates were more stable than calcitriol and 25-hydroxyvitamin D3, with RMSD convergence at ~2.9 Å and ~2.8 Å versus ~3.6 Å and ~4.5 Å, respectively CID131623 formed persistent H-bonds with Ser390 and Ser334, while 10467 established H-bond and water-bridge networks with Arg128 and Arg460.The protein's secondary structure remained intact throughout.
Conclusion: The integrated workflow established CYP24A1 as a RIF-associated target and highlights CID131623 (F. vulgare) and 10467 (N. sativa) as promising phytochemical inhibitors. Suitable binding stability in molecular dynamics simulations compared with endogenous vitamin D metabolites, support fur-

ther experimental evaluation as therapeutic candidates to restoring vitamin D homeostasis endometrial receptivity in RIF.

Keywords: Recurrent Implantation Failure, Endometrial Transcriptomics, Molecular Dynamics Simulation, CYP24A1, Iranian Traditional Medicin

P-116: The Effect of Cinnamon Supplementation on PI3K Gene Expression in Granulosa Cells in Infertile Women with Polycystic Ovary Syndrome Undergoing Assisted Reproductive Technology: A Double Blind Randomized Controlled Clinical Trial

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Background: Polycystic ovary syndrome (PCOS) is one of the most common hormonal disorders in women of reproductive age, affecting approximately 15–20% of women. insulin resistance (IR) is one of the factors that contributing to the development of this syndrome, which is observed in 50–70% of patients with this disorder. PI3K contribute to insulin resistance by affecting insulin signaling, glucose metabolism. The use of natural sources and herbal supplements has been recommended as a practical solution to reduce insulin resistance. Among these, cinnamon has been particularly emphasized due to its key role.

Materials and Methods: thirty infertile women with PCOS who referred to Royan Institute and met the inclusion criteria were recruited. After obtaining informed consent, participants were randomly assigned into two groups. The intervention group received 500 mg cinnamon supplement, while the control group received three placebo per day for six weeks: four weeks prior to the new *in vitro* fertilization (IVF) cycle and two weeks during the ovarian stimulation process. On the day of oocyte retrieval, follicular fluid was collected alongside oocytes by a specialist. Granulosa cells were immediately isolated from the follicular fluid and stored at –70°C. RNA was extracted from granulosa cells, and RNA quality was assessed using a Nanodrop spectrophotometer. After cDNA synthesis, RT-PCR was performed to evaluate the relative expression of PI3K gene, with GAPDH as the housekeeping gene, using the ΔΔCT method.

Results: Cinnamon supplementation did not significantly alter the expression of PI3K in granulosa cells of PCOS patients. Trends in expression were observed, but differences were not statistically significant, likely due to sample variability and limited size.

Conclusion: This finding is consistent with evidence suggesting that although cinnamon and its main metabolite, cinnamaldehyde, can improve metabolic profiles and insulin resistance in PCOS, these systemic effects may not translate into specific transcriptional changes in granulosa cells. Mechanistically, the PI3K/Akt pathway plays a key role in granulosa cell signaling and PCOS pathophysiology, yet modulation of this pathway by cinnamaldehyde has been primarily observed in tumor or non-granulosa cell models, limiting generalization to human granulosa cells

Keywords: Gene Expression, PCOS, Cinnamon, Infertilty, PI3K

P-117: The Protective Effect of Coumarin on Gene Expression of the Epigenetic Modifier Lsd1 in A Cyclophosphamide-Induced Mouse Model of Mremature Ovarian Failure

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Background: Cyclophosphamide (CTX), a widely used chemotherapeutic agent, is recognized for its gonadotoxic effects and its potential to induce premature ovarian failure (POF) in women younger than 40. Epigenetic regulators such as lysine-specific demethylase 1 (Lsd1) play pivotal roles in cellular homeostasis and gene expression regulation and may contribute to the pathogenesis of POF. On the other hand, natural antioxidants such as coumarin may mitigate the deleterious effects of CTX on molecular signaling pathways and epigenetic regulation. This study aimed to investigate the protective effect of coumarin on gene expression of the epigenetic modifier Lsd1 in a cyclophosphamide-induced mouse model of premature ovarian failure.

Materials and Methods: Forty female NMRI mice (6–8 weeks old) were randomly divided into four groups (n=10): (1) Control, (2) POF, (3) Coumarin, and (4) POF + Coumarin. Control and coumarin groups received intraperitoneal normal saline, while POF and POF + coumarin groups received CTX at a cumulative dose of 600 mg/kg in three injections. Oral gavage of saline was given to control and POF groups, whereas coumarin and POF + coumarin groups received 40 mg/kg for 7 days before and 7 days after CTX administration. Two weeks after the final coumarin gavage, mice were sacrificed, ovarian RNA was extracted, and Lsd1 expression was quantified by Real-Time PCR using specific primers.

Results: The results demonstrated that cyclophosphamide administration significantly decreased Lsd1 gene expression compared to the control ($P<0.0001$) and coumarin ($P<0.001$) group. In contrast, treatment with coumarin in the POF + coumarin

group significantly increased Lsd1 expression relative to the POF group (P<0.01).
Conclusion: These findings suggest that coumarin, likely through its antioxidant properties, can ameliorate CTX-induced epigenetic alterations and contribute to the restoration of ovarian function.
Keywords: Premature Ovarian Failure, Cyclophosphamide, Coumarin, Lsd1.

P-118: Protective Effect of Coumarin on Dnmt3a Expression in A Cyclophosphamide Induced Mice Model of Premature Ovarian Failure

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Background: Premature ovarian failure (POF) is a major complication of chemotherapy, particularly with cyclophosphamide, characterized by oxidative stress–induced damage and disruption of epigenetic regulation, including DNA methylation. Dnmt3a, a key de novo DNA methyltransferase, plays a critical role in regulating gene expression associated with folliculogenesis. This study aimed to evaluate the protective effect of coumarin on Dnmt3a gene expression in a cyclophosphamide-induced mouse model of POF.
Materials and Methods: In this experimental study, 40 adults female NMRI mice were randomly assigned into four groups (n=10): control, POF, coumarin, and POF+coumarin. POF was induced by intraperitoneal injection of cyclophosphamide (600 mg/kg in three doses). Coumarin (40 mg/kg) was administered orally for 14 days. Ovarian tissues were collected, and Dnmt3a gene expression was quantified using real-time PCR. Statistical analysis was performed using the Kruskal–Walli's test.
Results: Cyclophosphamide significantly increased Dnmt3a expression compared to the control group (P<0.01). Coumarin administration significantly reduced Dnmt3a expression compared to the POF group (P<0.05).
Conclusion: These findings suggest that coumarin exerts a protective effect on ovarian function, potentially through modulation of oxidative stress–induced epigenetic alterations and regulation of Dnmt3a expression. Coumarin may represent a promising epigenetic-targeting agent for mitigating chemotherapy-induced gonadtoxicity.
Keywords: Premature Ovarian Failure, Coumarin, Dnmt3a, DNA Methylation, Cyclophosphamide

P-119: The Clinical Characteristics of Women with Premature Ovarian Insufficiency Referring to Royan Institute

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Background: Premature ovarian insufficiency (POI) occurs when a woman's ovaries are no longer working before 40 years of age. POI affects almost 1% of women under 40 years old and almost 0.1% of women under 30 years old. The cause of spontaneous POI remains unknown in 90% of cases, but may be caused by chromosomal abnormalities, gene mutations, autoimmunity disorder, metabolic disorders, and infections. As this condition has life-changing physiological and psychological consequence in young women of reproductive age.
Materials and Methods: This descriptive study aimed to investigate demographic, psychological and clinical information on all premature ovarian insufficiency patients referring to Royan institute since June 2018.
Results: This study was conducted on 402 patients with primary ovarian insufficiency (POI) who referred to Royan Institute. The age range of the patients was 17 to 49 years. median age of 36 (IQR=32-41). Most of our patients (n=334; 83.1%) presented with secondary amenorrhea, while 68 patients (16.9%) presented with primary amenorrhea. and almost 61% of these patients were overweight (BMI>24.9). The median for the menarche age was 13 (Q1=12, Q3=14) and for menopause was 28 (Q1=22, Q3=34). The number of patients with primary amenorrhea was 54 (16%) and the number of patients with secondary amenorrhea was 283(84%). The results for waist circumference (WC) showed that about 50% of patients was at high risk (mean=84.47). Overall, 33% of the marriages were consanguineous. Hot flashes were reported to be the most common symptom (56.7%) before menopause among these patients. The median for FSH level was 59.8 (interquartile range=51.3) and median for LH was 31.5(Q1=16.2, Q3=47.8). Among these patients 88.55% had normal karyotype. Between patient with chromosomal abnormalities Mosaic was diagnosed in 46% cases. The results didn't show a significant association between patient's menopause age and their mothers menopause age. Psychology section of questionnaire showed that PHQ total score for 38.4% patients was less than 2 (Normal), for 30.6% patients were between 3 and 5 (mild), for 20.4% patients were between 6 and 8(moderate), and for 10.5% more than 9 (severe).
Conclusion: The results of this study may help to improve our understanding of the etiology, diagnosis and optimal intervention strategies for this condition.
Keywords: Premature Ovarian Insufficiency, Royan Institute,

Descriptive

P-120: Melatonin and Calcitonin Promote Embryo Implantation via Endometrial HB-EGF Upregulation in Mice

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Background: Implantation failure is a significant obstacle in assisted reproductive technology (ART). Calcitonin is involved in uterine receptivity and implantation of the embryo, and melatonin enhances embryo quality and implantation. The present study assessed the impact of melatonin-pretreated blastocysts and calcitonin administration on heparin binding-epidermal growth factor (HB-EGF) expression in murine endometrium.
Materials and Methods: Two-cell embryos were recovered from 1.5-day pregnant NMRI mice and cultured to blastocyst stage in GTM medium with or without 10-9 M melatonin. Pregnant and pseudo-pregnant mice were given intraperitoneal injections of 2 IU calcitonin, and 24 hours later, the blastocysts were transferred into pseudo-pregnant mice uteri. Two days later, the implantation sites were counted, and HB-EGF mRNA and protein were detected by qRT-PCR and Western blot. Statistics were analyzed with one-way ANOVA and Tukey's post hoc test. Melatonin pretreatment with calcitonin significantly enhanced HB-EGF expression ($P<0.001$) in pseudo-pregnant mice, while calcitonin alone increased HB-EGF levels in naturally pregnant mice ($P<0.001$).
Results: The number of implantation sites was greater in the melatonin group (4.6 ± 0.5, $P<0.05$), calcitonin group (7 ± 1, $P<0.001$), and combined group (8.6 ± 0.5, $P<0.001$) as compared to controls (2.6 ± 0.5). Additionally, calcitonin increased calcitonin receptor mRNA ($P<0.001$) and protein ($P<0.05$) expression in naturally pregnant and pseudo-pregnant mice.
Conclusion: In summary, melatonin-pretreated blastocysts along with calcitonin enhanced implantation through increasing HB-EGF expression, which may be a potential tool for improving ART success.
Keywords: Blastocyst, Calcitonin, HB-EGF, Implantation, Melatonin

P-121: Integrated Bioinformatics Identification and Experimental Validation of CircBNC2 as A Candidate Biomarker in Blood and Ectopic Tissue Samples of Endometriosis Patients

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Background: Endometriosis is a chronic estrogen-dependent inflammatory disorder characterized by the ectopic growth of endometrial tissue and remains associated with delayed diagnosis and limited non-invasive diagnostic approaches. Circular RNAs (circRNAs) have gained considerable attention as potential biomarkers due to their high stability and regulatory roles in gene expression. This study aimed to identify and evaluate candidate circRNAs associated with endometriosis using integrated bioinformatics analysis and experimental validation.
Materials and Methods: Multiple transcriptomic datasets associated with endometriosis (GSE192636, GSE105764, GSE247695, GSE120103, GSE141549, and GSE86534) were analyzed to identify differentially expressed transcripts and construct circRNA–microRNA (miRNA) regulatory networks. Differential expression analysis, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, and protein–protein interaction network construction were performed to prioritize candidate molecules. CircBNC2 and its associated miRNAs (miR-107 and miR-103-3p) were selected for experimental validation using quantitative real-time polymerase chain reaction (qRT-PCR). Validation was conducted on ectopic endometrial tissues from patients with endometriosis (n=30), peripheral blood samples from patients with endometriosis (n=30), and blood samples from healthy controls (n=30).
Results: CircBNC2 showed significant upregulation in endometriosis samples, while miR-107 and miR-103-3p demonstrated inverse expression patterns. Among the evaluated miRNAs, miR-107 exhibited more consistent expression changes across tissue and blood samples, suggesting a stronger association with CircBNC2. Functional enrichment analysis highlighted significant involvement of pathways related to cell cycle regulation, mitotic processes, chromosome segregation, and cell division.
Conclusion: The findings suggest that CircBNC2 may contribute to the molecular pathogenesis of endometriosis through circRNA-mediated regulatory mechanisms and may represent a promising candidate biomarker for future diagnostic applications.
Keywords: Endometriosis, Bioinformatics, CircBNC2, Diagnostic Biomarker, miR-107

P-122: Modulatory Effects of Black Garlic Extract on Ovarian Follicular Dynamics in a Mouse Model of Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common cause of infertility characterized by disrupted folliculogenesis and anovulation. Impaired follicular development is a key feature of PCOS. Black garlic (*Allium sativum L.*) possesses potent antioxidant and protective properties that may improve ovarian function. Therefore, this study aimed to investigate the effect of ethanolic extract of black garlic on ovarian follicular development in mice with induced PCOS.
Materials and Methods: PCOS was induced by daily subcutaneous injections of testosterone enanthate (1 mg/100 g) for 5 weeks. NMRI mice (3 weeks old) were randomly divided into

six groups (n=6): Control, PCOS, PCOS+BG1, PCOS+BG2, BG1, and BG2. The ethanolic extract of black garlic was administered by gavage at two different doses, BG1 (100 mg/kg) and BG2 (200 mg/kg), for 4 weeks. At the end of the experimental period, mice were sacrificed and ovarian tissues were collected. Histological evaluation was performed using hematoxylin and eosin (H&E) staining. The number of ovarian follicles at different developmental stages (primordial, primary, preantral, antral) as well as cystic follicles were counted and analyzed. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test, with $P<0.05$ considered statistically significant.

Results: The number of normal follicles at different developmental stages significantly decreased in the PCOS group compared to the control group, while the number of cystic follicles significantly increased. Treatment with black garlic extract at both doses (PCOS+BG1 and PCOS+BG2) significantly increased the number of developing follicles and reduced cystic follicles compared to the PCOS group. No significant differences were observed between the BG-only groups and the control group.

Conclusion: Ethanolic extract of black garlic improves ovarian follicular development and reduces cystic follicle formation in mice with induced PCOS, suggesting its potential therapeutic role in restoring normal ovarian function.

Keywords: Polycystic Ovary Syndrome, Black Garlic Extract, Folliculogenesis, Ovarian Follicles,

P-123: Metabolic and Hormonal Effects of Ethanolic Extract of Black Garlic in a Mouse Model of Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a multifactorial disorder characterized by insulin resistance, dyslipidemia, and hormonal imbalance. These metabolic and endocrine disturbances contribute significantly to the pathophysiology of the syndrome. Black garlic (*Allium sativum L.*) is known for its beneficial metabolic and antioxidant properties. Therefore, this study aimed to evaluate the effects of ethanolic extract of black garlic on insulin resistance, lipid profile, and reproductive hormones in a mouse model of PCOS.

Materials and Methods: PCOS was induced by daily subcutaneous injections of testosterone enanthate (1 mg/100 g) for 5 weeks. NMRI mice (3 weeks old) were randomly divided into six groups (n=6): Control, PCOS, PCOS+BG1, PCOS+BG2, BG1, and BG2. The ethanolic extract of black garlic was administered by gavage at two different doses, BG1 (100 mg/kg) and BG2 (200 mg/kg), for 4 weeks. PCOS was induced by daily subcutaneous injections of testosterone enanthate (1 mg/100 g) for 5 weeks. NMRI mice (3 weeks old) were randomly divided into six groups (n=6): Control, PCOS, PCOS+BG1, PCOS+BG2, BG1, and BG2. The ethanolic extract of black garlic was administered by gavage at two different doses, BG1 (100 mg/kg) and BG2 (200 mg/kg), for 4 weeks. At the end of the experimental period, blood samples were collected. Serum glucose and insulin levels were measured, and insulin resistance was assessed using the HOMA-IR index. Lipid profile, including total cholesterol, LDL, and HDL, was evaluated using standard biochemical assays. Serum levels of testosterone and progesterone were also measured. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test, with $P<0.05$ considered statistically significant.

Results: The PCOS group showed a significant increase in insulin resistance (HOMA-IR), total cholesterol, LDL, and testosterone levels, along with a significant decrease in HDL and progesterone levels compared to the control group. Treatment with black garlic extract at both doses (PCOS+BG1 and PCOS+BG2) significantly improved insulin sensitivity, reduced total cholesterol and LDL levels, and increased HDL levels compared to the PCOS group. In addition, black garlic extract significantly decreased serum testosterone levels and increased progesterone levels toward normal values. No significant differences were observed between the BG-only groups and the control group.

Conclusion: Ethanolic extract of black garlic improves metabolic and hormonal disturbances associated with PCOS, including insulin resistance, dyslipidemia, and sex hormone imbalance. These findings suggest its potential as a therapeutic agent for managing PCOS-related metabolic dysfunction.

Keywords: PCOS, Black Garlic Extract, Insulin Resistance, lipid Profile, Progesterone, Mouse

P-124: Effect of Ethanolic Extract of Black Garlic on Serum and Ovarian Oxidative Stress in Mice with Induced Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common cause of infertility due to anovulation. Oxidative stress plays a crucial role in the pathogenesis of PCOS, affecting both systemic and ovarian environments. Black garlic (*Allium sativum L.*) is known for its potent antioxidant properties. Therefore, this study aimed to evaluate the effect of ethanolic extract of black garlic on serum and ovarian oxidative stress in a mouse model of PCOS.

Materials and Methods: PCOS was induced by daily subcutaneous injections of testosterone enanthate (1 mg/100 g) for 5 weeks. NMRI mice (3 weeks old) were randomly divided into six groups (n=6): Control, PCOS, PCOS+BG1, PCOS+BG2, BG1, and BG2. The ethanolic extract of black garlic was administered by gavage at two different doses, BG1 (100 mg/kg) and BG2 (200 mg/kg), for 4 weeks. At the end of the experimental period, the mice were sacrificed. Serum and ovarian oxidative stress were evaluated by measuring malondialdehyde (MDA) levels and antioxidant capacity using thiobarbituric acid (TBA) and ferric reducing antioxidant power (FRAP) assays. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test, and $P<0.05$ was considered statistically significant.

Results: Serum and ovarian levels of MDA significantly increased in the PCOS group compared to the control group, indicating elevated oxidative stress. Treatment with black garlic extract at both doses (PCOS+BG1, PCOS+BG2) significantly

reduced MDA levels in both serum and ovarian tissues compared to the PCOS group. Additionally, antioxidant capacity in serum and ovary significantly decreased in the PCOS group, while it significantly increased in the treatment groups (PCOS+BG1 and PCOS+BG2), approaching control levels. No significant differences were observed between the BG-only groups and the control group.

Conclusion: Ethanolic extract of black garlic effectively reduces both systemic and ovarian oxidative stress and im≤roves antioxidant capacity in mice with induced PCOS. These findings suggest its potential as a therapeutic agent for managing oxidative stress-related aspects of PCOS.

Keywords: Polycystic Ovary Syndrome, Black Garlic Extract, Oxidative Stress.

P-125: Cervical Assessment for Preterm Birth Prediction: Role of Uterocervical Angle and Cervical Length at Two Gestational Time Points in Singleton and Multiple Pregnancies

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Background: Preterm birth is defined as delivery occurring before 37 completed weeks of gestation and remains a major contributor to neonatal morbidity and mortality worldwide. Sonographic assessment of cervical length (CL) has been widely used for predicting the risk of preterm birth. More recently, the uterocervical angle (UCA) has been proposed as an additional noninvasive ultrasonographic parameter for evaluating this risk.

Materials and Methods: This retrospective study was conducted at the Royan Institute and included medical records from 2021 to 2024. Cervical length and uterocervical angle were measured using transvaginal ultrasonography at two gestational intervals: 15–20 weeks and 21–27 weeks.

Results: A total of 171 pregnant women were included, of whom 67 delivered preterm and 104 delivered at term. Multiple pregnancies accounted for 70% of the preterm grou≤compared with 10.6% of the term group. Measurements of UCA and CL obtained before 20 weeks of gestation did not show a significant difference between the preterm and term groups. However, during 20–27 weeks of gestation, the UCA was significantly larger in the preterm group, while no significant difference was observed in CL measurements. In singleton pregnancies, no significant association was found between UCA or CL and preterm birth. In contrast, in multiple pregnancies, UCA measured at 20–27 weeks was significantly larger in the preterm group, and CL was consistently shorter compared with term pregnancies.

Conclusion: These findings suggest that assessment of the UCA and CL after 20 weeks of gestation may have greater predictive value for preterm birth, particularly in multiple pregnancies.

Keywords: Cervical length, Uterocervical Angle, Preterm labor.

P-126: Cardio Metabolic Risk Factor in Women with Premature Ovarian Insufficiency: A Cross-Sectional Study

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Background: Premature ovarian insufficiency (POI) is characterized by ovarian dysfunction before the age of 40 years and consequent hypoestrogenism, which may increase susceptibility to metabolic and cardiovascular disorders. However, evidence regarding the association between POI and metabolic syndrome (MetS) remains inconclusive. This study aimed to determine the prevalence of MetS and cardiovascular risk factors in women with POI compared with healthy controls.

Materials and Methods: This cross-sectional study included 238 women diagnosed with POI and 158 age-comparable women with regular menstrual cycles as controls. Demographic, anthropometric, clinical, and biochemical parameters were extracted from medical records and standardized questionnaires at Royan Institute, Iran. Mets was defined using the combined diagnostic framework of the International Diabetes Federation (IDF) and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) (NCEP-ATP III approach).

Results: The prevalence of MetS did not differ significantly between women with POI and controls (10.9% vs. 11.4%, P=0.88). Women with POI exhibited significantly higher low-density lipoprotein cholesterol and lower high-density lipoprotein cholesterol levels compared with controls. In multivariable analysis restricted to the POI group, elevated fasting blood glucose (OR=1.047, P=0.026), increased waist circumference (OR=1.126, P=0.003), and elevated triglyceride levels (OR=1.022, P <0.001) were independently associated with MetS. The majority of women with POI (97.7%) had a low 10-year Framingham cardiovascular risk score (<10%), and the overall cardiovascular risk distribution was comparable between groups (P=0.70).

Conclusion: Although overall MetS prevalence was not increased, women with POI demonstrated a cardiometabolic susceptibility phenotype, even in the absence of increased overall MetS prevalence, warranting early surveillance. Their significant lipid dysregulation poses a risk for future ischemic heart disease if not promptly managed.

Keywords: Premature Ovarian Insufficiency, Metabolic Syndrome, Cardiovascular Disease, Early Menopause, Fasting Blood Glucose

P-127: A Case Report of a Successful Twin Pregnancy Following Intrauterine Platelet-Rich Plasma Therapy in a Woman with Recurrent Implantation Failure, Thin Endometrium, and Endometriosis

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Background: Recurrent implantation failure (RIF) is a significant challenge in assisted reproductive technology (ART), often linked to thin endometrium, endometriosis, and diminished ovarian reserve. These conditions impair endometrial receptivity despite the transfer of high-quality embryos. Novel regenerative approaches, such as intrauterine platelet-rich plasma (PRP) therapy, have been proposed to improve endometrial thickness and implantation outcomes.
Materials and Methods: We report a 29-year-old woman with primary infertility, endometriosis, refractory thin endometrium, and diminished ovarian reserve. She underwent multiple laparoscopic surgeries for endometriomas and cervical polyps. Despite six failed frozen embryo transfer (FET) cycles using high-quality embryos and adjunct treatments, including hormone replacement therapy (HRT), granulocyte colony-stimulating factor (G-CSF), and intravenous immunoglobulin (IVIG), pregnancy was not achieved. Endometrial thickness remained persistently thin (6.0–6.5 mm). Intrauterine PRP was prepared from autologous blood and administered 48 hours before embryo transfer.
Results: Endometrial thickness increased to 7.8 mm, and subsequent FET resulted in a confirmed twin pregnancy without adverse events.
Conclusion: This case demonstrates the potential of PRP therapy to restore endometrial receptivity in complex infertility cases with RIF and thin endometrium. Individualized multimodal strategies incorporating PRP may offer benefit when conventional treatments fail. Further controlled studies are required to establish optimal protocols, patient selection criteria, and long-term outcomes.
Keywords: Platelet-Rich Plasma, Endometrium, Embryo Implantation, Recurrent Implantation Failure, Infertility

P-128: Ameliorative Effects of Black Garlic Extract on Doxorubicin-Induced Ovarian Damage: Evidence from A Mouse Model

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Background: Doxorubicin (DOX) is a widely used chemotherapeutic agent known to induce ovarian toxicity, follicular atresia, and impaired reproductive function. Oxidative stress induced by chemotherapy can disrupt follicular development and impair ovarian structure and function. Therefore, the use of natural antioxidants as protective agents has gained increasing attention. This study aimed to investigate the protective effects of black garlic extract on doxorubicin-induced histological and histomorphometric alterations in mouse ovaries
Materials and Methods: Thirty-six female mice were randomly divided into six groups: control, black garlic extract 100 mg/kg (BG-100), black garlic extract 200 mg/kg (BG-200), DOX+BG-100, DOX+BG-200, and DOX alone. Ovarian tissues were collected at the end of the experiment and examined histologically using light microscopy. Histomorphometric analysis was performed to determine the mean number of primordial, primary, secondary, mature, cystic, atretic follicles, and corpus luteum. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test and expressed as Mean ± SEM.
Results: Histological examination of the control and BG-treated groups revealed normal ovarian architecture with follicles at various developmental stages. The BG-200 group demonstrated improved follicular development, showing increased numbers of primordial (6.7 ± 0.3), secondary (5.2 ± 0.0), and tertiary follicles (4.2 ± 0.1) compared with the control group ($P \leq 0.05$). Co-administration of black garlic extract markedly attenuated doxorubicin-induced histopathological alterations in a dose-dependent manner. Both treatment groups (DOX+BG-100 and DOX+BG-200) showed partial restoration of normal ovarian architecture compared with the DOX group; however, the protective effects were more pronounced at the higher dose of black garlic extract (200 mg/kg). In the DOX+BG2 group, the numbers of primordial (5.9 ± 0.2), secondary (4.4 ± 0.1), and tertiary follicles (3.3 ± 0.5) were significantly increased compared with the DOX group ($P<0.05$). In addition, the number of corpus luteum (3.6 ± 0.2) showed a significant improvement in this group relative to the DOX-treated group. Histological observations also revealed reduced follicular degeneration, decreased vascular congestion, and improved granulosa cell organization in the DOX+BG2 group.
Conclusion: These findings indicate that the 200 mg/kg dose of black garlic extract provided the strongest protective and restorative effects against doxorubicin-induced ovarian damage.
Keywords: Ovarian, Antioxidant Activity, Follicular Damage, Oxidative Stress.

P-129: Zinc Oxide Nanoparticles and L-Carnitine Synergistically Attenuate Ovarian Oxidative Stress and Improve Ovulation in a Rat Model of PCOS

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women, associated with oxidative stress, impaired folliculogenesis, and infertility. This study aimed to evaluate the effects of zinc oxide nanoparticles (ZnO-NPs) and L-carnitine (LC) on oxidative stress markers and ovarian histology in a rat model of PCOS.
Materials and Methods: Thirty adult female Wistar rats were randomly divided into five groups (n=6): Control, PCOS (daily oral gavage of letrozole 1 mg/kg for 21 consecutive days), PCOS+ZnO-NPs (10 mg/kg, intraperitoneal), PCOS+LC (200 mg/kg, intraperitoneal), and PCOS+ZnO-NPs+LC. Treatments were administered intraperitoneally for 14 days. Ovarian levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and nitrite were measured. Histological assessments were performed to quantify follicles and corpora lutea.
Results: The PCOS group showed a significant increase in MDA (P<0.001) and decreases in SOD (P0.01), CAT (P<0.01), and nitrite (P<0.001) vs. controls. Treatment with ZnO-NPs (10 mg/kg) or L-carnitine (200 mg/kg) alone significantly reduced MDA, increased SOD and CAT (P0.05), and elevated nitrite levels compared to the PCOS group (P< 0.05). In the combination group (ZnO-NPs 10 mg/kg + L-carnitine 200 mg/kg), nitrite levels returned to control levels (P >0.05 vs. control), indicating restoration of nitric oxide balance. Histology showed reduced secondary, antral, and Graafian follicles in PCOS (P0.05). L-carnitine (200 mg/kg) significantly increased Graafian follicles (P=0.042). The number of corpora lutea was significantly increased in the combination group (ZnO-NPs + L-carnitine), suggesting improved ovulation.
Conclusion: Co-administration of ZnO-NPs (10 mg/kg) and L-carnitine (200 mg/kg) synergistically reduced oxidative stress and partially restored ovarian histology and nitrite levels, suggesting their potential as a therapeutic strategy for the management of PCOS.
Keywords: Polycystic Ovary Syndrome, Zinc Oxide Nanoparticles, L-Carnitine, Oxidative Stress,

P-130: Evaluating Pregnancy Outcomes: A Comparison of Single vs. Combined Transfer of High- and Low-Quality Embryos in ICSI Cycles

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Background: The role of including poor-quality embryos (PQEs) in conjunction with a top-quality embryo (TQE) during embryo transfer is a subject of ongoing debate in assisted reproductive technologies. This research sought to determine if the addition of PQEs alongside a TQE impacts the rates of pregnancy and live birth in cycles utilizing intracytoplasmic sperm injection (ICSI).
Materials and Methods: This cross-sectional study involved an analysis of data from 400 women who underwent fresh ICSI cycles at the Mehr Medical Institute between 2022 and 2023. Participants were categorized into nine distinct groups based on the number of embryos transferred (single, double, or triple) and the quality of the transferred embryos (various combinations of TQE and PQE). Comparisons of biochemical, clinical, and live birth rates were conducted using one-way ANOVA, chi-square tests, and multivariate logistic regression, with adjustments made for maternal age, endometrial thickness, and the day of embryo transfer.
Results: Initial analyses revealed significant variations in biochemical (P =0.001), clinical (P=0.004), ongoing (P=0.006), and live birth (P=0.003) rates across the different groups. However, subsequent multivariate analysis indicated that the embryo quality group did not have an independent influence on these outcomes. A negative correlation was observed between maternal age and the live birth rate, whereas a thicker endometrium was associated with a higher likelihood of success.
Conclusion: The findings suggest that transferring one or more poor-quality embryos in addition to a top-quality embryo does not enhance live birth rates or increase the incidence of multiple pregnancies in fresh ICSI cycles. These results lend support to the adoption of an elective single top-quality embryo transfer (eSET) strategy as a means to maximize success rates while simultaneously reducing the occurrence of multiple gestations.
Keywords: Intracytoplasmic Sperm Injection, Live Birth, Multiple Pregnancy, Embryo Transfer

P-131: Dose-Dependent Effects of Zinc Oxide Nanoparticles on Ovarian Function, Adipose Tissue Morphology, and Anti-Müllerian Hormone Levels in a Rat Model of Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, characterized by ovulatory dysfunction, hyperandrogenism, and multiple ovarian cysts. This study aimed to evaluate the effects of different doses of zinc oxide nanoparticles (ZnO-NPs) on ovarian function, adipose tissue morphology, and anti-Müllerian hormone (AMH) levels in a letrozole-induced PCOS rat model.
Materials and Methods: In this experimental study, animals were randomly divided into five groups (n=6/group): control, PCOS model (daily oral gavage of letrozole 1 mg/kg for 21 days), and three treatment groups receiving ZnO-NPs at doses of 5, 15, and 25 mg/kg (intraperitoneally for 14 consecutive

days). Following PCOS induction, animals were treated with ZnO-NPs. Serum levels of AMH, estradiol, and testosterone were measured using ELISA. Histological changes in the ovary and adipose tissue, as well as potential toxicity in the liver, kidneys, and spleen, were evaluated using hematoxylin-eosin staining.

Results: A dose-dependent reduction in AMH and testosterone levels was observed, which was significant at ZnO-NPs doses of 15 and 25 mg/kg (P0.05). Estradiol levels significantly increased in the 15 and 25 mg/kg ZnO-NPs groups compared to the PCOS group (P0.01 and P0.05, respectively), while the increase at 5 mg/kg ZnO-NPs was not statistically significant. Ovarian histopathology: The lowest dose of ZnO-NPs (5 mg/kg) reduced the number of cystic follicles and significantly increased the number of corpora lutea compared to the PCOS group (P=0.034). The 15 mg/kg dose of ZnO-NPs significantly increased the number of Graafian follicles (P0.05). Adipose tissue: Adipocyte size decreased in all ZnO NP treated groups, with the most pronounced reduction observed at 15 mg/kg ZnO-NPs (P=0.021). Toxicity: No histological damage was observed in the liver, kidneys, or spleen after treatment with any dose of ZnO NPs.

Conclusion: ZnO-NPs at a low dose (5 mg/kg) improved ovarian structure without inducing toxicity, while higher doses (15 and 25 mg/kg) were more effective in regulating hormonal profiles. The 15 mg/kg dose of ZnO-NPs is suggested as the optimal dose for simultaneous improvement of hormonal profile and adipose tissue morphology in the PCOS model.

Keywords: Polycystic Ovary Syndrome, Zinc Oxide Nanoparticles, Anti-Müllerian Hormone, Folliculogenesis, Adipose Tissue

P-132: Protective Role of Black Garlic Extract on Serum and Ovarian Oxidative Stress Induced By the Chemotherapy Drug Doxorubicin in Female Mice

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Background: Doxorubicin (DOX) is an effective chemotherapeutic agent widely used in cancer treatment; however, its clinical application is limited by severe side effects, particularly ovarian toxicity. Chemotherapy-induced gonadotoxicity can result in hormonal imbalance, follicular depletion, and long-term fertility complications. Therefore, identifying natural compounds with protective properties is of considerable importance. Black garlic contains high levels of antioxidant and anti-inflammatory compounds, suggesting a potential protective role against oxidative damage. This study aimed to evaluate the protective effects of black garlic ethanolic extract on doxorubicin-induced ovarian toxicity in mice.

Materials and Methods: In this experiment, thirty-six female mice were randomly assigned to six groups: control (C), extract black garlic100 mg/kg (BG-100); extract black garlic 200 mg/kg (BG-200); doxorubicin + extract black garlic100 mg/kg (DOX+BG-100); doxorubicin + extract black garlic 200 mg/kg (DOX+BG-200), and doxorubicin alone. At the end of the experiment, serum biochemical parameters, including oxidative stress markers and indicators of ovarian function, were assessed and data were analyzed using one-way ANOVA followed by Tukey's post hoc test, and $P<0.05$ was considered statistically significant.

Results: Serum and ovarian levels of MDA significantly increased in the DOX group compared to the control group, indicating elevated oxidative stress. Treatment with black garlic extract at both doses (DOX+BG100, DOX+BG200) significantly reduced MDA levels in both serum and ovarian tissues compared to the DOX group. Additionally, antioxidant capacity in serum and ovary significantly decreased in the DOX group, while it significantly increased in the treatment groups (DOX+BG100 and DOX+BG200), approaching control levels. No significant differences were observed between the BG-only groups and the control group. Overall, black garlic extract, particularly at 200 mg/kg, effectively reduced oxidative stress and improved serum biochemical parameters.

Conclusion: In conclusion, black garlic ethanolic extract exhibits significant protective effects against doxorubicin-induced ovarian toxicity, likely due to its antioxidant properties. These findings support its potential as a complementary therapeutic agent to reduce chemotherapy-associated reproductive damage

Keywords: Black Garlic Extract, Doxorubicin, Malondialdehyde (MDA), Ovarian,

P-133: Optimization of a Non-Invasive EV Isolation Method from Low-Volume Uterine Fluid: A Cost-Effective Approach for Clinical Fertility Research

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Background: Extracellular vesicles (EVs) are small, membrane-enclosed structures, facilitating cell-to-cell communication and making them important tools in reproductive research. Uterine lavage fluid (UFL) is a valuable sample that reflects the state of the endometrium and the environment required for embryo implantation. Previous studies have reported the isolation of EVs from UFL as a non-invasive approach. However, this fluid is usually available in very small volumes. Therefore, a simple, low-cost, and non-invasive method is needed to isolate EVs even from very small volumes. In this study, we optimized a method for isolating extracellular vesicles from low-volume UFL.

Materials and Methods: UFL was collected using a noninvasive aspiration method. After centrifugation, the samples were concentrated using 5-kDa Amicon filters to facilitate process-

ing of small volumes. EVs were then extracted using the Exo-Cib kit (Cib Biotech, Iran). Minor changes in reagent volumes and incubation times were made to improve performance. Finally, the total protein concentration extracted from EVs was measured by the bicinchoninic acid assay (BCA assay), protein expression patterns were measured by SDS-PAGE, particle size distribution was confirmed by dynamic light scattering (DLS), and EV markers (CD63, CD81, TSG101, Calnexin) were confirmed by Western blotting according to MISEV2023.
Results: SDS-PAGE analysis demonstrated low albumin contamination (a weak band around 66 kDa) in the samples. DLS analysis revealed a dominant EV population among the isolated particles (mean Z=377.7 nm, PDI=0.226), indicating an acceptable size distribution and sufficient homogeneity. Western blot analysis also showed expression of the markers CD81, CD63, and TSG101 in the isolated EVs, with no detectable calnexin (an endoplasmic reticulum marker).
Conclusion: The main advantage of this protocol is its simplicity, cost-effectiveness, and accessibility, making it suitable for small volumes of uterine fluid and providing an effective alternative to expensive methods such as ultracentrifugation or commercial chromatography systems, especially since ultracentrifugation facilities are not available in all infertility centres. It could therefore be a useful method for fertility research.
Keywords: EV Isolation, Uterine Fluid, Non-Invasive EV Isolation.

P-134: Transplantation of Engineered Uterine Scaffolds into Rat

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Background: Given the importance of the uterus in reproduction, new tissue engineering methods using host body cells were explored.
Materials and Methods: Uterine pieces of 1.5 cm were decellularized with (Sodium Dodecyl Sulfate) SDS and TritonX-100 solutions at different concentrations and times. The quality of decellularization and the scaffold structures were assessed using (Hematoxylin-Eosin) H&E and Hoechst stainings, Scaning Electron Microscopy and Raman Spectrometry. The normal uterine horn of 1 cm was removed and replaced with a sterile decellularized scaffold while the other horn remained intact. Both uterine horns were removed after 10, 20, and 30 days and their tissue sections were prepared. In another groups, 20 and 30 days after transplantation, female rats were mated with same strain male rats. When sperm were seen in the vaginal smear, day zero of pregnancy was determined. On the 13th day of pregnancy, the fetuses were examined.
Results: The results showed that the uterine scaffolds were compeletly decellurized and their structure and extracellular matrix components were well preserved using SDS and Triton X-100 solutions. The mesenchymal stem cells of host body migrated into the grafted decellularized scaffolds. They nested and differentiated in the uterine glands, blood vessels, myometrium and endometrium layers of the uterus. The regeneration capacity of uterus maintained after 20 to 30 days of transplantation whereas between 4 and 6 embryos implanted in the normal uterine horns. The transplanted uterine horn didn't show any inflammation but no embryos implanted there. It seems that the engineered uterus needs more time to prepare for embryo implantation and their blood supply.
Conclusion: The engineering of the uterus as an important organ involved in reproduction is a suitable and inexpensive method of tissue engineering. This method did not cause an immune response.
Keywords: Decellularization, Uterine Scaffolds, Transplantation, SDS, Triton X-10

P-135: XIST May Promote PCOS Pathogenesis Through a ceRNA Axis Targeting ETS2

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Background: Long non-coding RNAs (lncRNAs) regulate diverse physiological processes through their function as competitive endogenous RNAs (ceRNAs). Although dysregulation of the lncRNA X-inactive specific transcript (XIST) has been reported in various human diseases, its role in polycystic ovary syndrome (PCOS) remains unclear. This study aimed to elucidate the mechanism by which XIST contributes to PCOS pathogenesis through integrative analysis of functional datasets.
Materials and Methods: We systematically analyzed GEO datasets related to PCOS, including RNA-seq, microarray, and miRNA-seq data derived from granulosa cells (GCs) and blood samples. The analytical workflow included enrichment analysis, construction of lncRNA–miRNA–mRNA interaction networks, and validation by qRT-PCR.
Results: Compared with healthy controls, PCOS patients showed significant dysregulation of 1,131 mRNAs, 30 miRNAs, and XIST in granulosa cells. Among the 120 genes upregulated and correlated with XIST, 25 genes were enriched in inflammation-associated pathways. Five miRNAs were identified as negative regulators of XIST-associated genes, allowing construction of a ceRNA network comprising XIST–miRNA–mRNA interactions. Six genes—*AQP9, ETS2, PLAU, PLEK, SOCS3*, and *TNFRSF1B*—were identified as dual biomarkers in both granulosa cells and blood. Network analysis highlighted ETS2 as the central node in the ceRNA network, suggesting a novel regulatory axis involving XIST, hsa-miR-146a-5p, hsa-miR-144-3p, hsa-miR-1271-5p, and ETS2. qRT-PCR confirmed the upregulation of both XIST and ETS2.
Conclusion: These findings provide new insights into the molecular mechanisms underlying PCOS and identify potential diagnostic biomarkers and regulatory targets for future research.
Keywords: Polycystic Ovary Syndrome, XIST, Biomarker, Drug Repositioning.

P-136: Endometrial Receptivity Recovery After Laparoscopic Salpingectomy: A Comparative Molecular Study

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Background: Given that HOX genes act as biomarkers for endometrial receptivity, getting the timing right between salpingectomy and the commencement of an IVF/ICSI cycle becomes especially critical. In the present study, the researchers identified the most favorable interval for reestablishing endometrial receptivity after laparoscopic salpingectomy in patients with hydrosalpinx. This was achieved by measuring HOX genes and HOTAIR expression before and after surgery across two different recovery periods (three versus four menstrual cycles) and then matching these molecular profiles against those of healthy controls.
Materials and Methods: This study included 20 infertile women (aged 20–37) with sonographic hydrosalpinx (10–20 mm diameter) and normal hormones, BMI, regular cycles, and confirmed ovulation (day 21 progesterone). All underwent salpingectomy. Post-surgery, mid-luteal endometrial biopsies were taken in cycle 3 (Group A, n=10) and cycle 4 (Group B, n=10). Fifteen healthy, fertile women served as controls. Endometrial HOX gene and lncRNA HOTAIR expression were measured by qPCR before and after surgery and compared to controls. Gene expression results were expressed as fold change.
Results: Before salpingectomy, HOXA and HOXD paralogs were downregulated, while HOXC10/C11 and HOTAIR were upregulated compared to controls ($P \leq 0.05$). After salpingectomy, Group A showed restored physiological expression of HOXA10/A11/D10, but not HOXA9. In Group B, HOXA and HOXD10 expression significantly increased from pre-surgery levels ($P \leq 0.05$). HOTAIR was elevated pre-surgery in both groups, and normalized post-surgery ($P \leq 0.05$).
Conclusion: Salpingectomy restores key gene expression to physiological levels, enhancing receptivity. Our findings further highlight the necessity of at least four post-surgery menstrual cycles to eliminate hydrosalpinx fluid toxicity and optimize HOX gene expression. These results support salpingectomy as the standard treatment and warrant further research into patient gene expression profiles.
Keywords: Hydrosalpinx, Salpingectomy, Endometrial Receptivity.

P-137: Uterine Fluid Exosomal Noncoding RNAs for Window of Implantation Prediction in Recurrent Implantation Failure: A Systematic Review of Diagnostic Test Accuracy

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Background: Recurrent implantation failure (RIF) affects 10 15% of couples undergoing assisted reproductive technology (ART) and is often attributed to a displaced window of implantation (WOI). Current WOI assessment requires invasive endometrial biopsy (e.g., ERA test). Uterine fluid exosomal non coding RNAs (ncRNAs) – including microRNAs (miRNAs), long non coding RNAs (lncRNAs) and circular RNAs (circRNAs) – offer a minimally invasive alternative. This systematic review evaluates their diagnostic accuracy for predicting receptive WOI in women with RIF
Materials and Methods: We searched PubMed, Scopus, Web of Science, Embase, CENTRAL, and ClinicalTrials.gov from inception to 28 may 2026. We included non randomized human studies comparing women with RIF to fertile/receptive controls, reporting sensitivity, specificity, or AUROC of uterine fluid exosomal ncRNA signatures. Two reviewers independently screened records, extracted data, and assessed risk of bias using QUADAS 2. A narrative synthesis was performed; meta-analysis was not feasible due to substantial heterogeneity in index tests, reference standards, and EV isolation methods. The protocol was registered in PROSPERO.
Results: Of 1,168 identified records, after deduplication and screening, six studies (total 476 women, range 22 87 per study) met inclusion criteria. All six reported exosomal miRNAs; two studies examined lncRNAs, and no study investigated exosomal circRNAs. The most consistently upregulated miRNAs in receptive WOI were miR 21 5p, miR 30d 5p, let 7 family and miR 200c. For miRNA signatures, sensitivity ranged 68 87%, specificity 70 89% and AUROC 0.79 0.92 (four studies provided threshold data). The two lncRNA studies (H19, MALAT1) reported AUROC of 0.78 0.81 without threshold based diagnostic accuracy. Risk of bias was high or unclear in four of six studies, mainly due to patient selection and lack of pre specified thresholds.
Conclusion: Uterine fluid exosomal miRNAs show promising diagnostic accuracy (AUC ~0.8) for WOI prediction in RIF. However, the complete absence of circRNA data, limited lncRNA evidence, and substantial methodological heterogeneity prevent clinical application. Large, prospective validation studies with standardized protocols (MISEV2023) are urgently needed.
Keywords: Recurrent Implantation Failure, Endometrial Receptivity, Liquid Biopsy, CeRNA Network.

P-138: Identification of Endometrial Cancer Stem Cells Using Convolutional Neural Networks on Histopathological Images: A Systematic Review

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Background: Endometrial cancer stem cells (ECSCs) are rare

subpopulations responsible for tumor initiation, metastasis, recurrence, and treatment resistance in endometrial cancer. Traditional methods for identifying ECSCs, such as flow cytometry and immunohistochemistry based on surface markers (such as CD133, ALDH1, and SOX2), are time-consuming, expert-dependent, and lack automated detection capabilities. Convolutional neural networks (CNNs) have emerged as a powerful tool in the automated analysis of histopathological images and the detection of subtle micromorphological patterns. This systematic review aims to systematically review studies that have used CNNs to directly or indirectly identify cancer stem cells in histopathological specimens of endometrial cancer.

Materials and Methods: A systematic search was conducted in PubMed, Scopus, Web of Science, and IEEE Xplore databases from January 2019 to December 2025. Combined keywords included "Endometrial Cancer Stem Cells", "Convolutional Neural Networks" or "Deep Learning", "Histopathology," and "Image Classification". Original studies in English that used CNN architectures (such as ResNet, Inception, and EfficientNet) to identify ECSCs or correlate with the expression of ECSC markers in H&E or immunohistochemical images were included.

Results: Of the total 193 articles found, 18 studies met the inclusion criteria. 13 (72%) studies used pretrained architectures such as ResNet50 and InceptionV3 with transfer learning techniques. The sensitivity of the models in detecting areas containing ECSCs was reported to be between 84 and 96%, and the specificity was between 79 and 91%. The studies showed that CNNs were able to detect specific nuclear patterns, including fine-grained chromatin, high nuclear-to-cytoplasmic ratio, and the presence of prominent nucleoli, which were significantly correlated with high expression of ALDH1 and CD133 ($P<0.001$). Moreover, network activation maps showed that CNNs prioritized areas around blood vessels in the tumor stroma, known as stem cell niches.

Conclusion: Convolutional neural networks have great potential for automated identification of endometrial cancer stem cells from histopathology images and can serve as an auxiliary tool for pathologists. However, the lack of standardization in network architecture, the lack of large labeled datasets, and the lack of multicenter validation are major barriers to clinical adoption of this method.

Keywords: Automated Detection, Convolutional Neural Networks, Endometrial Cancer Stem Cells, Histopathological Images.

P-139: Unraveling the Molecular Network of Endometriosis: An Integrative Bioinformatics Analysis of SERPINE1 and VWF Genes

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Background: Endometriosis (EM) is a chronic gynecological condition characterized by the presence of endometrium-like tissue outside the uterine cavity. Delays between the appearance of symptoms and definitive diagnosis often postpone appropriate treatment, which may subsequently increase infertility-related complications. Consequently, identifying reliable EM associated genes is essential for improving timely diagnosis and clinical management.

Materials and Methods: For this purpose, EM-associated genes were first retrieved from the GeneCards database. Subsequently, differential gene expression analysis was performed using GSE7305 and GSE25628, and genes with log2FC > 1 and adj. $P<0.05$ were selected. Through Venn diagram analysis Serpin Family E Member 1 (SERPINE1) and Von Willebrand Factor (VWF) genes were identified at the intersection of the three gene sets.

Results: These genes also demonstrated significant expression in the endometrium in the Human Protein Atlas database. Furthermore, they showed higher expression in EM compared to control groups in the Gene Expression Omnibus Profiles database. The *SERPINE1* gene encodes plasminogen activator inhibitor-1 (PAI-1), which inhibits fibrinolysis and contributes to the formation of EM adhesions. Therefore, excessive expression of *SERPINE1* may disrupt the fibrinolytic system, predisposing women to EM. Some studies have shown elevated *SERPINE1* expression levels in EM patients, and PAI-1 has been found to be increased in deep EM compared to normal endometrium. VWF plays a key role in blood coagulation, and increased VWF levels can affect platelet adhesion. Studies have suggested that women with impaired fibrinolysis may exhibit a hypercoagulable state associated with increased VWF expression, suggesting the potential role of VWF in the development of EM. In addition, VWF has been associated with several inflammatory pathways through its interactions with leukocytes, platelets, and inflammatory mediators. Considering that inflammation is a major characteristic of EM, the inflammatory properties of VWF may contribute to the pathogenesis and progression of the disease. Despite the growing interest, the role of these factors in EM development remains unclear.

Conclusion: In conclusion, evidence suggests that SERPINE1 and VWF may simultaneously contribute to the inflammatory and angiogenic processes involved in EM, contributing to the stability of endometriotic lesions. However, further investigation could enhance our understanding of EM pathogenesis and provide new opportunities for earlier diagnosis.

Keywords: Endometriosis, Inflammation, Fibrinolysis, VWF, SERPINE1

P-140: The Role of Altered MUC1 Gene Expression in Impaired Endometrial Receptivity in Women with Grade 3 Isthmocele

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Background: Isthmocele is a pouch-like defect in the anterior wall of the uterine at the site of the previous cesarean section. This defect may contribute to secondary infertility through

impaired endometrial receptivity and possible alterations in the expression of key genes involved in embryo implantation. Endometrial receptivity is essential for successful embryo implantation and requires a cross-talk between the endometrium and the embryo. Mucin-1 (MUC1), a transmembrane glycoprotein, plays a key role in embryo-endometrium interaction. This glycoprotein creates a protective barrier that must be removed during embryo implantation to allow embryo attachment. This study aimed to evaluate the expression level of MUC1 in the endometrium of women with grade 3 isthmocele compared to fertile women during the window of implantation (WOI).

Materials and Methods: This study enrolled 10 women with secondary infertility due to grade 3 isthmocele and regular menstrual cycle (case group) and 10 fertile women referred for sex selection (control group). Endometrial samples were collected during the WOI (days 17-21 of the menstrual cycle). Expression of MUC1 gene was analyzed using quantitative Real-time PCR.

Results: Real-time PCR analysis showed that MUC1 gene expression was higher in the case group compared to the control group; however, this increase was not statistically significant (P0.31).

Conclusion: As it has been reported, MUC1 gene expression is locally downregulated during the window of implantation to facilitate embryo attachment. Therefore, inadequate downregulation of this gene may impair trophoblast-endometrial adhesion, disrupt implantation, and lead to infertility. Higher expression of MUC1 observed in women with grade 3 isthmocele may be related to a local inflammatory environment. Increased expression of this gene may inhibit embryo attachment to the endometrium, impairing implantation and contribute to secondary infertility.

Keywords: Isthmocele, Endometrial Receptivity, Implantation Failure, MUC1, Secondary Infertility

P-141: The Association Expression of MicroRNA-126 and lncRNA-XIST with Risk of Diabetes in PCOS Patients

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 4–21% of women in reproductive age. This condition often manifests during adolescence and is accompanied by hormonal disturbances, particularly in insulin metabolism and function, highlighting its association with chronic diseases such as cardiovascular disorders, hypertension, and type 2 diabetes mellitus. Insulin resistance is a metabolic disorder in which cellular sensitivity to insulin is reduced, leading to an impaired ability of body cells to utilize insulin as a regulator of blood glucose. This dysfunction increases the risk of developing type 2 diabetes in patients through disruption of the key IRS/PI3K/AKT signaling pathway. microRNA-126 has been proposed as a regulator of the PI3K pathway by targeting insulin receptor substrate-1 (IRS-1), a mediator of insulin-facilitated glucose uptake. In addition, lncRNA-XIST may function as a regulatory factor in the PI3K/Akt signaling pathway. This study aimed to assess association of miR-126 and lncRNA-XIST with glucose metabolism indicators.

Materials and Methods: Serum samples were collected from 25 PCOS patients diagnosed according to Rotterdam criteria and 25 age matched healthy controls. Quantitative RT-PCR was performed to measure the relative expression of miR-126 and lncRNA XIST. Expression levels were then analyzed in relation to glucose metabolism parameters

Results: The expression levels of both miR-126 and lncRNA XIST were significantly upregulated in the serum of PCOS patients compared to healthy individuals ($P<0.05$). When the expression levels were stratified based on glucose metabolism indicators, miR-126 showed a significant association, whereas lncRNA XIST did not demonstrate a meaningful correlation with these metabolic parameters.

Conclusion: Our findings suggest that miR-126 may play an important role in the pathophysiology of insulin resistance and the risk of developing type 2 diabetes in PCOS patients. In contrast, despite its upregulation in PCOS, lncRNA XIST was not associated with diabetes related indices. These results highlight miR-126 as a potential diagnostic biomarker and therapeutic target for metabolic complications in PCOS.

Keywords: Polycystic Ovary Syndrome, Insulin Resistance, Type 2 Diabetes, MicroRNA-126, lncRNA-XIST

P-142: Integrated Transcriptomic and Protein-Level Analysis Identifies ZEB2 within MAPK-Centered EMT Networks in PCOS

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Background: Polycystic ovary syndrome (PCOS) is associated with impaired folliculogenesis, chronic low-grade inflammation, and endometrial dysfunction. However, the molecular crosstalk between mitogen-activated protein kinase (MAPK) signaling and epithelial-mesenchymal transition (EMT) across ovarian and endometrial compartments remains incompletely understood. This study integrated transcriptomic and network analyses to characterize MAPK-centered EMT signatures in granulosa cells and endometrium, with particular emphasis on ZEB2, and explored preliminary protein-level variation in granulosa cells according to IVF outcome.

Materials and Methods: Public microarray/RNA-seq datasets from granulosa cells and endometrial biopsies of women with PCOS and controls were re-analyzed. Differentially expressed genes were identified and examined by pathway-enrichment and protein-protein interaction network analyses. MAPK-related genes and EMT-regulating transcription factors were interrogated across granulosa and endometrial datasets. To provide preliminary experimental support, ZEB2 protein abundance was assessed by Western blotting in representative granulosa-cell protein samples from PCOS patients with contrasting IVF outcomes and normalized to β-actin. The Western blot compari-

son was interpreted descriptively.
Results: In granulosa cells, PCOS-related genes were enriched in MAPK, Rap1, and PI3K-Akt signaling, together with TNF, NF-κB, and chemokine pathways, indicating an inflammatory and stress-responsive follicular environment. In the endometrium, epithelial-stromal analysis revealed enrichment of focal adhesion, extracellular matrix-receptor interaction, Wnt, TGF-β, and MAPK pathways, consistent with extracellular-matrix remodeling and EMT-like alterations. Network analysis positioned ZEB2 as a connector linking MAPK, TGF-β, and Wnt modules to EMT-related gene programs. In the preliminary Western blot assessment, β-actin-normalized ZEB2 abundance was approximately 2.1-fold higher in the unfavorable IVF-outcome group than in the favorable-outcome group. This observation was descriptive and requires confirmation across the complete biological cohort.
Conclusion: Integrated transcriptomic and network findings support MAPK-centered EMT signaling as a shared mechanistic axis connecting granulosa-cell dysfunction and endometrial remodeling in PCOS. ZEB2 emerged as a candidate regulatory node at the intersection of MAPK, TGF-β, and Wnt signaling. The preliminary protein-level pattern in granulosa cells further supports prioritizing ZEB2 for cohort-level validation as a potential molecular correlate of impaired reproductive outcome in PCOS.
Keywords: Polycystic Ovary Syndrome, ZEB2, Epithelial–Mesenchymal Transition, Granulosa Cells, MAPK Signaling

P-143: Probiotic Supplementation Improves Oocyte Quality and Pregnancy Rates in Women with Unexplained Infertility: Gut-Ovary Axis Modulation in a Randomized Clinical Trial

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Background: Unexplained infertility (UI) remains a significant challenge in reproductive medicine. Given the emerging evidence that oxidative stress and the reproductive microbiome influence oocyte competence, we investigated whether oral probiotic supplementation could improve oocyte quality and *in vitro* fertilization (IVF) outcomes in women with UI.
Materials and Methods: In a randomized, double-blind, placebo-controlled trial, 56 women undergoing IVF were assigned to receive either oral Biofem probiotic capsules containing Lactobacillus (1×10^9 CFU, every 12 h; n=28) for 8 weeks before the IVF cycle, or placebo (n=28). Follicular fluid (FF) was analyzed for oxidative stress markers, microbiota composition, and protein expression. Reactive oxygen species (ROS) and total antioxidant capacity (TAC) were assessed via ELISA; Lactobacillus and Bifidobacterium levels were quantified by qRT-PCR; and key oocyte competence proteins (BMP15, GDF9, IGF1, and MAPK) were measured by Western blot. Clinical outcomes included oocyte maturation, fertilization, embryo quality, and pregnancy rates.
Results: Probiotic supplementation resulted in a significantly higher fertilization rate compared with the control group (80.0% vs. 50.0%, P<0.001), as well as a significantly higher cleavage rate (100.0% vs. 66.7%, P<0.001). Regarding embryo quality, the number of good-quality embryos per cycle was significantly greater in the probiotic group (Z=3.70, P<0.001). The chemical pregnancy rate was also significantly higher in the probiotic group during the transfer cycle (46.4% vs. 21.4%, P<0.05). Follicular fluid analysis revealed significantly reduced ROS levels and increased TAC in the probiotic group (P<0.01). In addition, probiotic supplementation was associated with increased abundance of Lactobacillus and Bifidobacterium species and upregulation of BMP15, GDF9, IGF1, and MAPK expression (P<0.001).
Conclusion: Oral probiotic supplementation enhances the follicular fluid microenvironment by reducing oxidative stress, enriching beneficial microbiota, and increasing oocyte competence–related protein expression. These molecular changes are associated with improved fertilization, embryo quality, and pregnancy outcomes in women with unexplained infertility. Beyond redox homeostasis, the observed effects may partly reflect modulation of local immune–inflammatory signaling within the follicular milieu, a process increasingly recognized as integral to oocyte competence and implantation. Probiotics may represent a promising and safe adjunctive strategy to improve reproductive success.
Keywords: Unexplained Infertility, Probiotics, Microbiota, Fertilization

P-144: Protective Effect of Piperine on Cyclophosphamide-Induced Histopathological Changes in the Ovary of Female Mice

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Background: Cyclophosphamide is a widely used chemotherapeutic agent that can induce severe ovarian damage and infertility through oxidative stress. Piperine, the main active component of black pepper, possesses antioxidant and anti-inflammatory properties. This study aimed to investigate the protective effect of piperine on ovarian histopathological changes induced by cyclophosphamide in female mice.

Materials and Methods: In this experimental study, 20 female mice were randomly divided into four groups (n=5): control group, cyclophosphamide-treated group (5 mg/kg), piperine-treated group (10 mg/kg), and combination group receiving cyclophosphamide (5 mg/kg) plus piperine (10 mg/kg). Treatments were administered intraperitoneally for three weeks during the first five days of each week. After the treatment period, ovarian tissues were collected and fixed in 10% formalin for histopathological evaluation. Serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were also assessed.
Results: Cyclophosphamide treatment caused marked destruction of normal ovarian follicular structure, reduced the number of primary and developing follicles, and induced ovarian tissue damage. Administration of piperine reduced histopathological injuries and improved follicular integrity in cyclophosphamide-treated mice. In addition, piperine helped preserve serum levels of LH and FSH compared to the cyclophosphamide group
Conclusion: Piperine may attenuate cyclophosphamide-induced ovarian damage through its antioxidant properties and could be considered a protective adjunct during chemotherapy to reduce reproductive side effects.
Keywords: Piperine, Cyclophosphamide, Oxidative Stress, Ovarian Follicles, Ovarian Histopathology

P-145: Association of Follicular Fluid-Derived Exosomal Profiles with BAX and BCL-2 Gene Expression in Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age. Accumulating evidence suggests that alterations in the follicular microenvironment contribute significantly to impaired folliculogenesis in PCOS. Follicular fluid (FF), contains bioactive molecules and extracellular vesicles, particularly exosomes, that mediate intercellular communication between granulosa cells and oocytes. Exosomes transport proteins, lipids, and nucleic acids capable of modulating gene expression and cellular signaling pathways. Oxidative stress and dysregulated apoptosis are recognized contributors to ovarian dysfunction in PCOS. The present study aimed to investigate the association between follicular fluid–derived exosomal characteristics and the expression of apoptosis-related genes, BAX and BCL-2 in women with PCOS.
Materials and Methods: In this experimental study, granulosa cells and follicular fluid samples were obtained from 50 women undergoing assisted reproductive procedures, including patients diagnosed with PCOS and age-matched healthy controls, classified according to World Health Organization (WHO) criteria. Exosomes were isolated from follicular fluid using standard differential centrifugation methods and characterized by dynamic light scattering (DLS) to determine particle size distribution. Total exosomal protein concentration was quantified using the Bradford assay. Relative mRNA expression levels of BAX and BCL-2 in granulosa cells were assessed using quantitative real-time polymerase chain reaction (qRT-PCR). Statistical analyses were performed using independent-samples t-tests, with P0.05 considered statistically significant.
Results: The PCOS group demonstrated a significant increase in the mean diameter of isolated exosomes and total exosomal protein concentration compared with controls (P0.05). Molecular analysis revealed a significant upregulation of BAX expression in granulosa cells from patients with PCOS. Although BCL-2 expression showed a downward trend in the PCOS group, the reduction did not reach statistical significance. The altered BAX/BCL-2 expression pattern suggests a shift toward a pro-apoptotic state in the ovarian microenvironment of PCOS patients.
Conclusion: These findings indicate a significant association between altered exosomal profiles and increased apoptotic signaling in PCOS. The enlargement and elevated protein content of follicular fluid–derived exosomes may reflect disrupted cellular communication and enhanced oxidative stress. Collectively, the data underscore the multifactorial pathophysiology of PCOS and highlight the potential role of extracellular vesicles as biomarkers and mediators of ovarian dysfunction.
Keywords: Polycystic Ovary Syndrome, Exosomes, Apoptosis, Extracellular vesicles

P-146: Effects of Conditioned Media Derived from Human Wharton's Jelly Mesenchymal Stem Cells on the Expression of Genes and Proteins Related to the Regulation of NF-κB Associated-Inflammatory Signaling Pathways and Mitochondrial Oxidative Stress in Human Granulosa Cells of Normal and Polycystic Ovaries

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Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age, affecting more than 10% of this population. It is characterized by oxidative stress and inflammatory dysregulated pathways. Mesenchymal stem cell derived conditioned medium (CM) contains a variety of growth factors, cytokines, and bioactive molecules that can regulate cellular processes and exert therapeutic effects in different disease models. This study aimed to investigate the potential modulatory effects of conditioned medium derived from human umbilical cord Wharton's jelly

mesenchymal stem cells (h WJMSCs CM) on NF-κB associated-inflammatory signaling pathways and mitochondrial oxidative stress in human granulosa cells of normal and polycystic ovaries.

Materials and Methods: Human granulosa cells were obtained during oocyte retrieval from 50 healthy women undergoing IVF/ICSI treatment and 50 women diagnosed with PCOS. Following purification and characterization using flow cytometry, granulosa cells from normal and polycystic ovaries were divided into two experimental groups and cultured in 6 well plates for 48 hours. Cells were treated either with standard culture medium (control) or with a non-toxic concentration of h WJMSCs CM (200 µg/mL). The expression levels of key targeted genes including NFKB, SIRT3, and PPARGC1A were examined using qRT-PCR and the concentrations of the inflammatory cytokines including IL-6 and IL-1β was were measured using ELISA.

Results: The results showed that granulosa cells obtained from patients with PCOS exhibited a significant reduction in the expression of protective genes involved in the regulation of oxidative stress and cellular homeostasis, including SIRT3 and PPARGC1A compared with cells from healthy individuals. In contrast, the expression of the pro inflammatory transcription factor NFKB and the production of inflammatory cytokines IL 6 and IL 1β were markedly increased in PCOS granulosa cells, indicating an enhanced inflammatory state and oxidative stress within the follicular microenvironment. Treatment with h-WJMSCs CM significantly improved these alterations.

Conclusion: These findings suggest that hWJ MSCs CM can modulate inflammatory and oxidative stress pathways and partially restore the molecular balance of granulosa cells in PCOS.

Keywords: Granulosa Cells, Polycystic Ovary, Mesenchymal Stem Cells, Oxidative Stress

P-1:47: Do NSAIDs Affect Uterine Healing Following Cesarean Section? Evidence from a Rat Model

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Background: Cesarean section (CS) initiates a significant healing and remodeling response in the uterus, with postoperative pain primarily managed by non-steroidal anti-inflammatory drugs (NSAIDs). While effective for pain and inflammation via cyclooxygenase inhibition, NSAIDs may simultaneously impair healing by suppressing critical growth factors. This study investigated ibuprofen's effects on post-cesarean uterine repair in a rat model. This study investigated ibuprofen's effects on post-cesarean uterine repair in a rat model.

Materials and Methods: Thirty-six female Wistar rats were allocated to six groups: Control, NSAID-only, CS at 2 (CS-2W) and 4 weeks (CS-4W), and corresponding CS groups receiving ibuprofen (CS-2W+NSAID, CS-4W+NSAID). Uterine tissues were analyzed for histomorphometry and mRNA expression of growth factors (bFGF, VEGF), cytokines (IL-6, IL-10), and caspase-3 via qRT-PCR.

Results: In the CS groups, ibuprofen administration significantly reduced bFGF and VEGF expression. In addition, IL-6 was elevated in CS-2W, CS-4W, and CS-2W+NSAID groups ($P<0.001$), but reduced in CS-4W+NSAID versus CS-4W ($P<0.01$). IL-10 increased in both NSAID-treated CS groups, with highest expression in CS-4W+NSAID versus CS-4W ($P<0.05$). Caspase-3 was elevated across all experimental groups ($P< 0.0001$). Myometrial thickness decreased in all CS groups, with further reduction in CS-4W+NSAID versus CS-4W ($P<0.01$).

Conclusion: Ibuprofen treatment following cesarean delivery effectively controls inflammation but simultaneously impairs the expression of crucial regenerative growth factors and fails to mitigate apoptosis. This altered healing microenvironment is associated with worsened thinning of the uterine wall. The results indicate that post-cesarean NSAID use requires careful consideration of its potential impact on long-term uterine integrity.

Keywords: Cesarean Section, NSAID, Ibuprofen, Wound healing,

P-148: Polycystic Ovary Syndrome and the Outcome of Assisted Reproductive Techniques

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Background: This cross-sectional study investigates the association of Polycystic Ovary Syndrome (PCOS), Body Mass Index (BMI), and the number of retrieved oocytes with assisted reproductive technique (ART) outcomes in infertile Iranian women.

Materials and Methods: ART outcomes were evaluated in 508 infertile women with PCOS (aged 18–40) referred to Arash Women's Hospital, Tehran, Iran, between 2016 and 2025. Embryological data and clinical outcomes were analyzed across a total of 1000 frozen and 402 fresh embryo transfers. Statistical correlations were determined using the Eta coefficient test.

Results: The number of retrieved oocytes was the primary factor positively affecting the pregnancy rate ($P< 0.001$), showing a positive correlation with clinical pregnancy ($n=0.07$). Both the number of oocytes ($P<0.001$) and maternal age ($P<0.05$) were significantly higher in the fresh transfer group. Pregnant women exhibited a significantly lower BMI ($P=0.029$) and a higher number of retrieved oocytes ($P<0.001$) than non-preg-

nant patients. Notably, the clinical pregnancy rate was significantly higher in the frozen embryo transfer group compared to the fresh group (412 vs. 137 successful pregnancies; P<0.05). Furthermore, BMI correlated with the duration, type (n=0.33), and cause of infertility (n=0.35).
Conclusion: The number of retrieved oocytes and a lower BMI are key determinants of ART success in PCOS patients. Frozen embryo transfers yield significantly better pregnancy rates than fresh cycles. Preconception lifestyle modifications for weight reduction represent an effective strategy to optimize fertility outcomes in this cohort.
Keywords: Polycystic Ovary Syndrome, Body Mass Index, Intracytoplasmic Sperm Injection, Embryo Transfer

P-149: Comparative Molecular Docking of Potent COX-2 Inhibitors versus Celecoxib for Endometriosis Management

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Background: Endometriosis is a chronic inflammatory disease in which COX-2 plays a key pathogenic role. Selective COX-2 inhibitors such as celecoxib have therapeutic potential but cardiovascular concerns. This study aimed to evaluate the binding affinity of ten potent COX-2 inhibitors (selected from ChEMBL with IC_{50} 0.006–0.3 nM) compared to celecoxib using molecular docking.
Materials and Methods: Ten compounds with reported IC_{50} values against COX-2 (range 0.006–0.3 nM) were selected from the ChEMBL database. The three-dimensional structure of COX-2 (PDB ID: 5KIR) was retrieved. Molecular docking was performed using AutoDock Vina. Celecoxib served as the reference drug. Binding affinities (kcal/mol) were calculated and compared.
Results: Binding affinities of the ten compounds ranged from -8.1 to -10.0 kcal/mol. The highest binding affinity (-10.0 kcal/mol) was observed for Boc-4-(CHF2)-Phe-OH. Three additional compounds showed -9.6 kcal/mol each. Celecoxib exhibited a binding affinity of -9.2 kcal/mol. Thus, four compounds demonstrated superior binding to COX-2 compared to celecoxib (range -9.6 to -10.0 vs. -9.2). One compound with -9.3 kcal/mol was comparable to celecoxib. The remaining four compounds showed lower affinities (-9.1, -8.9, -8.9, -8.1 kcal/mol). All RMSD values were zero, indicating stable docking conformations.
Conclusion: Four ChEMBL-derived COX-2 inhibitors with low nanomolar IC_{50} values, particularly Boc-4-(CHF2)-Phe-OH, showed docking affinities superior to celecoxib, suggesting their potential as alternative anti-inflammatory agents for endometriosis management. Further experimental validation is recommended.
Keywords: COX-2, Molecular Docking, Endometriosis, ChEMBL, Celecoxib

P-150: MicroRNA Expression Profiles in Premature Ovarian Insufficiency Patients Undergoing Intra Cytoplasmic Sperm Injection (ICSI)

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Background: Premature Ovarian Insufficiency (POI) is a condition affecting women under the age of 40 and is characterized by primary or secondary amenorrhea lasting longer than four months. POI can result in infertility due to a diminished ovarian follicle reserve and impaired follicular function. Recent studies have shown that abnormal expression of microRNAs (miRNAs) plays an important role in the development of reproductive disorders. In the present study, we investigated whether the serum expression levels of miR-21, miR-155, and miR-146 differ in POI patients and whether the expression of these microRNAs in cumulus cells (CCs) is related to the maturation of oocytes obtained during ICSI procedures.
Materials and Methods: In this study, 120 women diagnosed with POI were compared to 150 women presenting infertility due to other female factors. All participants were <40 years old and underwent ICSI cycles at the Arak Infertility Treatment Center (ACECR). The miRNA expression level was determined by using real-time PCR. In both the POI and control groups, serum and CC samples were collected and analyzed in pairs to compare miRNA expression levels. Additionally, the association between miRNA expression levels and oocyte maturation was evaluated in each group.
Results: There was a significant reduction in the expression level of miR-21 in the serum and the CCs samples of the POI group compared with the control group (T-test, P<0.05). No significant differences were observed for miR-155 and miR-146 (P>0.05). Moreover, reduced miR-21 expression in CC samples was associated with decreased oocyte maturity in this group (Pearson correlation).
Conclusion: These findings revealed that ovarian dysfunction in women with POI was associated with reduced miR-21 expression, which was positively correlated with oocyte maturity. Therefore, miR-21 may be considered a potential biomarker involved in the pathogenesis of POI.
Keywords: MicroRNA, Oocyte Maturation, Premature Ovarian Insufficiency.

P-151: Effects of Green Tea Extract Supplementation on Nrf2-Mediated Antioxidant Signaling in Peripheral Blood Mononuclear Cells of Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) affects 6–20% of reproductive-aged women. Insulin resistance and metabolic syndrome are common in PCOS, increasing the risk of cardiovascular disease, diabetes, and reduced quality of life. Inflammation and oxidative stress contribute to PCOS pathogenesis, including elevated reactive oxygen species production by peripheral blood leukocytes. Green tea extract supplementation has been suggested as a potential anti-inflammatory intervention for women with PCOS. This study assessed the effects of green tea tablets and metformin on mRNA expression Nrf2, HO-1 and,GCLC in women with polycystic ovary syndrome.
Materials and Methods: In this clinical trial, 94 women with PCOS were enrolled and allocated to green tea (n=33), metformin (n=29), or placebo (n=32) groups. Participants were recruited from referrals to Fatemieh Hospital through convenience sampling. Inclusion criteria included reproductive-aged women with PCOS, insulin resistance, BMI ≥25, oligoovulation, ovarian cysts (2–6 mm), and irregular menstrual cycles. Women with heart or renal failure, hyperthyroidism, anxiety disorders, stomach sensitivity, gastrointestinal intolerance to metformin, or pregnancy were excluded. Green tea extract (500 mg twice daily for one week and then daily) or metformin (1000 mg/day) was administered for three months. Expression of Nrf2, HO-1, and GCLC was measured using quantitative real-time PC.
Results: In this study, the average age of participants in the green tea, metformin, and control groups was 27.3 ± 4.11, 27.75 ± 4.23, and 27.06 ± 4.18 years, respectively (P 0.808). All three study groups were not significantly different in terms of body mass index, mean age, menstrual cycle parameters, ovary, endometrium, antioxidant biomarkers, leptin, insulin and fasting blood sugar. Green tea could alleviate the deleterious effects of PCOS through the enhancement of Nrf2, HO-1 and GCLC levels (P0.05, P0.005, P=0.02 respectively).
Conclusion: The results showed that green tea consumption in patients with polycystic ovary syndrome was associated with better outcomes in regulating the Nrf2 pathway in these patients.
Keywords: Green Tea, Nuclear Factor (erythroid-derived 2)-like 2, Human, Polycystic Ovary Syndrome.

P-152: Ovo-Protective Effects of Platelet-Rich Plasma and Pentoxifylline Co-administration on Ovarian Recovery and IVF Outcomes in Cyclophosphamide-Induced Premature Ovarian Failure (POF) in BALB/c Mice

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Background: Premature ovarian failure (POF) induced by chemotherapeutic agents such as cyclophosphamide (CY) can impair fertility by disrupting ovarian function. Platelet-rich plasma (PRP), rich in growth factors, and pentoxifylline (PTX), a methylxanthine derivative with antioxidant properties, may exert preventive or regenerative effects. This study investigated the effect of PRP and PTX[FT1.1] on ovary and *in vitro* fertilization (IVF) outcomes.
Materials and Methods: 25 mature female mice were assigned into five groups: control, POF (75 mg/kg of CY, intraperitoneal [ip] on days 1 and 10 to induce POF), POF-PRP (200 µl, ip, half an hour after CY injection on day 1 and 10), POF-PTX (50 mg/kg, orally, half an hour after CY injection daily for 21 day [FT2.1]), and POF-PRP-PTX group was treated similarly to POF-PTX and POF-PRP groups[FT3.1]. At the end of experiments on day 21, to evaluate the effect of PRP and PTX on IVF outcomes, PMSG was injected intraperitoneally and after 48 hours followed by HCG injection to release oocytes. Then the ova were obtained from fallopian tubes fertilized with sperm in culture medium for 5 hours[FT4.1] and then was assessed fertilization rate, and embryo development. After that, the serum level of anti-Mullerian hormone (AMH), estradiol (E2), as well as biochemical assessments including malondialdehyde (MDA), and superoxide dismutase (SOD) activity were performed. Moreover, ovarian parameters (total ovarian volume, cortex and medulla volume, follicular count) were assessed.
Results: CY significantly decreased AMH and E2 level while increasing MDA level and decreasing SOD activity. Our results also indicated that CY -induced POF negatively affected ovarian parameters. A significant decrease in the number of retrieved oocytes, fertilization and cleavage rates[FT5.1], while increase in abnormal oocytes was also observed. Furthermore, our results demonstrated the potential of both treatments (PRP and PTX) in improving hormone level, oxidative damage, ovarian parameters and IVF results.
Conclusion: It can be concluded that the concurrent use of PRP and PTX can recover ovarian function and IVF outcomes from CY -induced POF.
Keywords: Cyclophosphamide, Premature Ovarian Failure, Platelet-Rich Plasma, Pentoxifylline.

P-153: Development of an Endometrial mRNA Diagnostic Panel for Hydrosalpinx Stratification and Salpingectomy Prediction

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Background: Hydrosalpinx is a common cause of female infertility that impairs endometrial receptivity and reduces implantation and IVF success rates through chronic inflammatory changes. However, reliable molecular biomarkers for identifying patients who may benefit from salpingectomy are still lacking. This study aimed to design and validate an endometrial mRNA-based diagnostic panel capable of stratifying patients with hydrosalpinx and predicting candidates who may achieve improved outcomes following salpingectomy.

Materials and Methods: This case-control study included 20 infertile women with confirmed hydrosalpinx, who underwent endometrial biopsy before and after laparoscopic salpingectomy, and 20 fertile women as healthy controls. In parallel, publicly available transcriptomic datasets (GSE106602, GSE111974, GSE58144, GSE178101, and GSE223639) were integrated to identify differentially expressed genes (DEGs) associated with hydrosalpinx and endometrial dysfunction. Candidate mRNAs were prioritized based on differential expression patterns, biological relevance, and consistency across datasets. qRT-PCR was then validated on selected transcripts, and diagnostic performance was evaluated using ROC curve analysis.
Results: Our results indicated that the proposed mRNA panel can ability to differentiate hydrosalpinx-related samples from controls. Several transcripts showed consistent dysregulation before and after salpingectomy, suggesting their involvement in the molecular signature of the disease and their potential utility as response-associated biomarkers.
Conclusion: Endometrial mRNA profiling may represent a promising strategy for the evaluation of hydrosalpinx. The proposed diagnostic panel may assist clinicians in identifying patients who are more likely to benefit from salpingectomy and support personalized treatment decision-making.
Keywords: Hydrosalpinx, mRNA Diagnostic Panel, Endometrial Biopsy, Infertility, RNA Sequencing

P-154: From Molecules to Medicine: Computational Discovery of Novel COX- Inhibitors for Endometriosis

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Background: Endometriosis is a chronic inflammatory disease in which endometrial-like tissue grows outside the uterus, leading to pelvic pain, infertility, and reduced quality of life. Evidence suggests that beyond inflammation, neurogenic mechanisms and neuroinflammation also contribute to persistent pain in this disorder. COX-2 (PTGS2), a key enzyme in prostaglandin synthesis, is highly upregulated in endometriosis and plays an important role in inflammation and pain signaling. Therefore, inhibition of COX-2 is considered a promising strategy for reducing disease progression and associated pain. Molecular docking provides a powerful in silico approach to evaluate potential COX-2 inhibitors and identify novel therapeutic candidates targeting endometriosis-related inflammatory and neurobiological pathways. The present study employed molecular docking analysis to screen Mefenamic acid and Diclofenac and their analogs, aiming to identify improved inhibitors with higher binding affinity to COX-2.
Materials and Methods: In this study, a computational approach was used to identify potential COX-2 inhibitors for endometriosis. The COX-2 protein structure was prepared from the Protein Data Bank, and selected anti-inflammatory ligands were optimized before analysis. Molecular docking was performed to evaluate ligand binding affinity and interactions with the COX-2 active site. Pharmacokinetic properties and drug-likeness were assessed using SwissADME. Candidate compounds were then compared based on docking scores, protein–ligand interactions, complex stability, and ADME profiles to identify the most promising inhibitors.
Results: Molecular docking of Mefenamic acid (PubChem CID: [4044]) and Diclofenac (PubChem CID: [3033]) against COX-2 yielded a binding energy of -91.2335 kcal/mol and -114.893. Among Mefenamic acid's 1607 analogs, 2-[[3-(4-fluorophenyl) cyclobutanecarbonyl] amino]-6-methylbenzoic acid (PubChem CID: [167977142]) exhibited one of the strongest affinities (-142.542 kcal/mol) but was excluded due to violations in drug-likeness filters. The strongest affinity belongs to the diclofenac analogue, 2-[2-(dihydroxy amino) ox ethoxy] ethyl 2-[2-(2,6-dichloroanilino) phenyl] acetate with 431.27 g/mol Molecular weight, (which showed a binding energy of -164.483 kcal/mol, Auto Dock vina: -8.108 kcal/mol) with favorable ADME properties. InterAction mapping revealed seven hydrogen bonds with Tyr385, Ser530, Asn375 and Phe529 supported by multiple steric contacts with Tyr348, Leu352, Tyr385, Ser530and Gly 533 stabilizing the ligand within the COX-2 pocket.
Conclusion: Among all tested ligands 2- [2-(dihydroxy amino) ox ethoxy] ethyl 2-[2-(2,6-dichloroanilino) phenyl] acetate emerged as the most promising in silico candidate for COX-2 inhibition. While these findings highlight potential molecular leads for Endometriosis's therapy, they are preliminary and require rigorous *in vitro* and *in vivo* validation before any translational or therapeutic implications can be established.
Keywords: COX-2, Endometriosis, Mefenamic Acid, Diclofenac.

Genetic

P-155: Segregation Analysis Reveals Limited Disease Association of Candidate Variants Identified by Whole-Exome Sequencing in Endometriosis

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Background: Endometriosis is a chronic inflammatory disorder affecting approximately 10–15% of women of reproductive age and is a major cause of pelvic pain and infertility. The disease is considered multifactorial, resulting from complex interactions between genetic and environmental factors. Familial aggregation and twin studies have highlighted the importance of genetic susceptibility in the development of endometriosis. Recent advances in next-generation sequencing technologies, particularly whole-exome sequencing (WES), have enabled the identification of genetic variants potentially associated with the disease. The present study aimed to investigate candidate genetic variants in a family affected by endometriosis.
Materials and Methods: We evaluated a patient diagnosed

with endometriosis who was referred to the Royan Institute. Whole-exome sequencing (WES) was performed to identify potential disease-associated variants. Candidate variants were subsequently validated by Sanger sequencing and assessed in available family members, including an affected sister, a mother with uterine fibroids, and an unaffected father.
Results: WES analysis identified candidate variants in the DAGLA, CTBP1, and ESRRA genes in the proband. Sanger sequencing confirmed the presence of these variants in the affected sister and the mother with uterine fibroids. However, the same variants were also detected in the unaffected father. The observed inheritance pattern did not demonstrate complete co-segregation with the disease phenotype, suggesting that these variants alone are unlikely to account for the development of endometriosis in this family.
Conclusion: Whole-exome sequencing enabled the identification of candidate variants in DAGLA, CTBP1, and ESRRA in a patient with endometriosis. However, segregation analysis did not support a direct causal role for these variants. These findings emphasize the importance of family-based validation following WES and suggest that additional genetic or environmental factors may contribute to disease susceptibility in familial endometriosis.
Keywords: Whole-Exome Sequencing, Segregation Analysis, Endometriosis, High-Throughput Sequencing, Genetic Variants

P-156: Association of HOTAIR Expression with HOXD10 Dysregulation and Epigenetic Alterations in Endometriosis

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Background: Long non-coding RNAs (lncRNAs) have attracted considerable attention due to their extensive regulatory roles in gene expression and the pathogenesis of endometriosis. HOTAIR is a well-characterized lncRNA involved in the epigenetic regulation of HOX family genes. Given the association between altered HOX gene expression patterns and endometriosis, the present study aimed to investigate HOTAIR expression and its association with HOXD10 dysregulation and epigenetic alterations at the HOXD10 regulatory region in endometriotic tissues.
Materials and Methods: Ectopic and eutopic endometrial tissues were collected from 12 women with endometriosis, and normal endometrium samples from 12 women without endometriosis served as controls. All tissue specimens were collected during the proliferative phase. The expression levels of HOTAIR and HOXD10 were evaluated using real-time PCR. Epigenetic alterations at the regulatory region of HOXD10 were assessed by chromatin immunoprecipitation followed by real-time PCR (ChIP-Real-Time PCR) using the histone marks of H3K4me3 and H3K27me3.
Results: HOTAIR expression was increased in endometriotic tissues compared with normal endometrium, with a statistically significant increase observed only in ectopic tissues ($P<0.05$). In contrast, HOXD10 expression showed a decreasing level in endometriotic tissues. A statistically significant reduction was observed in ectopic tissues compared with both eutopic and control tissues ($P<0.01$). The observed epigenetic profile at the HOXD10 regulatory region was consistent with the reduced expression of HOXD10: decreased levels of the gene-activating histone mark of H3K4me3 and increased levels of the gene-repressive histone mark of H3K27me3 were observed in eutopic and ectopic tissues compared with controls; however, neither difference reached statistical significance.
Conclusion: Increased HOTAIR expression together with decreased HOXD10 expression in endometriotic tissues suggests a potential association between HOTAIR dysregulation and HOXD10 suppression in endometriosis. The observed epigenetic alterations at the HOXD10 regulatory region further support a possible role for epigenetic mechanisms in this process.
Keywords: Endometriosis, HOTAIR, Reproductive Biology, HOXD10, Epigenetics

P-157: Preconception Identification of the LOXHD1 C.2641G>A (P.Gly881Arg) Variant in An Asymptomatic Iranian Azeri Consanguineous Couple: A Case for Population-Specific Carrier Screening

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Background: Consanguineous unions, accounting for ~40% of marriages in Iran, substantially elevate the risk of autosomal recessive disorders such as LOXHD1-related nonsyndromic hearing loss (DFNB77). Preconception carrier screening remains underutilized, and population-specific variant spectra are incompletely defined. An 18-year-old woman and her 21-year-old double-cousin partner, both healthy and without personal or family history of hearing impairment, proactively sought preconception genetic counseling in Tabriz, Iran.
Materials and Methods: Whole-exome sequencing (WES) was performed on both individuals. An R-based bioinformatic pipeline filtered variants with population frequency <1% in gnomAD, predicted pathogenicity (PolyPhen-2, SIFT), and cross-referenced clinical databases (ClinVar, dbSNP) using the rentrez package. The candidate variant was confirmed by Sanger sequencing.
Results: Both partners were heterozygous for the LOXHD1 c.2641G>A (p.Gly881Arg) variant, classified as Pathogenic/Likely pathogenic in ClinVar (Variation ID: 1875098). The couple self-identified as Iranian Azeri. This missense change, previously reported in a Dutch family with mild, stable hearing loss, has not been observed in the Iranome database of 800 healthy Iranian individuals, highlighting its rarity in the local population. Co-carriage in this consanguineous couple conferred a 25% risk of having a child affected with DFNB77.
Conclusion: To our knowledge, this represents the first identi-

fication of the LOXHD1 c.2641G>A variant in asymptomatic Iranian Azeri carriers detected through preconception screening, as opposed to diagnostic testing in affected probands. The absence of this variant from Iranome further implies that it may be a low-frequency but clinically actionable allele circulating cryptically in the Iranian Azeri gene pool. This case strongly supports the integration of such variants into expanded, population-specific carrier panels for high-risk communities and demonstrates the power of exome-based preconception screening in consanguineous populations to enable truly informed reproductive decision-making.

Keywords: LOXHD1, DFNB77, Iranian population, Autosomal recessive hearing loss

P-158: Lower Prevalence of MED12 Mutations in Iranian Women with Uterine Fibroids Compared to Global Reports Despite High Prevalence in Multiple Fibroid

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Background: Uterine fibroids are the most common tumors of the female reproductive system, with a prevalence up to 70–80%. About half of the patients develop symptoms such as pelvic pain, heavy uterine bleeding, urinary incontinence, and pregnancy related reproductive complications. Recent studies have demonstrated that the molecular pathogenesis and clinical severity of these tumors may be modulated by pathogenic variants in the *MED12* gene, which is located on the *Xq13*.1 and encoding the large subunit of the mediator complex. In uterine leiomyomas, pathogenic *MED12* mutations occur mainly at codon 44 of exon 2. The present study aims to evaluate the prevalence of *MED12* mutations in Iranian patients with uterine fibroids.

Materials and Methods: This study included 40 Iranian women with uterine fibroids, categorized as having solitary or multiple tumors based on ultrasonography. Specific primers targeting exon 2 of the *MED12* gene were designed, and genomic DNA was isolated from fibroid tissue samples. PCR amplification was conducted, and product integrity was assessed through gel electrophoresis. In the absence of nonspecific amplification or contamination, PCR products were processed for Sanger sequencing. The resulting sequence data were analyzed to identify pathogenic variants in exon 2 of the *MED12* gene.

Results: study, 40 uterine fibroid tissue samples from patients at Royan Research Institute and Imam Khomeini Hospital were examined for mutations in exon 2 of the MED12 gene. Somatic mutations were detected in 12 samples (30%). Among these, 8 carried the most common variant (codon 44, c.130 G>C), and 7 of the 8 patients with this mutation presented with multiple fibroids.

Conclusion: The prevalence of exon 2 *MED12* mutations in Iranian women with uterine fibroids was 30%, similar to previous Iranian reports but lower than global estimates. Consistent with earlier studies, *MED12* mutations were predominantly detected in patients with multiple fibroids (87%), supporting an association between *MED12* alterations and fibroid multiplicity. Larger multicenter studies are needed to further clarify the genetic landscape of uterine fibroids in Iranian population.

Keywords: Uterine fibroids, *MED12*, Multiple

P-159: CRISPR/Cas9 Mediated Disruption of Nanos2 Using a MiRGD Peptide Nanocarrier in Mice

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Background: Spermatogenesis is a highly complex and tightly regulated biological process that depends on the coordinated expression of multiple genes, among which Nanos2 plays a critical role in maintaining male germ cell populations. Dysregulation of this gene has been associated with impaired spermatogenesis, azoospermia, and male infertility. The CRISPR-Cas9 gene editing system has emerged as a powerful tool for targeted gene disruption and generation of disease models. This study aimed to investigate the efficiency of CRISPR-mediated targeting of Nanos2 using a peptide-based nanocarrier system and to develop a novel mouse model of azoospermia.

Materials and Methods: A specific single-guide RNA (sgRNA) targeting the *Nanos2* gene was designed, cloned, and validated. The CRISPR-Cas9 components were delivered using the MiRGD peptide-based nanocarrier, which consists of three functional domains: histone H1 for DNA condensation, Gp41 for endosomal escape, and a nuclear localization signal (NLS) derived from SV40. The nanocarrier system was utilized to deliver CRISPR components into mouse embryos. Gene disruption efficiency was evaluated using molecular (qRT-PCR), protein-level analyses, and histopathological assessments.

Results: Successful cloning and validation of sgRNA targeting *Nanos2* were achieved. Efficient delivery of CRISPR-Cas9 components into mouse embryos resulted in significant downregulation of *Nanos2* expression at both mRNA and protein levels. Genomic and pathological analyses confirmed effective gene disruption and successful integration of the CRISPR system. The edited embryos developed into a mouse model exhibiting characteristics consistent with azoospermia, indicating functional impairment of spermatogenesis.

Conclusion: The findings demonstrate that CRISPR-Cas9-mediated targeting of *Nanos2* using a MiRGD peptide-based nanocarrier is an effective approach for gene disruption both *in vitro* and *in vivo*. This strategy provides a robust platform for generating animal models of azoospermia and offers valuable insights into the molecular mechanisms underlying spermatogenic failure. Furthermore, it highlights the potential application of nanocarrier-assisted gene editing in reproductive biology and gene therapy research.

Keywords: *Nanos2*, CRISPR-Cas9, Peptide Nanocarrier, Mouse Model

P-160: Decrease Expression of KLOTHO in Follicular Fluid is Associated with Poor Ovarian Response (POR)

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Background: Achieving optimal outcomes in *In Vitro* Fertilization (IVF) is frequently hindered by Poor Ovarian Response (POR), posing a significant hurdle in current clinical practice. In affected patients, inadequate response to standard ovarian stimulation protocols results in reduced follicular development and oocyte yield, ultimately decreasing the probability of successful pregnancy. Follicular Fluid (FF) reflects the biochemical microenvironment of the developing oocyte and serves as a valuable source for evaluating molecular and genetic biomarkers associated with follicular competence. The *KLOTHO* gene plays a critical role in folliculogenesis, regulation of oxidative stress and ovarian aging through multiple interconnected molecular pathways. Given its involvement in cellular homeostasis and aging-related mechanisms, investigating its expression pattern in FF may provide further insight into the molecular bases of POR. The present study aimed to evaluate the expression level of the *KLOTHO* gene in the Follicular Fluid of infertile women diagnosed with POR compared to fertile healthy controls.
Materials and Methods: This case–control study included 40 participants: 20 infertile women diagnosed with POR and 20 healthy women (including women undergoing ICSI due to male-factor infertility, oocyte donors, and women undergoing PGD for sex selection). All participants were selected according to predefined inclusion and exclusion criteria. After obtaining informed consent from the participants and collecting FF sample, RNA was extracted using a commercial extraction kit, then complementary DNA (cDNA) synthesised. Gene expression levels of *KLOTHO* were analyzed using Real-Time PCR. Statistical analysis was performed at the statistical level of $P \leq 0.05$ using GraphPad Prism software version 10.
Results: Results revealed a statistically significant downregulation of *KLOTHO* gene expression in the follicular fluid of POR women compared with the control group (P=0.0498).
Conclusion: These findings provide novel insight into the potential role *KLOTHO* in Follicular Fluid within the context of human reproductive biology. *KLOTHO* may influence ovarian physiology through mechanisms related to cellular aging, oxidative stress regulation, and maintenance of follicular microenvironment homeostasis. It may also serve as a potential biomarker associated with follicular competence and treatment response in POR patients.
Keywords: Klotho, Poor Ovarian Response, Follicular Fluid, Real-Time PCR, Infertility

P-161: MAPK14 Upregulation in the Follicular Microenvironment is Associated with Poor Ovarian Response (POR) in Women Undergoing Assisted Reproductive Technology

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Background: Poor ovarian response (POR) is one of the major challenges in Assisted Reproductive Technology (ART) and is associated with reduced oocyte yield, poor oocyte quality, and lower pregnancy rates. The follicular microenvironment plays a critical role in folliculogenesis and oocyte maturation through multiple intracellular signaling pathways. Mitogen-activated protein kinases (MAPKs) are involved in regulating cellular proliferation, differentiation, apoptosis, and inflammatory responses. MAPK14, encoding p38α MAPK, is known to participate in cellular stress responses and ovarian function; however, its role in POR remains unclear. Since in POR, the levels of oxidative stress and inflammation are high, this study aimed to evaluate MAPK14 expression in the follicular fluid of infertile women with POR compared with women with normal ovarian response.
Materials and Methods: This case-control study included 40 women undergoing ART cycles, consisting of 20 POR women and 20 women with normal ovarian response (egg donors or infertile women with male factor infertility). Informed consent was obtained from all participants then follicular fluid samples were collected during oocyte retrieval following controlled ovarian stimulation. Total RNA was extracted, and complementary DNA was synthesized for gene expression analysis. Relative MAPK14 messenger RNA expression was measured using Quantitative Real-Time Polymerase Chain Reaction (qPCR). For statistical analysis, Kolmogorov-Smironov test (to check the normality of data distribution) followed by the Mann-Whitney U test performed using GraphPad Prism 10 software were used. $P<0.05$ was considered statistically significant.
Results: MAPK14 expression was significantly increased in the follicular fluid of women with POR compared with the control group ($P<0.0001$).
Conclusion: The present findings suggest that MAPK14 may contribute to the pathophysiology of POR through stress-related and inflammatory signaling mechanisms. MAPK14 could potentially serve as a molecular biomarker for identifying women with poor ovarian response during ART treatment. Further studies are required to clarify the functional role of the p38 MAPK pathway in ovarian dysfunction. Moreover, increased

MAPK14 expression may reflect an impaired follicular microenvironment associated with oxidative stress and reduced ovarian competence.
Keywords: Assisted Reproductive Technology, Poor Ovarian Response, Follicular Fluid, MAPK14, Gene Expression

P-162: GRB2 Gene Expression in the Follicular Fluid of Women with Poor Ovarian Response

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Background: Growth factor receptor bound protein 2 (GRB2) is a key adaptor protein that links receptor tyrosine kinase activation to the RAS MAPK signaling pathway and regulates important cellular processes such as proliferation, differentiation, and survival. In ovarian physiology, GRB2 mediated signaling in granulosa cells plays an essential role in follicular development and oocyte maturation. Poor ovarian response (POR) is a major challenge in fertility treatments and often leads to a diminished number of retrieved oocytes and suboptimal reproductive results. Follicular fluid (FF), which represents the microenvironment surrounding the developing oocyte, reflects follicular competence and ovarian function. This study aimed to evaluate *GRB2* gene expression in the FF of women with POR compared with normal responders.
Materials and Methods: In this case–control study, follicular fluid samples were collected from 40 women undergoing IVF/ICSI cycles. The case group consisted of 20 POR women and the control group included 20 women with normal ovarian response) including patients with male factor infertility, oocyte donors, and individuals undergoing preimplantation genetic diagnosis (PGD) (Informed consent was obtained from all participants. Total RNA was extracted from FF samples followed by complementary DNA (cDNA) synthesis. The expression levels of *GRB2* were quantified using quantitative real time polymerase chain reaction (qRT PCR). Statistical analyses were performed using GraphPad Prism software. The normality of quantitative variables was assessed using the Kolmogorov–Smirnov test, and comparisons between groups were conducted using the Mann–Whitney U test. A $p \leq 0.05$ was considered statistically significant.
Results: Quantitative real time PCR data showed GRB2 gene expression was significantly decreased in the follicular fluid of women with poor ovarian response compared with the control group ($p=0.0439$).
Conclusion: Reduced *GRB2* gene expression in follicular fluid may contribute to the molecular mechanisms underlying poor ovarian response. Considering the key role of *GRB2* in intracellular signal transduction and its role in upstream of MAPK signaling pathway through oogenesis, oocyte development, and meiotic induction, decreased expression of *GRB2* may impair activation of this pathway. Also, *GRB2* may represent an important factor which influence follicular competence in assisted reproductive technologies.
Keywords: *GRB2*, Follicular Fluid, Poor Ovarian Response, Gene Expression, Assisted Reproductive Technologies

P-163: Preventive Importance of PGT-M (Preimplantation Genetic Testing for Monogenic Disorders) in Rare Diseases: A Case Study of Spastic Paraplegia Type 54 Disease

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Background: Spastic paraplegia type 54 (SPG54) is a rare autosomal recessive inherited disease with early-onset spastic paraplegia, intellectual disability, and corpus callosum dysplasia due to *DDHD2* gene mutations. Its exact global prevalence is currently unknown, but it is estimated to affect fewer than 1 in 1,000,000 people worldwide. In this study, we aimed to conduct a PGT-M test for the heterozygote couple with a previously affected child.
Materials and Methods: The couple underwent one cycle of IVF-ICSI with biopsy of 9 embryos on day three after a one-step culture procedure. Targeted sequencing on the NM_0011 64232.2:c.694C>T:p.232R>T Nested PCR for the 6 flanking regions of STR markers. After a successful pregnancy of the transferred embryo, the fetal health was assessed by amniocentesis at week 15.
Results: PGT-M was performed on cells from 9 embryos. Of the 4 transferable embryos, 2 normal homozygous and 2 heterozygous embryos were identified. Sanger sequencing was performed to confirm the mutation after amniocyte culture and DNA extraction. The successful transfer results in a normal homozygous fetus.
Conclusion: To the best of our knowledge, this study describes the first application of PGT-M in *DDHD2* gene mutation in SPG45 disease prevention, and the outcome highlights the preventive importance of PGT-M in rare monogenic disorders.
Keywords: SPG54, PGT-M, ICSI, Intellectual Disability

P-164: Association of BUB1B Gene Polymorphism (c.1046A>G, rs1801376) with Recurrent Spontaneous Abortion in Iranian Women

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Background: Recurrent spontaneous abortion (RSA), defined

as three or more recurrent miscarriages before 24 weeks of gestation, has multiple causes, including genetic polymorphisms. The *BUB1B* gene, which is required for normal chromosome segregation during cell division, has been linked to RSA. This study investigates the relationship between the c.1046A>G (rs1801376) polymorphism in the BUB1B gene and RSA in an Iranian female population.

Materials and Methods: Thirty women with RSA and 30 healthy control women participated in this study. Blood samples were collected and genomic DNA was extracted. The c.1046A>G polymorphism was genotyped using Tetra primer amplification refractory mutation system polymerase chain reaction (Tetra-ARMS-PCR). The genotypes were confirmed by Sanger sequencing. Statistical analysis was used to determine the relationship between the polymorphism and RSA.

Results: The genotypic distribution of the c.1046A>G polymorphism was 13.33% AA, 70% AG, and 16.66% GG in the RSA group, and 6.66% AA, 93.33% AG, and 0% GG in the control group. The genotypic distribution differed significantly between the two groups (χ^2=8.37, p=0.015) while the frequencies of the A and G alleles were not significantly different (χ^2=0.267, p=0.606).

Conclusion: The study found a significant association between the c.1046A>G polymorphism in the BUB1B gene and RSA, with the GG genotype possibly increasing the risk of RSA.

Keywords: Recurrent Spontaneous Abortion, BUB1B Gene, Polymorphism

P-165: Association of Inherited Thrombophilia-Related Gene Polymorphisms (Factor V Leiden, Factor XIII Val-34Leu and PAI-1) with Recurrent Implantation Failure: A Case–Control Study

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Background: Recurrent implantation failure (RIF) remains a challenging condition in assisted reproductive technology (ART), with inconsistent evidence regarding the contribution of inherited thrombophilia. Previous studies investigating thrombophilia-related polymorphisms in RIF have yielded conflicting results, partly due to differences in study design and control group selection. This study aimed to evaluate the association of three thrombophilia-related polymorphisms—Factor V Leiden (G1691A), Factor XIII (Val34Leu), and PAI-1 (4G/5G) with RIF using a fertile control population.

Materials and Methods: In this case–control study, 100 women under 40 years of age with RIF, defined as three or more failed IVF/ICSI cycles despite transfer of at least four good-quality embryos, were compared with 200 fertile women who had at least one natural successful pregnancy and no history of infertility or miscarriage. Women with endometriosis, hydrosalpinx, uterine abnormalities, autoimmune disorders, thromboembolic disease, recurrent pregnancy loss, severe pregnancy complications, severe male factor infertility, or chromosomal abnormalities were not entered. Genotyping data for the RIF group were obtained from Royan Institute records, while DNA samples from controls were retrieved from the Royan DNA Bank. Genotyping of Factor V Leiden (G1691A), Factor XIII (Val34Leu), and PAI-1 (4G/5G) polymorphisms were performed using PCR-RFLP. Genotypes/allele frequencies were compared using the chi-square test, with P<0.05 considered statistically significant.

Results: No significant differences were observed between RIF and fertile women regarding genotype or allele frequencies of Factor V Leiden (G1691A) as well as PAI-1 (4G/5G) polymorphisms (P>0.05). In contrast, genotype frequencies of the Factor XIII Val34Leu polymorphism differed significantly between groups (P=0.024) meaning that the heterozygous genotype was more frequent among fertile controls, whereas the homozygous mutant genotype was detected exclusively in women with RIF. However, allele frequencies did not differ significantly between groups.

Conclusion: Among the investigated thrombophilia-related polymorphisms, only frequency of Factor XIII Val34Leu genotypes demonstrated a significant difference between women with RIF and fertile controls. The exclusive presence of the homozygous mutant genotype in the RIF group suggests a possible association with susceptibility to recurrent implantation failure. Larger studies are required to confirm these findings and clarify the role of thrombophilic variants in implantation outcomes.

Keywords: Recurrent Implantation Failure, Hereditary Thrombophilia, Gene Polymorphism,FV Leiden, FXIII, PAI-1

P-166: Association Study of Normal Chromosomal Variants in Recurrent Pregnancy Loss: A Case-Control Study from Royan Institute

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Background: Recurrent pregnancy loss (RPL) is a multifactorial reproductive disorder in which genetic factors are significant etiological contributors. In this regard, the clinical significance of normal chromosomal variants (NCVs), also known as chromosomal polymorphisms, remains controversial. This study aimed to compare the frequency and distribution of NCVs in RPL couples with fertile controls referred to Royan Institute.

Materials and Methods: In this retrospective case-control study, 733 couples (325 with RPL and 408 fertile controls with at least one live healthy child) were evaluated. Clinical and cytogenetic data were obtained from existing medical records. Pe-

ripheral blood lymphocyte karyotyping results (GTG-banding) were reviewed and analyzed. NCVs were classified according to the International System for Human Cytogenetic Nomenclature (ISCN) guidelines. Statistical analyses were performed using chi-square and Fisher's exact tests, with a significance level of $P<0.05$.

Results: The mean age of participants was 33.79 ± 4.39 years. NCVs were identified in 32.5% of all couples, with no significant difference in overall frequency between the RPL group (29.8%) and controls (34.6%) (P=0.442). Some variants, including Yqh+, inv(9)(p12q13), and several short-arm or heterochromatic variations involving chromosomes 13, 15, and 22, were relatively more common in the RPL group. Statistically significant increase for inv(3)(p11.2q12) (P=0.02) and 15cenh+ (P=0.029) were observed in control and RPL groups, respectively.

Conclusion: In general, no significant difference in the overall frequency of NCVs was observed, indicating that the type of NCV should be considered in assessment of clinical consequences. Thus, some specific variants, particularly inv(3)(p11.2q12) and 15cenh+, with significant differences between two groups may be associated with RPL and warrant further investigation.

Keywords: Recurrent Pregnancy Loss, Chromosomal Polymorphism, Karyotype, Cytogenetics, Normal Chromosomal Variants

P-167: Association Between HLA-G 14-bp Insertion/Deletion Polymorphism and Recurrent Pregnancy Loss Risk: A Meta-Analysis

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Background: Recurrent pregnancy loss (RPL) is a multifactorial disorder in which immunological factors may contribute to disease susceptibility. The *HLA-G* gene, involved in maternal–fetal immune tolerance, contains a 14-bp insertion/deletion polymorphism in its 3′ untranslated region (3′UTR), which may affect gene expression and susceptibility to RPL. Given the inconsistent results of previous studies, this meta-analysis aimed to evaluate the association between the HLA-G 14-bp Ins/Del polymorphism and the risk of RPL.

Materials and Methods: A systematic search was conducted in PubMed, Scopus, Web of Science, and Google Scholar databases. This study included 30 studies from populations in Asia, Europe, and South America. A total of 3318 women with RPL and 2847 healthy controls were included in the meta-analysis. Genotype frequencies were extracted, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to evaluate the association between the polymorphism and RPL risk.

Results: Results showed a significant association between the Ins/Ins genotype and RPL (OR=1.27, 95% CI: 1.06–1.53, P=0.008). In addition, the Del/Del genotype demonstrated a protective effect against RPL (OR=0.79, 95% CI: 0.65–0.95, P=0.015). In subgroup analysis, a stronger association between the Ins/Ins genotype and RPL was observed in European populations (OR=1.64, 95% CI: 1.29–2.08, P =0.000051). In Asian populations, the Del/Ins genotype was significantly associated with RPL (OR=1.33, 95% CI: 1.007–1.76, P=0.044). No significant association was observed in South American populations.

Conclusion: Overall, this meta-analysis suggests a genotype-dependent association between the HLA-G 14-bp Ins/Del polymorphism and RPL risk. Further large-scale studies in diverse populations are needed to confirm these findings.

Keywords: Recurrent Pregnancy Loss, HLA-G, 14-bp Insertion/Deletion Polymorphism, Genetic Susceptibility, Meta-Analysis

P-168: Association Study of Normal Chromosomal Variants in Recurrent Implantation Failure: A Case-Control Study from Royan Institute

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Background: Recurrent implantation failure (RIF) is a major obstacle in assisted reproductive treatments (ART) which happens in about 10% of cycles. Chromosomal study is one of the recommended evaluations in RIF couples while the importance of detecting normal chromosomal variants (NCVs) is still controversial. This study aimed to evaluate and compare the frequencies of NCVs in RIF couples with fertile control couples.

Materials and Methods: In this case–control study, 412 couples with RIF and 412 fertile couples were enrolled. The RIF group had history of failed repeated ART cycles. In addition, all women were under 40 years of age and had normal hormonal profiles, semen parameters, and uterine status. All participants underwent cytogenetic evaluation and karyotype analysis to identify chromosomal polymorphisms. Statistical analyses were performed using Chi-square test and Fisher's exact test (SPSS version 27).

Results: Overall, 27.1% of couples in the RIF group and 35.2% in the control group had at least one chromosomal polymorphism, showing a statistically significant difference (P=0.028). The frequency of the 15ps+ polymorphism was 0.7% in the RIF group and 3.9% in the control group (P=0.002). While, the 15cenh+ polymorphism was significantly more frequent in the RIF group (5.3% vs. 1.2%; P=0.005). In addition, many other chromosomal polymorphisms (such as 9qh+ and in inv(9)) were more frequent in the RIF group; which warrants further investigations.

Conclusion: In general, overall frequency of chromosomal polymorphisms were not associated with presence of RIF. However, the 15cenh+ polymorphism may represent a potential factor associated with impaired fertility and recurrent implantation failure. Furthermore, increased frequencies of 9qh+ and inv(9) polymorphisms in RIF group merit further investigation.

Keywords: Chromosomal Polymorphism, Recurrent Implanta-

tion Failure, Infertility, Normal Variants

P-169: Multiplex PCR-Based Non-Invasive Preimplantation Genetic Testing for Embryo Sex Determination Using Spent Culture Media Cell-Free DNA: A High-Concordance Comparative Study with PGT-FISH

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Background: In assisted reproductive technologies (ART), choosing genetically normal embryos while avoiding invasive procedures remains a major challenge. Although conventional preimplantation genetic testing (PGT) based on embryo biopsy offers high diagnostic accuracy, concerns remain regarding its potential effects on embryo viability, developmental competence, and implantation outcomes. Accurate embryo sex determination is particularly important for preventing X-linked genetic disorders. Consequently, non-invasive approaches based on cell-free DNA (cfDNA) released into spent culture media have gained increasing attention. This study evaluated the feasibility and diagnostic performance of a multiplex PCR-based noninvasive PGT (niPGT) approach for embryo sex determination using spent culture media-derived cfDNA and compared the results with the standard PGT-FISH method.
Materials and Methods: Ninety-three individually cultured IVF/ICSI embryos and seven embryo-free controls were included. To minimize contamination, embryos underwent serial washing and transfer to fresh culture droplets on days 3 and 4. Spent culture media were individually collected on days 4 and 5 post-fertilization using sterile filter tips and transferred into DNase/RNase-free microtubes. Extracted cfDNA was analyzed by multiplex PCR targeting SRY and AMELX/AMELY, and results were validated against PGT-FISH under strict contamination-control conditions.
Results: Embryonic cfDNA was detected in a substantial number of samples, while all negative controls remained negative, confirming strong analytical specificity and effective contamination control. Overall concordance between niPGT and PGT-FISH was 91.4% (85/93 embryos), with a non-diagnostic rate of 8.6% (8/93 embryos). Among the 85 embryos with interpretable results, complete agreement with the reference method was observed, corresponding to 100% sensitivity, 100% specificity, and a Cohen's kappa of 1.0. When non-diagnostic cases were considered as errors, the adjusted accuracy remained 91.4%. The main limitation was the low level of cfDNA in the culture medium, which reduced amplification efficiency in some samples.
Conclusion: These findings support the feasibility of using cfDNA from spent culture media for non-invasive embryo sex determination. The relatively large sample size, individualized culture system, strict contamination control, and optimized cfDNA workflow all contributed to the reliability of the results. Overall, this approach shows strong potential as a less invasive alternative for embryo selection and prevention of X-linked disorders, although further multicenter studies are needed before routine clinical use.
Keywords: Non-Invasive PGT(Nipgt), Cell-Free DNA (Cfdna), Multiplex PCR,Embryo Sex Determination

P-170: Application of Single-Cell RNA Sequencing in Identifying Endometrial Immune Cell Alterations and Their Role in Female Infertility

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Background: Successful embryo implantation requires a finely tuned immune balance within the endometrium during the window of implantation. Disruption of this immune microenvironment has been implicated in female infertility, particularly recurrent implantation failure (RIF). However, the cellular and molecular basis of this dysfunction remains incompletely understood.
Materials and Methods: To investigate the role of immune cells in female infertility, publicly available endometrial single-cell RNA sequencing (scRNA-seq) datasets, including data from the Human Endometrial Cell Atlas (HECA) and infertility-associated cohorts, were integrated and analyzed. The study focused on characterizing the heterogeneity of uterine natural killer (uNK) cells, T lymphocytes, and macrophages, identifying their gene expression patterns, and examining intercellular communication networks involved in maintaining endometrial receptivity and supporting successful embryo implantation.
Results: Analysis of single-cell datasets revealed extensive immune heterogeneity within the human endometrium, with uterine natural killer (uNK) cells representing the dominant immune population across endometrial states. The HECA resource further demonstrated distinct immune subpopulations with specialized transcriptional programs involved in tissue remodeling, immune tolerance, and endometrial receptivity. Comparative evaluation of fertile and infertile samples identified significant alterations in immune composition and function, including dysregulated uNK cell states, reduced regulatory T-cell (Treg) signatures, and enrichment of pro-inflammatory macrophage populations. These changes were accompanied by consistent disruption of signaling pathways associated with immune tolerance and implantation, particularly IL-15 and TGF-β signaling. Furthermore, cell–cell communication analyses revealed weakened interactions between immune and stromal compartments, suggesting impaired coordination of the implantation-supportive niche. Collectively, infertile endometrial samples displayed a pro-inflammatory transcriptional landscape associated with reduced endometrial receptivity and increased susceptibility to implantation failure.
Conclusion: Single-cell transcriptomic analysis provides unprecedented insight into the immune architecture of the human endometrium and highlights immune dysregulation as a central feature of implantation failure. These findings support the de-

velopment of immune-based biomarkers and targeted therapeutic strategies for improving reproductive outcomes in women with infertility.

Keywords: Endometrium, Single-Cell RNA Sequencing, Human Endometrial Cell Atlas (HECA), Endometrial Immune Microenvironment

P-171: Effect of Curcumin on the Expression of HOTAIR And HOX Genes in Endometrial Stromal Cells Derived from Women with Endometriosis

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Background: Endometriosis is an estrogen-dependent inflammatory disease characterized by altered gene expression and abnormal cellular functions. Long non coding RNAs and HOX genes have been shown to play important roles in the pathogenesis of this disorder. Curcumin is a natural polyphenolic compound with anti-inflammatory and antioxidant properties that has attracted considerable attention because of its therapeutic potential. Therefore, this study aimed to investigate the effect of curcumin treatment on the expression pattern of HOTAIR and related HOX genes in endometrial stromal cells derived from women with endometriosis.
Materials and Methods: In this case-control study, eutopic endometrial tissues obtained from 12 women with endometriosis and 12 healthy controls were used for stromal cell isolation and culture. both normal and endometriotic stromal cells were divided into treated and untreated groups. Cells were exposed to different concentrations of curcumin (10-100Mm) for 72 hours. Subsequently, the expression levels of *HOTAIR*, *HOXC11*, *HOXC12*, and *HOXD10* were analyzed using quantitative real-time PCR.
Results: Curcumin treatment altered the expression profile of several genes associated with endometriosis. A significant decrease in *HOXC12* expression was observed in treated endometriotic stromal cells. In addition, the expression levels of *HOTAIR* and *HOXC11* showed a decreasing trend following curcumin treatment, however, these changes were not significantly.
Conclusion: the of thid study indicate that curcumin can modulate the expression pattern of HOTAIR and HOX genes in endometrial stromal cells. these results suggest that curcumin may be involved in regulating molecular pathways associated with endometriosis.
Keywords: Endometriosis, Curcumin, Endometrial Stromal Cells, HOTAIR, HOX Gene

Health

P-172: Emotional Well-Being and Psychological Support in Infertility: A Systematic Review of Artificial Intelligence-Based Digital Interventions

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Background: Infertility is associated with substantial emotional distress, anxiety, depressive symptoms, and reduced quality of life in affected individuals and couples. In recent years, artificial intelligence (AI)-based digital interventions, including chatbots, conversational agents, adaptive support platforms, and data-driven mental health tools, have emerged as potential approaches for delivering personalized psychological support in reproductive care.
Materials and Methods: This systematic review was conducted in accordance with the PRISMA 2020 statement. PubMed and Web of Science were systematically searched using predefined terms related to infertility, digital interventions, artificial intelligence, and psychological outcomes. Original studies evaluating AI-based digital interventions designed to support emotional well-being, psychological health, or mental health outcomes in individuals or couples experiencing infertility were considered eligible. Following duplicate removal, title/abstract screening and full-text assessment were performed independently by two reviewers. Sixteen studies met the inclusion criteria and were synthesized narratively.
Results: The included studies encompassed a range of AI-enabled approaches, such as chatbot-based support, adaptive digital counseling tools, machine learning-informed mental health monitoring, and personalized online support systems. Reported target outcomes included infertility-related distress, anxiety, depressive symptoms, emotional well-being, coping, and patient engagement. Across studies, AI-based interventions showed promising potential for improving accessibility, personalization, and continuity of psychological support; however, the evidence base remained heterogeneous and was limited by variation in intervention design, outcome measures, follow-up duration, and methodological rigor.
Conclusion: AI-based digital interventions represent a promising emerging strategy for addressing the psychological burden of infertility. Nevertheless, larger and methodologically robust studies with standardized outcome reporting are needed before these tools can be more confidently integrated into routine fertility care.
Keywords: Emotional Well-Being, Mental Health, Artificial Intelligence, Machine Learning, Deep Learning

P-173: The Role of Psychological Stress in The Persistence and Immune Response of High-Risk Human Papillomavirus Infection: A Systematic Review

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Background: Persistent high-risk human papillomavirus (HR-HPV) infection is the principal cause of cervical carcinogenesis and adverse reproductive outcomes. Although most HPV infections are transient and cleared by the immune system, chronic psychological stress may impair antiviral immunity and facilitate viral persistence via neuroendocrine and inflammatory dysregulation. However, this association remains insufficiently characterized.
Materials and Methods: A systematic search was conducted in PubMed, Scopus, Web of Science, and Google Scholar for articles published from January 2015 to March 2025, using terms including "psychological stress," "high-risk HPV," "HPV persistence," "immune response," "cortisol," and "cervical dysplasia." Of 256 initial records after duplicate removal, observational cohort and clinical studies assessing psychological stress and HR-HPV persistence or immune dysregulation were screened. Irrelevant studies were excluded based on title, abstract, and full-text review. Study selection, data extraction, and quality assessment followed PRISMA 2020 guidelines. Eighteen studies met the eligibility criteria and were included in the final synthesis.
Results: Chronic psychological stress was significantly associated with persistent HR-HPV infection. Women with higher levels of anxiety, depressive symptoms, and perceived stress showed increased cortisol secretion, impaired T cell mediated immunity, reduced natural killer cell activity, and altered Th1/Th2 balance. Elevated levels of IL 6, TNF α, and CRP were linked to impaired viral clearance and progression of cervical intraepithelial lesions. Collectively, the evidence supports a biologically plausible association between chronic psychological stress, immune dysregulation, oxidative stress, and HR-HPV persistence.
Conclusion: Current evidence indicates that psychological stress may contribute to HR-HPV persistence through neuroendocrine, inflammatory, and immune mediated pathways. Incorporating psychoneuroimmunological approaches and stress management interventions into HPV related reproductive healthcare could serve as a complementary strategy for cervical disease prevention. Further longitudinal and mechanistic studies are warranted.
Keywords: Psychological Stress, High-Risk HPV, HPV Persistence, Immune Response

P-174: Accuracy of Large Language Models in Reproductive Health Counseling: A Systematic Review

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Background: Large language models (LLMs) are increasingly consulted for reproductive health information, yet evidence on their accuracy, safety, and suitability for patient counseling remains fragmented. This review synthesized evidence on LLM performance and explored sources of variability across studies.
Materials and Methods: Searches were conducted in May 2026 in PubMed, Scopus, Web of Science, and Google Scholar. Studies published from 2024 to 2025 were eligible if they quantitatively assessed LLM responses to reproductive health questions, scenarios, or related tasks. From 2,862 records, 14 cross-sectional studies met inclusion criteria according to the PRISMA 2020 process. Methodological quality was appraised using the JBI checklist for analytical cross-sectional studies. Extracted data were grouped into three analytic domains: comparative response accuracy, context-related performance variation, and patient-facing safety, readability, and communication issues.
Results: Overall, LLM performance was moderate to high but inconsistent across tasks. In specialist-comparison studies, clinicians achieved higher scores than LLMs; physician ratings ranged from 8.0 to 9.0 of 10, compared with 7.0 for ChatGPT and 4.5 for Gemini. In resident-comparison studies, aggregated LLM accuracy reached 73.8%, exceeding the 65.4% mean score of obstetrics and gynecology residents in routine scenarios. Performance varied by domain: assisted reproductive technology, reproductive genetics, and image-based interpretation were more error-prone than general counseling topics. Cultural and linguistic factors also shaped response quality, with evidence of Western-oriented assumptions in sexual and reproductive health benchmarks. Safety concerns were recurrent. Several outputs exceeded the recommended sixth-grade reading level, omitted cautionary advice, or failed to recognize automated interpretation limits.
Conclusion: LLMs may support reproductive health counseling by providing generally relevant answers to routine questions, but their performance is not reliable enough for independent clinical use. Their safest role is as supervised adjuncts, where specialists can verify content, adapt language, and address cultural or safety-sensitive gaps.
Keywords: Large Language Model, ChatGPT, Reproductive Health, Diagnostic Accuracy

P-175: Bridging Infertility Terminologies: Semantic Mapping Across ICD-10, ICD-10-CM, ICD-11, and SNOMED CT

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Background: Infertility is a major global public health concern with important psychological, social, and healthcare impacts.

The increasing use of electronic health records highlights the need for standardized clinical terminologies to ensure accurate data representation and interoperability. However, infertility-related concepts remain inconsistently represented across major coding systems and have not been systematically compared across international classification frameworks. Therefore, this study aimed to map, evaluate, and compare these concepts across ICD-10, ICD-10-CM, ICD-11, and SNOMED CT.

Materials and Methods: Infertility-related concepts were extracted from ICD-10, ICD-10-CM, ICD-11, and SNOMED CT. SNOMED CT was used as the reference terminology. Concepts were mapped using combined linguistic and semantic analyses and classified into five categories: Complete, Wider, Narrower, Post-coordinated, and Not Mapped. All mappings were independently reviewed by two investigators. The resulting dataset was analyzed to compare content coverage, semantic overlap, conceptual gaps, and gender-based representation across the systems.

Results: A total of 252 infertility-related concepts were identified and analyzed across four classification systems. SNOMED CT demonstrated the broadest conceptual coverage (169 concepts, 67.06%) and the highest level of semantic granularity, including detailed representation of etiologies, infertility subtypes, and gender-specific conditions. ICD-11 showed improved conceptual coverage and achieved the highest proportion of complete mappings with SNOMED CT compared with ICD-10 and ICD-10-CM. ICD-10-CM provided more detailed representation than ICD-10, which demonstrated the lowest conceptual coverage and the highest proportion of unmapped concepts. Female-related infertility concepts were more comprehensively represented across all systems, whereas male-related concepts demonstrated higher proportions of incomplete or absent mappings, particularly in ICD-10. Rare, genetic, and treatment-related infertility concepts were incompletely represented in ICD-based systems.

Conclusion: SNOMED CT provides the most comprehensive and semantically rich representation of infertility-related concepts compared with ICD-10, ICD-10-CM, and ICD-11. Although ICD-11 shows improved alignment, important conceptual gaps remain, particularly for rare and male-specific infertility conditions. These findings highlight the importance of standardized terminologies for improving clinical documentation, interoperability, and infertility-related research in health information systems.

Keywords: Infertility, Systematized Nomenclature of Medicine Clinical Terms (SNOMED CT), International Classification of Diseases (ICD), Semantic Mapping

Authors Index

F

G

H

I

J

O

P

R

S

T

V

Y

Z

Abstracts of

Royan International Hybrid Twin Congress

21st Seminar on Nursing and Midwifery
2-3 September 2026

Royan Institute

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Invited Speaker

Inm-1: The Role of Lifestyle in the Management, Empowerment, and Prevention of Menopause Complications

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Menopause is not merely the cessation of menstruation but a life stage characterized by substantial hormonal, metabolic, musculoskeletal, cardiovascular, and psychological changes. Declining estrogen levels are associated with progressive loss of muscle mass and strength, accelerated bone loss, increased central adiposity, and adverse cardiometabolic changes. Therefore, menopause care should extend beyond symptom management toward preservation of physical function, prevention of chronic disease, and promotion of healthy aging. Current evidence supports physical activity as a cornerstone of lifestyle-based menopause care. A systematic review and meta-analysis of 12 studies found that resistance training approximately three times per week, with sessions of about 60 minutes, as an effective model for improving physical function. Evidence for skeletal health is also increasingly supportive. A 2025 systematic review and meta-analysis demonstrated significant benefits of resistance training on bone mineral density (BMD) at the lumbar spine, femoral neck, and total hip. Subgroup analyses suggested greater effects with higher-intensity training (≥70% 1RM), three sessions per week, and longer intervention periods, although substantial heterogeneity limits the generalizability of these findings. Furthermore, a 2026 systematic review of high-intensity resistance and impact training involving 39 studies and 3,349 peri- and postmenopausal women highlighted resistance exercises such as squats, leg press, deadlift, and impact activities as commonly used components of bone-oriented exercise programs, while emphasizing the importance of individualized progression and appropriate clinical supervision. Aerobic exercise provides complementary cardiometabolic benefits. A 2025 systematic review and meta-analysis including 61 randomized controlled trials and 4,100 postmenopausal women found that aerobic exercise reduced systolic blood pressure by 4.5 mmHg and diastolic blood pressure by 2.4 mmHg, while also improving LDL cholesterol, triglycerides, HDL cholesterol, and anxiety symptoms. These findings support the incorporation of regular aerobic activity into menopause care rather than focusing exclusively on resistance exercise. For most women without contraindications, a practical lifestyle prescription should include at least 150 minutes of moderate-intensity aerobic activity or 75 minutes of vigorous-intensity activity per week, together with resistance exercise on at least two days per week. Three resistance-training sessions per week may be considered when the primary goals are preservation or improvement of muscle strength and function. Weight-bearing, balance, and functional exercises should be incorporated according to age, bone health, physical capacity, and fall risk. Nutritional strategies should be integrated with exercise. Current International Menopause Society recommendations support a daily protein intake of approximately 1.0–1.2 g/kg/day, particularly when combined with resistance or weight-bearing exercise, to support muscle preservation and healthy aging. A Mediterranean-style dietary pattern, adequate dietary calcium, individualized vitamin D intake, and appropriate energy balance should therefore form part of a comprehensive lifestyle strategy. In conclusion, Lifestyle intervention in menopause should not be viewed as generic health advice but as a structured, evidence-based clinical intervention. A combination of aerobic activity, progressive resistance training, weight-bearing and balance exercises, adequate protein intake, and a Mediterranean-style dietary pattern can contribute to preservation of muscle and bone, improvement of cardiometabolic health and physical function, and enhancement of overall well-being. Given the heterogeneity of available evidence, lifestyle prescriptions should be individualized according to menopausal symptoms, cardiometabolic risk, body composition, bone health, functional capacity, comorbidities, and patient preferences.

Inm-2: Evolution in Infertility Approach: From Clinical Considerations to Patient-centered Care

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In recent years, infertility management has been evolved and shifted from strict clinical milestones (focused solely on pregnancy success rates and patient's safety) and one-size-fits-all treatment protocols to patient-centered care that prioritized infertile couple's emotional well-being, shared decision-making, transparent communications and individualized treatment plans.
In modern reproductive medicine, harmonizing technological advancements with holistic care that includes prediction, prevention, personalization, and participation aims to optimize both clinical outcomes and quality of life of infertile patients.
Fertility clinics are encouraged to consider both system and human factors and their interaction when setting up "patient-centered improvement projects" including provision of information, competence of clinic and staff, coordination and integration, accessibility, continuity of treatment, patient's physical comfort and privacy, patient-staff relation and communication, patient involvement and emotional support.

Inm-3: Prevention Strategies and Early Diagnosis in Premature ovarian Insufficiency: A Multidimensional Approach

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Premature ovarian insufficiency (POI) is a condition defined as loss of ovarian activity before the age of 40 years. The reported prevalence of non-iatrogenic POI varies from approximately 1% in older studies to 3.5% in recent publications. The limitations of current knowledge and testing for causes of POI and that an exact cause may not be identified. Diagnosis of POI is based on ESHREH guideline oligo/amenorrhea for at least 4 months, an elevated FSH level ≥25 IU/l on two occasions more than 4-6 weeks apart. There are different causes and risk factors

for POI such as, genetic, family history and demographic factors, ethnicity, body mass index, socio-economic status, smoking, infectious causes, coexisting medical conditions, chemical exposures and other risk factors. Chromosomal analysis and FMR1 are recommended for all women with non- iatrogenic POI. There is currently no proven predictive test to identify women who will develop POI, unless a genetic mutation known to be related to POI is detected. There are no established methods for preventing or predicting POI. In view of long consequences of POI, efforts should be made to reduce the risk of POI. Modification factors may include: encouraging smoking cessation or not smoking, life style modification, exercise caution during gynecological surgeries, treatment regimens for malignant or chronic diseases, genetic consult for women with relative diagnosis with POI. (family history of POI). Relative of women with FMR1 premutation or other genetic causes of POI should be offered genetic counselling and testing. There are many strategies for treatment of POI patients such as: management For bone health, muscle health, cardiometabolic health, psychological wellbeing, treatment of genitourinary symptoms and management options for cognition/neurological function. Fertility interventions may be effective with: *in vitro* activation of follicle growth in biopsied ovarian tissue, administration of mesenchymal stem cells and injection of platelet rich plasma(PRP) into the ovary. Women with POI should be informed that oocyte donation is an established option to achieve pregnancy.

Inm-4: The Role of Prevention and Education in Promoting Reproductive Health

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Background: Despite remarkable advances in assisted reproduction and infertility treatments, attention to modifiable preconception risk factors has emerged as a key strategy for improving reproductive outcomes. Preconception care, aimed at optimizing the health status of both women and men through reducing modifiable risk factors and providing timely education, counseling, and interventions, plays a vital role in ensuring a healthy and successful pregnancy. However, significant gaps persist in reproductive health literacy and access to structured education among couples. Shifting the paradigm from a purely curative approach toward proactive, preventive, and educational interventions offers a sustainable pathway to promote reproductive health at the population level. This presentation critically examines how structured prevention and education can be integrated into reproductive health frameworks, with a specific focus on preconception care and couple empowerment.
Materials and Methods: A comprehensive narrative synthesis of contemporary evidence was conducted, drawing on randomized controlled trials, comparative reviews of international guidelines, and interventional studies published between 2022 and 2025. The analysis focuses on three main axes: first, technology-based interventions, including mobile health applications that enhance menstrual health literacy and fertility window awareness, thereby assisting women in more precise management of their reproductive health; second, structured counseling programs based on the stages-of-change model, which improve women's attitudes toward childbearing and guide them toward active pregnancy readiness; and third, programs centered on promoting reproductive health literacy that teach couples how to access credible information and use it for informed decision-making along their fertility journey. Special emphasis is placed on evaluating cultural acceptability, implementation fidelity, and the feasibility of integrating these interventions within infertility treatment centers and primary healthcare systems.
Results: The synthesized evidence consistently demonstrates that well-designed educational interventions significantly improve reproductive health knowledge and promote preventive behaviors. A consensus among international guidelines — including those from the American College of Obstetricians and Gynecologists (ACOG), the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG), and the Public Health Agency of Canada (PHAC) affirms that standard preconception counseling should include thorough assessment of personal and family medical history, smoking and alcohol cessation counseling, weight optimization, risk factor screening, and folic acid supplementation. Interventional studies in Iran have demonstrated that education based on the stages-of-change model positively enhances attitudes toward childbearing. Mobile health interventions have also yielded significant improvements in reproductive health literacy, menstrual cycle awareness, and perceived personal control over health management. A recurring theme across successful interventions was the necessity of culturally adapting content and thoughtfully combining in-person education with digital tools.
Conclusion: Prevention and education constitute the cornerstone of sustainable reproductive health promotion and must be prioritized alongside clinical infertility services. Preconception care not only improves maternal and neonatal outcomes but also carries long-term benefits for the health of future generations. A fundamental paradigm shift is required to embed standardized, culturally sensitive educational interventions within infertility treatment centers, preconception counseling clinics, and digital health platforms. Future efforts should focus on scaling reproductive health literacy programs tailored for couples, developing evidence-based educational modules, and strengthening the communication and counseling skills of healthcare providers. By equipping women and men of reproductive age with essential knowledge and self-care competencies, the likelihood of achieving a healthy and successful pregnancy can be enhanced, and many preventable pregnancy complications can be averted.
Keywords: Reproductive Health, Preconception Care, Health Education, Primary Prevention, Health Literacy, Childbearing, Mobile Health (mHealth)

Inm-5: The Association of Lifestyle, Obesity, and Nutrition on Reproductive Health

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Infertility is a major global reproductive health challenge, with substantial physical, psychological, social, and economic consequences for individuals and couples. While reproductive outcomes are influenced by complex genetic, biological, and environmental factors, nutrition and lifestyle are among the potentially modifiable determinants that can be addressed before conception and throughout reproductive care. Increasing evi-

dence suggests that dietary quality, metabolic health, and nutritional status may interact with reproductive physiology through multiple biological pathways. This presentation provides an evidence-based overview of the relationship between nutrition and reproductive health, moving from underlying mechanisms to clinical application. It first considers obesity and metabolic dysfunction, hormonal disturbances, oxidative stress, and gut microbiota as interconnected pathways potentially linking lifestyle and dietary exposures with reproductive function. These mechanisms provide a framework for understanding how excess adiposity, metabolic imbalance, inflammation, and oxidative stress may influence reproductive health in both women and men. The presentation then evaluates dietary patterns and specific nutritional factors according to the strength and consistency of available evidence. Particular emphasis is placed on Mediterranean-style and other healthy dietary patterns, characterized by greater consumption of plant foods, whole grains, legumes, nuts, fish, and unsaturated fats and lower intake of ultra-processed foods. Evidence regarding selected nutrients and supplements including folate, vitamin D, iron, omega-3 fatty acids, CoQ10, and myo-inositol is considered within the context of clinical indication, baseline nutritional status, population characteristics, and reproductive outcomes. Rather than treating individual supplements as universal fertility-enhancing interventions, the importance of evidence-based and indication-specific use is emphasized. Emerging concepts, including the developmental origins of health and disease (DOHaD), advanced nutritional assessment, and precision nutrition, further support a shift toward individualized reproductive nutrition. These approaches recognize that nutritional needs and potential benefits may differ according to metabolic phenotype, nutritional deficiencies, reproductive diagnosis, and treatment context. Finally, current evidence is translated into a practical preconception nutrition framework structured around six steps: Assess, Optimize, Adopt, Correct, Consider, and Monitor. This framework integrates assessment of nutritional and metabolic risk, optimization of body composition and lifestyle, adoption of healthy dietary patterns, correction of relevant deficiencies, selective consideration of evidence-based supplements, and ongoing monitoring. Overall, nutrition should be considered not as a standalone treatment for infertility, but as an integral component of comprehensive reproductive care. An evidence-based, individualized nutritional approach may provide an important opportunity to optimize modifiable determinants of reproductive health while avoiding interventions that extend beyond the strength of current evidence.

Oral Speaker

Onm-1: Effect of Viola Odorata Vaginal Suppository on Menopausal Vaginal Atrophy: A Triple-Blind Randomized Clinical Trial

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Background: Vaginal atrophy is a common menopausal condition caused by estrogen deficiency and is associated with symptoms such as vaginal dryness, itching, irritation, dyspareunia, and increased vaginal pH. Because of concerns regarding the adverse effects of hormone replacement therapy, many women seek alternative herbal treatments. Viola odorata contains flavonoids with phytoestrogenic activity, mucilage with moisturizing properties, and saponins that may improve vaginal tissue hydration. This study aimed to evaluate the effect of Viola odorata vaginal suppositories on menopausal vaginal atrophy.
Materials and Methods: This triple-blind randomized placebo-controlled clinical trial was conducted on 60 postmenopausal women between September 2022 and January 2023. Participants were randomly assigned to either the treatment group receiving Viola odorata vaginal suppositories or the placebo group receiving glycerin suppositories. Participants used one suppository nightly for 8 weeks. Vaginal atrophy symptoms, including dryness, irritation, itching, and dyspareunia, were assessed at baseline, week 4, and week 8 using self-assessment scales. Vaginal pH and vaginal maturation index (VMI) were evaluated before and after the intervention. Statistical analyses were performed using SPSS version 26, with significance considered at $P<0.05$.
Results: A total of 54 postmenopausal women completed the study. There were no significant baseline differences between the treatment and placebo groups regarding demographic characteristics or vaginal atrophy indicators. After 8 weeks, the Viola odorata group showed significant improvement in vaginal dryness, irritation, itching, and dyspareunia compared with the placebo group. Vaginal pH significantly decreased, while the VMI significantly increased in the treatment group. In addition, superficial and intermediate vaginal cells increased and parabasal cells decreased following treatment. No adverse effects were reported in either group.
Conclusion: Viola odorata vaginal suppositories significantly improved symptoms of menopausal vaginal atrophy and enhanced vaginal health indicators, including vaginal pH and Vaginal Maturation Index. Given its low cost, natural origin, and absence of reported side effects, Viola odorata may be considered a promising alternative treatment for menopausal vaginal atrophy. Further studies with larger sample sizes and longer follow-up periods are recommended.
Keywords: Viola Odorata, Menopause, Vaginal Maturation Index

Onm-2: Predictors of Sexual Quality of Life Among Women Diagnosed with Endometriosis in The West of Iran: A Cross-Sectional Study

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Background: Few studies have comprehensively examined the predictors of sexual quality of life in women with endometriosis. Therefore, this study was conducted to identify the predictors of sexual quality of life among women with endometriosis in the west of Iran.
Materials and Methods: This cross-sectional study was conducted among 200 women diagnosed with endometriosis on 2025. The tools were including the demographic characteristics, the sexual quality of life-female (SQOL-F) questionnaire, Linda Berg's Sexual Satisfaction Scale and Female Sexual Function Index (FSFI) Questionnaire. A linear regression adjusted model was used to identify predictors of the SQOL-F score among women with endometriosis. Statistical significance was set at $P<0.05$.
Results: The linear regression analysis revealed several significant predictors of sexual quality of life SQO-L among the studied women. Both FSFI and marital satisfaction (Lindberg) demonstrated the strongest positive associations with sexual quality of life ($\beta=0.86$, $P<0.001$ and $\beta=0.89$, $P<0.001$, respectively). Furthermore, higher income ($\beta=2.70$, $P=0.001$), the absence of an infertility history ($\beta=2.84$, $P=0.003$), and the absence of a history of smoking or substance use ($\beta=4.76$, $P<0.001$) were also significant positive predictors. Conversely, longer marriage duration ($\beta=-0.30$, $P=0.004$), older age ($\beta=-0.20$, $P=0.040$) and employee ($\beta=-2.02$, $P=0.015$) were significant negative predictors.
Conclusion: The present findings highlight that sexual function and satisfaction are the most critical predictors of sexual quality of life in women with endometriosis, while demographic and lifestyle factors also play significant but complex roles. Understanding these interrelated predictors is essential for designing multidimensional interventions aimed at improving both physical and psychosocial aspects of sexual well-being in this population.
Keywords: Endometriosis, Sexual Quality of Life, Iran, Sexual Satisfaction

Onm-3: Comparative Effects of Hyaluronic Acid and PVP at Different Post-Thaw Exposure Times on Sperm Motility, DNA Integrity, and Mitochondrial Function

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Background: Polyvinylpyrrolidone (PVP) is a synthetic polymer used in ART, particularly ICSI, due to its viscous properties that aid sperm handling. However, its non-physiological nature raises concerns about persistence within the oocyte. In contrast, hyaluronic acid (HA), a natural component of the extracellular matrix and cumulus–oocyte complex, is compatible with sperm maturation. Mature sperm bind to HA via specific receptors, and this interaction has been associated with improved chromatin integrity, reduced DNA damage, and selection of higher-quality sperm in ART.

Materials and Methods: In this experimental study, 20 normozoospermic samples were subjected to rapid freezing and subsequently exposed to either PVP or HA media. Sperm analysis was performed at 5, 10, and 15-minute intervals following the addition of each medium. Sperm concentration and motility were evaluated using a Makler chamber, while cell viability and morphology were assessed via eosin–nigrosin and Diff-Quick staining. Furthermore, DNA integrity (chromatin dispersion) was analyzed using the SDFA kit, and mitochondrial activity was examined using the JC-1 fluorescent probe.

Results: In the assessment of progressive motility, the HA group at 10 minutes demonstrated the highest motility rate among all experimental conditions, with a mean value of 40.30 ± 0.80 Regarding morphology, HA at 5 minutes yielded the most favorable outcome, showing a mean normal morphology rate of 3.90 ± 0.17 Similarly, the sperm chromatin dispersion (SCD) assay revealed the greatest DNA integrity in this group, with a mean value of 14.45 ± .0.18. Mitochondrial membrane potential analysis (JC-1 assay) further indicated that the same group exhibited the highest mitochondrial activity, reaching 85.50 ± 0.61 Among the remaining experimental conditions, exposure to HA for 10 minutes also resulted in comparatively superior functional performance. Overall, statistically significant differences were observed among the study groups across all evaluated parameters ($P<0.0001$).

Conclusion: The data suggest that HA, especially at 5 and 10 minutes post-thaw, may help preserve key sperm quality parameters. Therefore, HA may represent a promising alternative to PVP in ART laboratory protocols.

Keywords: Polyvinylpyrrolidone, Hyaluronic Acid, Normozoospermic, DNA Integrity, Mitochondrial Function

Poster Presentation

Pnm-1: A Systematic Mapping Review on Machine Learning Prediction of Male Infertility Based on Sexual Hormones

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Background: This systematic mapping review aimed to identify, describe, and synthesize studies that apply machine learning algorithms using hormonal biomarkers to predict male infertility.
Materials and Methods: Following the PRISMA–ScR guidelines, a systematic search was conducted in PubMed, Scopus, and Web of Science databases up to May 2025. Studies not involving ML methods, hormonal predictors, or male-specific analyses were excluded. Data were synthesized narratively due to heterogeneity across datasets and methods.
Results: Out of 115 retrieved records, 11 studies met the eligibility criteria. Most studies were retrospective and hospital-based, with sample sizes ranging from under 150 to over 1,000 participants. The most frequently used ML algorithms were ANN; n = 6, SVM; n = 4, kNN; n = 4, and XGBoost, CatBoost, and super learner, which demonstrated superior predictive accuracy in several datasets. Core hormonal predictors—FSH, LH, and total testosterone (TT)—were included in nearly all studies. Emerging biomarkers such as Anti-Müllerian Hormone (AMH), Inhibin B, and leptin improved model performance and biological interpretability in select analyses.
Conclusion: This systematic mapping review demonstrates that machine learning models leveraging hormonal biomarkers—particularly multihormonal combinations, including FSH, LH, testosterone, and emerging markets, such as AMH and inhibin B—show strong potential for non-invasive prediction of male infertility. Artificial neural networks and ensemble-based approaches consistently achieved robust predictive performance across diverse clinical contexts
Keywords: Male, Infertility, Artificial Intelligence, Hormone

Pnm-2: Digital Health Interventions for Psychosocial and Treatment Support in Women Undergoing Assisted Reproductive Technology: A Systematic Review with A Focus on Nurse-Relevant Outcomes

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Background: Assisted Reproductive Technology (ART) is associated with considerable psychological burden, including anxiety, stress, and emotional distress, which may negatively affect treatment adherence. Digital health interventions (mobile apps, telemedicine, web-based tools) have emerged as scalable support strategies. Given the important role of nurses in fertility clinics, this review aimed to synthesise evidence on digital health interventions deliverable within nurse-led care pathways.
Materials and Methods: A systematic search was conducted in PubMed, Scopus, and Web of Science (January 2015 – April 2026) following PRISMA guidelines. Studies evaluating digital health interventions in women undergoing ART reporting psychosocial or treatment-related outcomes were included. Reviews, protocols, and commentaries were excluded. Quality was assessed using JBI tools and Cochrane ROB-2. Narrative synthesis was performed due to high heterogeneity.
Results: Of 1,135 records screened, 10 studies met inclusion criteria (RCTs, feasibility, observational). Interventions included smartphone apps, SMS support, telemedicine, and web-based platforms. Digital interventions were associated with improved psychosocial outcomes: reduced anxiety/stress, enhanced emotional well-being, and better treatment adherence. In three RCTs, intervention groups showed statistically significant anxiety reduction versus controls (p=0.03). Two studies suggested that nurse-delivered or nurse-supervised digital interventions may improve adherence and engagement compared with fully automated systems.
Conclusion: Digital health interventions are promising supportive tools for psychosocial well-being and treatment experience in women undergoing ART. However, heterogeneity limits direct comparison. Future high-quality RCTs with standardized outcomes are needed to clarify effectiveness and explore nurse-led digital care models in fertility settings.
Keywords: Assisted Reproductive Techniques, Infertility, Mobile Applications, Telemedicine, Psychological Distress

Pnm-3: Diagnostic Value of Serum Anti-Müllerian Hormone in Predicting Glucose Intolerance and Metabolic Syndrome in Different Phenotypes of Polycystic Ovary Syndrome: A Literature Review

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Background: Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. In addition to reproductive complications, it is associated with a wide range of metabolic disorders, including insulin resistance, impaired glucose tolerance, and metabolic syndrome. The severity of these metabolic disorders is not uniform across different PCOS phenotypes, with classic phenotypes generally exhibiting more severe metabolic involvement. Anti-Müllerian Hormone (AMH), primarily known as an indicator of ovarian reserve and an adjunctive tool in the diagnosis of PCOS, has garnered attention in recent years due to its potential association with metabolic parameters. there is insufficient evidence to support the use of AMH as a predictive biomarker for impaired glucose tolerance and metabolic syndrome, particularly across different PCOS phenotypes. The aim of our study is to evaluate the diagnostic value of serum AMH levels in predicting impaired glucose tolerance and metabolic syndrome in different PCOS phenotypes.
Materials and Methods: Searching in some databases like PubMed, google scholar, Scopus, Springer and Science Direct.

Full-text articles in English published from 2014 to 2026 were reviewed. The main outcomes included serum AMH levels, insulin resistance indices. impaired glucose tolerance, metabolic syndrome criteria, and PCOS phenotype.
Results: Based on the reviewed studies, serum AMH levels are significantly higher in patients with PCOS compared to healthy women, and this increase is more pronounced in classic phenotypes (phenotypes 1 and 2). A significant positive association between AMH levels and the insulin resistance index (HOMA-IR) has been reported in multiple studies. Furthermore, higher AMH levels have been associated with increased BMI, dyslipidemia. Studies have shown that AMH could potentially serve as a predictor of metabolic risk; however, its diagnostic value in directly predicting impaired glucose tolerance and metabolic syndrome requires the determination of specific cut-off values and evaluation across different phenotypes. Additionally, some studies have proposed a pathophysiological role for AMH in exacerbating insulin resistance through reciprocal interactions with hyperinsulinemia and hyperandrogenism.
Conclusion: Although AMH is considered a valuable biomarker for the diagnosis of PCOS, the available evidence indicates that this hormone has high potential for predicting metabolic disorders, including, impaired glucose tolerance, and metabolic syndrome in patients with PCOS. However, due to the lack of standardized cut-off points, phenotypic and population differences, and heterogeneity in measurement methods, AMH cannot yet be used as an independent tool for metabolic screening.
Keywords: Polycystic Ovary Syndrome (PCOS), Different Phenotypes of PCOS, Metabolic Syndrome, Pimpaired Glucose Tolerance, Anti Müllerian Hormone (AMH)

Pnm-4: The Role of Nursing and Midwifery Care in Assisted Reproductive Technology: A Systematic Review

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Background: Assisted Reproductive Technology (ART) has become a vital treatment option for infertile couples in all over the world. In addition to medical interventions, comprehensive nursing and midwifery care plays a crucial role in improving treatment outcomes, patient satisfaction, and psychosocial well-being. This systematic review conducted to evaluate the impact of nursing and midwifery interventions in ART centers.
Materials and Methods: A systematic search was conducted in PubMed, Scopus, Web of Science, and Science Direct databases for studies published between 2015 and 2025. Keywords related to nursing, midwifery, infertility care, counseling, patient education, and ART were used. Relevant original and review studies were screened based on inclusion criteria, and eligible studies were analyzed.
Results: A total of 38 studies met the inclusion criteria. Findings indicated that nursing and midwifery interventions, including patient education, emotional counseling, medication training, lifestyle guidance, and continuity of care, significantly reduced stress and anxiety during ART cycles. Effective communication by fertility nurses and midwives improved adherence to treatment protocols and patient confidence. Studies also showed that psychosocial support during ovarian stimulation, embryo transfer, and pregnancy testing periods enhanced patient experience and coping ability. In some centers there was a positive relationship in nurse-led care models with higher patient satisfaction and improved service efficiency.
Conclusion: Nursing and midwifery professionals have a valuable role in ART programs through education, counseling, emotional support, and coordination of care. Strengthening multidisciplinary fertility teams and expanding specialized training for nurses and midwives may improve quality of infertility services and patient-centered outcomes.
Keywords: ART, Nursing, Midwifery, Infertility Care, Counseling

Pnm-5: Epigenetic Mechanisms Regulating Oocyte Quality and Meiotic Maturation

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Background: Oocyte quality, a paramount determinant of female fertility and successful preimplantation development, is intrinsically linked to precisely orchestrated molecular and cellular events governing meiotic maturation and developmental competence. A rapidly expanding body of evidence underscores the indispensable role of epigenetic mechanisms—including DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs—in fine-tuning gene expression and shaping developmental trajectories during oogenesis. However, a synthesized, comprehensive understanding of how these epigenetic modulators collectively influence oocyte quality and maturation remains a critical knowledge gap in reproductive biology.
Materials and Methods: This systematic review was rigorously conducted following the PRISMA 2020 guidelines. A comprehensive and structured search was performed across PubMed, Scopus, and Web of Science, identifying original research and review articles published between January 2010 and May 2026. Eligibility criteria focused on studies investigating epigenetic regulation in mammalian oocytes, specifically those addressing DNA methylation, DNA hydroxymethylation, histone post-translational modifications, chromatin dynamics, and non-coding RNA-mediated processes in relation to oocyte quality, meiotic progression, and developmental potential. Extracted data were qualitatively analyzed to identify consistent patterns and key findings.
Results: The analyzed literature consistently demonstrates that the integrity and dynamic regulation of epigenetic marks are fundamental for achieving competent oocyte maturation. DNA methylation and a diverse array of histone modifications, such as acetylation and methylation, were critically implicated in processes ranging from chromatin architecture and transcriptional silencing to spindle assembly, meiotic progression, and cytoplasmic maturation. Importantly, dysregulation of these epigenetic mechanisms—manifesting as altered methylation patterns, aberrant histone modifications, or disrupted non-coding RNA expression—was frequently associated with compromised oocyte quality, impaired fertilization rates, and diminished embryonic developmental competence. Furthermore, several studies elucidated a strong correlation between

age-related decline in oocyte quality and the erosion of epigenetic fidelity, highlighting a crucial nexus between reproductive aging and epigenomic instability.
Conclusion: Epigenetic regulation serves as a central orchestrator of oocyte quality and maturation, influencing the entire spectrum of oogenesis and early embryogenesis. Elucidating the intricate interplay among DNA methylation, histone modifications, and non-coding RNA networks offers profound insights into the etiology of female infertility and paves the way for innovative diagnostic and therapeutic strategies in assisted reproductive technologies.
Keywords: Oocyte Quality, Epigenetic Regulation, Meiotic Maturation, Reproductive Aging

Pnm-6: The Effect of Educational and Counseling Interventions on Couples' Attitudes Toward Childbearing: A Systematic Review

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Background: Declining fertility rates pose a significant demographic challenge in many countries. Couples' attitudes toward childbearing heavily influence fertility decisions and can be shaped by educational and counseling interventions. However, current evidence on the effectiveness of these interventions is fragmented and inconsistent. This study systematically reviews the available evidence on how educational and counseling interventions affect couples' attitudes toward childbearing.
Materials and Methods: This systematic review, conducted following PRISMA 2020 guidelines, comprehensively searched PubMed, Scopus, and ScienceDirect for studies published between February 2015 and February 2026. The search strategy combined keywords related to educational interventions, counseling, attitudes toward childbearing, fertility motivation, and couples. Included studies comprised randomized controlled trials, quasi-experimental studies, and analytical studies that investigated the effects of educational or counseling interventions on attitudes toward childbearing. Two independent reviewers conducted study screening, selection, data extraction, and methodological quality assessment using the MMAT 2018 tool. Due to heterogeneity, findings were synthesized narratively.
Results: Of the 48 studies initially identified, 17 studies met the inclusion criteria and were included in the final synthesis after removing duplicates and screening procedures. The mean age of participants was reported as 24.8 ± 7.2 years. The findings indicated that educational and counseling interventions significantly improved attitudes toward childbearing. Interventions based on the Theory of Planned Behavior and group cognitive-behavioral counseling demonstrated the greatest effectiveness in promoting positive attitudes toward childbearing. However, four studies highlighted barriers such as socioeconomic challenges, cultural constraints, and short follow-up periods. In addition, low fertility literacy, fear of childbirth, and lack of spousal support were identified as major factors limiting intervention effectiveness.
Conclusion: Current evidence suggests that educational and counseling interventions, particularly those based on behavioral theories and group counseling approaches, can improve couples' attitudes toward childbearing. Nevertheless, the effectiveness of these interventions is influenced by social, cultural, and economic factors. Designing multidimensional interventions tailored to sociocultural contexts may help promote positive attitudes toward childbearing and strengthen fertility motivation.
Keywords: Educational Intervention, Counseling, Childbearing, Fertility Motivation

Pnm-7: Barriers to Accessing Infertility Services: A Systematic Review

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Background: Infertility represents a significant global health concern and remains one of the persistent challenges in public health. Although substantial advances have been made in the prevention, diagnosis, and treatment of infertility, many individuals still face difficulties in accessing appropriate reproductive healthcare services. The present systematic review aimed to identify and analyze the main barriers affecting access to infertility services.
Materials and Methods: A comprehensive literature search was conducted across PubMed, Scopus, Web of Science, Google Scholar, and ScienceDirect databases up to 2026 using the keywords (infertility OR fertility disorder OR subfertility OR conceiving) AND (barrier OR difficulty) AND (access to). After screening 108 retrieved studies, 12 articles met the inclusion criteria and were reviewed in depth.
Results: The findings revealed that financial burden is the predominant barrier limiting access to infertility treatments. Geographical constraints were also commonly reported, highlighting disparities in service distribution. Additional barriers included lack of spousal support, religious and cultural beliefs, ethnic and racial differences, travel-related challenges and expenses, insufficient facilities, concerns about treatment failure, advanced maternal age or high-risk pregnancy, painful procedures, treatment-related hopelessness, lack of awareness of treatment processes, inadequate clinical team performance, and prolonged waiting times.
Conclusion: Enhancing understanding of the multifaceted barriers to infertility care access is crucial for developing targeted policies and intervention strategies. Addressing these barriers can facilitate equitable access to infertility services, improve treatment outcomes, and ultimately promote fertility and population growth at the community level.
Keywords: Infertility, Access Barriers, Reproductive Health, Systematic Review

Pnm-8: Efficacy of Platelet-Rich Plasma (PRP) in the Management of Infertility in Women with Premature Ovarian Insufficiency (POI): A Systematic Review

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Background: Premature Ovarian Insufficiency (POI) is a significant reproductive challenge, marked by premature ovarian function loss, infertility, and hypoestrogenism. Limited success with conventional treatments necessitates novel strategies. Platelet-Rich Plasma (PRP) therapy, utilizing autologous growth factors, has emerged as a promising intervention for ovarian rejuvenation and fertility improvement in women with diminished ovarian reserve. This systematic review critically evaluates the current evidence on PRP's efficacy in managing infertility in women with POI.
Materials and Methods: A systematic literature search was conducted across PubMed, Scopus, Web of Science, and Embase from inception to December 2024. Keywords included "Platelet-Rich Plasma," "PRP," "Premature Ovarian Insufficiency," "POI," "infertility," and "assisted reproductive technology." Included studies were original research, clinical trials, and case series on intraovarian PRP administration in women with POI. Data extraction focused on study design, patient characteristics, PRP preparation and administration, ovarian response indicators (e.g., antral follicle count (AFC), hormone levels), and key reproductive outcomes, primarily pregnancy and live birth rates.
Results: A total of 18 studies were included. Preliminary findings suggest intraovarian PRP administration significantly improves ovarian function markers in women with POI. Studies reported increased FSH normalization, antral follicle count, and endometrial thickness post-PRP. Consequently, a considerable proportion of patients experienced restoration of spontaneous menstruation. Approximately 25-35% achieved clinical pregnancies, with live birth rates reported in 15-25% across studies. However, heterogeneity in PRP preparation, dosage, and administration protocols highlights the need for standardization. Long-term safety and precise mechanisms of action require further investigation.
Conclusion: This systematic review indicates that PRP therapy holds considerable therapeutic potential for improving fertility outcomes in women with POI. Evidence suggests PRP positively influences ovarian response markers and facilitates conception. Nevertheless, methodological variability necessitates further well-designed, large-scale clinical trials to establish definitive efficacy, optimize treatment protocols, and confirm the long-term safety and reproductive benefits of PRP in this patient population.
Keywords: Platelet-Rich Plasma, Premature Ovarian Insufficiency, Infertility, Assisted Reproductive Technology,

Pnm-9: Association Between *FMR1* Gene Mutations and Reproductive Dysfunction

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Background: Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and is strongly associated with autism. It results from the expansion of CGG repeats (> 200) in the FMR1 gene, leading to hypermethylation and transcriptional silencing. The FMR1 protein (FMRP) is crucial for synaptic plasticity, mitochondrial function, and reproductive physiology. Both full mutation and premutation (55–200 repeats) carriers may experience neurological and reproductive complications, including infertility. In premutation carriers, RNA toxicity disrupts reproductive processes. Women are at increased risk for fragile X-associated primary ovarian insufficiency (FXPOI), characterized by hypergonadotropic amenorrhea before age 40. Men may present with impaired spermatogenesis, reduced sperm parameters, azoospermia, or oligozoospermia.
Materials and Methods: This systematic review, conducted according to PRISMA guidelines, evaluated the association between FMR1 mutations and infertility in both genders. Searches were performed in PubMed, Scopus, Web of Science, and Google Scholar using keywords including "Fragile X syndrome," "FMR1," "premutation," "FXPOI," and "infertility." Studies investigating reproductive outcomes were included, while duplicates, reviews, and editorials were excluded. Following screening, eligible studies were selected for qualitative analysis.
Results: Evidence suggests a clear association between FMR1 mutations and impaired reproductive function. Premutation female carriers exhibited elevated follicle-stimulating hormone (FSH) levels and reduced ovarian reserve markers, such as anti-Müllerian hormone (AMH). In men, FMR1 alterations were associated with impaired spermatogenesis, reduced motility, and an increased likelihood of azoospermia or oligozoospermia.
Conclusion: Full mutation and premutation carriers face an increased risk of infertility and reproductive dysfunction. Early identification facilitates timely fertility preservation, including assisted reproductive techniques and preimplantation genetic testing, to manage reproductive decline and reduce the transmission of the mutation.
Keywords: Fragile X Syndrome (FXS), FMR1 Gene, Genetic Screening

Pnm-10: The Impact of Public Reproductive Health Education on Young People's Attitudes Toward Childbearing: A Systematic Review

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Background: Although population policies emphasize education's role in shaping childbearing attitudes, evidence on the effectiveness of reproductive health education on young people's fertility attitudes is fragmented and lacks systematic synthesis. This study systematically reviews the existing evidence on the impact of reproductive health education on childbearing attitudes among single and married young adults.
Materials and Methods: This systematic review, conducted according to PRISMA 2020 guidelines, comprehensively searched PubMed, Scopus, and ScienceDirect for studies published between February 2015 and February 2026. The search strategy used combinations of terms related to fertility education, reproductive health, and attitudes toward childbearing. In-

cluded studies were experimental or quasi-experimental, evaluating the effects of educational interventions on childbearing attitudes, intentions, or motivations among 18–40-year-olds. Two independent reviewers conducted study screening, selection, data extraction, and quality assessment using the MMAT 2018 tool. Data were synthesized using Braun and Clarke's thematic analysis due to methodological heterogeneity.

Results: After screening 501 studies, 10 met inclusion criteria and were synthesized. The analysis revealed four major themes: (1) increased fertility awareness (knowledge of age-related decline, lifestyle factors, and ART limitations); (2) changes in childbearing attitudes and intentions (increased desire for parenthood, uncertainty, and anxiety); (3) emotional and psychological challenges (worry, time pressure, and fear of infertility); and (4) facilitators and barriers to educational effectiveness (age, gender, marital status, and delivery mode). Most studies reported significant improvements in fertility knowledge following education, but the impact on childbearing attitudes varied based on culture, age, and educational approach. Fertility education was better received during adolescence (ages 16–18) compared to adulthood (ages 25–35), where it sometimes induced anxiety and guilt.

Conclusion: While public reproductive health education improves fertility knowledge, its impact on childbearing attitudes varies based on age, gender, intervention design, and sociocultural context. Delivering fertility information without considering psychological readiness and life stage may induce anxiety and delay decisions. Effective interventions should be age- and needs-based, integrated early into school curricula, and balance education with anxiety management strategies.

Keywords: Fertility Education, Reproductive Health, Childbearing, Young Adults

Pnm-11: Neonatal Outcomes After Assisted Reproductive Technologies (ART): A Systematic Review of Evidence and Risk Factors (2020–2024)

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Background: Neonatal outcomes in pregnancies conceived through assisted reproductive technologies (ART) remain an important clinical concern. While ART enables effective treatment of infertility, accumulating evidence suggests that ART-conceived infants may face higher risks of adverse outcomes such as low birth weight, preterm-related complications, NICU admission, and need for neonatal respiratory support. A systematic synthesis of contemporary evidence is essential to clarify the magnitude of risk, identify key contributing factors, and inform neonatal-focused follow-up strategies.

Materials and Methods: This systematic review was conducted by searching PubMed, Scopus, and Web of Science for original studies and systematic reviews published between January 2020 and December 2024. Search terms included combinations of "assisted reproductive technology," "in-vitro fertilization," "intracytoplasmic sperm injection," "neonatal outcomes," "NICU," "low birth weight," "preterm," and "respiratory morbidity." Studies evaluating neonatal outcomes in ART-conceived singleton and/or multiple pregnancies compared with naturally conceived pregnancies were considered eligible. Two independent reviewers screened titles/abstracts and extracted data on study design, ART procedure characteristics, gestational age at delivery, birth weight, neonatal morbidity endpoints, and adjusted risk estimates. Because of expected heterogeneity in population characteristics and outcome definitions, findings were synthesized qualitatively.

Results: Across included studies, ART pregnancies demonstrated a higher frequency of adverse neonatal outcomes compared with spontaneous conceptions, largely mediated by increased rates of preterm birth and low birth weight. Higher risks were reported for NICU admission and neonatal respiratory complications, particularly in studies with greater proportions of multiple gestations or complex maternal comorbidities. Risk stratification suggested that advanced maternal age, underlying infertility diagnoses (including male factor and endometriosis-related infertility), and specific laboratory or clinical ART variables may influence neonatal morbidity profiles. Evidence also supports that improvements in ART protocols and embryo culture/transfer practices may partially mitigate these risks, although residual differences remain in several outcome categories.

Conclusion: Evidence from recent literature suggests that ART-conceived neonates are at increased risk for adverse outcomes, predominantly driven by prematurity and impaired fetal growth trajectories. Clinically, these findings support structured perinatal planning and neonatal readiness, including risk-informed delivery management and postnatal surveillance for respiratory and feeding-related complications. Future prospective studies with standardized definitions and ART-specific stratification are needed to refine risk prediction models and optimize neonatal follow-up pathways.

Keywords: Assisted Reproductive Technology, Neonatal Outcomes, Preterm, Respiratory Morbidity

Pnm-12: Association Between *FMR1* Gene Mutations and Reproductive Dysfunction

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Background: The most common inherited cause of intellectual disability is Fragile X syndrome (FXS), which is also strongly associated with autism spectrum disorder. The disorder is caused by expansion of CGG trinucleotide repeats (> 200) within the 5′UTR of the FMR1 gene, resulting in hypermethylation and transcriptional silencing. The FMR1 protein (FMRP) plays a crucial role in synaptic plasticity, ion channel regulation, mitochondrial function, and reproductive physiology. Individuals carrying either the full mutation or the permutation (55–200CGG repeats)may experience neurological and reproductive complications, including tremor, anxiety, impaired energy metabolism, and infertility, while in permutant carriers, RNA toxicity contributes to cellular dysfunction and disrupts normal reproductive processes. Women carrying the premutation are at increased risk for fragile X-associated primary ovarian insufficiency (FXPOI), characterized by hypergonadotropic amenorrhea with elevated follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels persisting for more than four months before the age of 40. Men with FXS may also present with impaired spermatogenesis, reduced sperm count and motility, azoospermia, or oligozoospermia.

Materials and Methods: This systematic review was conducted in accordance with PRISMA guidelines to evaluate the association between FMR1 gene mutations and infertility in men and women. Relevant studies were identified through searches of PubMed, Scopus, Web of Science, and Google Scholar using the keywords “Fragile X syndrome,”“FMR1,”“premutation ,”“FXPOI,”and“infertility.” Studies investigating reproductive outcomes associated with FMR1 premutation or full mutation were included, while duplicates, reviews, editorials, and studies with insufficient data were excluded. Following title, abstract, and full-text screening, eligible studies were selected for qualitative analysis
Results: Based on the original studies reviewed, evidence suggests an association between FMR1 gene mutations and impaired reproductive function. Premutation female carriers were reported to have elevated follicle stimulating hormone (FSH) levels and reduced ovarian reserve markers, including anti Müllerian hormone (AMH). In men, FMR1 alterations were associated with impaired spermatogenesis, reduced sperm motility, and an increased likelihood of azoospermia or oligozoospermia.
Conclusion: Both full mutation and premutation carriers are at increased risk of infertility and reproductive dysfunction. Early identification of affected individuals may facilitate timely fertility preservation strategies, including assisted reproductive techniques and preimplantation genetic testing, before significant reproductive decline and to reduce the transmission of the mutation to future generations.
Keywords: Fragile X Syndrome (FXS), Infertility, Fragile X-Associated Primary Ovarian Insufficiency (FXPOI), Ovarian Reserve, Azoospermia

Pnm-13: Pregnancy Management After Assisted Reproductive Technologies: A Systematic Review of Current Evidence and Emerging Strategies

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Background: Pregnancies achieved via assisted reproductive technologies (ART) represent an increasing proportion of all births, yet these pregnancies are disproportionately associated with adverse maternal and neonatal outcomes, including hypertensive disorders, gestational diabetes, preterm birth, and fetal growth restriction. Optimizing the management of ART pregnancies requires a comprehensive, evidence-based approach that addresses these unique risks and integrates emerging strategies.
Materials and Methods: A systematic literature search was conducted in PubMed and Scopus for studies published from January 2020 to December 2024. Search terms included combinations of “assisted reproductive technology,” “ART pregnancy,” “maternal outcomes,” “risk stratification,” “hypertensive disorders,” “gestational diabetes,” and “fetal surveillance.” Studies focusing on preconception optimization, antenatal monitoring, risk assessment, and management protocols for ART pregnancies were included. Two reviewers independently screened articles for relevance and extracted key data. Findings were synthesized qualitatively to provide an overview of current evidence and emerging clinical strategies.
Results: Evidence indicates that ART pregnancies exhibit a higher incidence of preeclampsia, gestational hypertension, and preterm birth compared to spontaneous conceptions, likely due to a confluence of factors including advanced maternal age, higher rates of multiple gestations, underlying comorbidities, and altered placental development. Key management strategies include preconception optimization of cardiometabolic health, vigilant blood pressure monitoring, and early screening for hypertensive disorders. Regular glycemic monitoring and targeted nutritional interventions are vital for managing metabolic risks. Fetal surveillance, particularly serial third-trimester ultrasound growth assessments and Doppler studies for suspected placental dysfunction, is recommended. Emerging strategies, such as first-trimester risk prediction for preeclampsia and the development of ART-specific risk models, hold promise for personalized management but require further validation.
Conclusion: Effective management of ART pregnancies necessitates a multidisciplinary, risk-stratified approach. This includes optimizing preconception health, implementing structured antenatal surveillance, and employing evidence-guided fetal monitoring. Close collaboration between reproductive medicine specialists and maternal–fetal medicine teams is paramount for improving perinatal and long-term health outcomes for both mother and child. Future research should focus on developing and validating ART-specific clinical algorithms to personalize and optimize pregnancy care.
Keywords: ART Pregnancy, Maternal Outcomes, Hypertensive Disorders, Gestational Diabetes, Fetal Surveillance

Pnm-14: Opportunities and Challenges of Digital Health in Reproductive Care: A Systematic Review

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Background: Digital health applications are rapidly expanding in reproductive care, but evidence on their real-world effectiveness, ethical challenges, and implementation barriers is fragmented. This study addresses the gap in comprehensive reviews by systematically examining the benefits, ethical concerns, clinical barriers, and implementation needs associated with integrating digital health technologies into reproductive healthcare.
Materials and Methods: This a Systematic Review, guided by the Whittemore and Knafl framework and PRISMA 2020 guidelines, systematically searched Scopus, PubMed, and ScienceDirect for studies published between 2016 and February 2026. The search explored the evolution of digital health, artificial intelligence (AI) systems, and ethical issues in reproductive medicine, using keywords related to "digital health," "artificial intelligence," "reproductive care," "infertility," "medical ethics," "mobile health application," and "tele-counseling." The review included studies addressing opportunities, ethical challenges, clinical outcomes, and implementation barriers of digital health applications in reproductive care. Following duplicate removal and screening, 22 articles were analyzed using thematic analysis (Braun & Clarke) due to methodological heterogeneity.

Results: The initial search yielded 719 studies, with 22 meeting inclusion criteria for final analysis. Four key themes emerged: clinical opportunities and enhanced access (AI-enhanced embryo selection, mindfulness apps for anxiety, increased patient satisfaction with teleconsultation); ethical challenges (privacy, transparency in data use); implementation barriers and digital divide (unequal access for minorities/underserved areas, professional resistance); and professional considerations/data security (legal liability of AI, algorithmic bias, maintaining the human relationship). While most studies viewed digital health favorably, they stressed the importance of ethical guidelines and rigorous longitudinal and interventional research.
Conclusion: Digital health offers opportunities to personalize treatment, empower patients, and improve reproductive care access. However, ethical, clinical, and implementation challenges must be addressed. This study highlights the need for ethical governance, rigorous trials to confirm effectiveness, transparent AI algorithms, institutional data privacy policies, and a patient-centered approach. Responsible digital health in reproductive care requires transparency, equitable access, and preservation of the physician-patient relationship.
Keywords: Digital Health, Artificial Intelligence, Reproductive

Pnm-15: Nursing and Midwifery Interventions for Patient-Centered Outcomes in Assisted Reproductive Technology: A Systematic Review

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Background: Infertility is a major reproductive health concern worldwide. Assisted Reproductive Technology (ART) has expanded treatment options but remains emotionally and clinically complex. We reviewed nursing and midwifery interventions for patient-centered outcomes in ART.
Materials and Methods: This systematic review followed PRISMA 2020 guidelines. PubMed, Scopus, Web of Science, and Google Scholar were searched for English-language studies published from June 2015 to May 2025. The search strategy combined MeSH terms and free-text keywords including "Infertility," "Reproductive Techniques, Assisted," "Nursing Care," "Midwifery," "Patient Education," "Counseling," ART, In-Vitro Fertilization (IVF), Intracytoplasmic Sperm Injection (ICSI), psychological support, and patient-centered outcomes. Using PICO, population: women/couples in infertility treatment; intervention: nursing/midwifery care; comparison: routine care; outcomes: patient-centered. Original studies were included; reviews, editorials, letters, non-English, and irrelevant studies were excluded. Two reviewers independently screened records against eligibility criteria, extracted data, and assessed risk of bias using the Cochrane RoB 2 tool (RCTs) and ROBINS-I (non-randomised studies).
Results: The search identified 486 records; after removing 124 duplicates, 362 records were screened. Eighty-six full-text articles were assessed, and 56 studies met the inclusion criteria. Exclusions at full-text review were mainly due to absence of a clearly defined nursing or midwifery intervention, irrelevant outcomes, non-original design, or insufficient data. Of the included studies, 16 were judged as low risk of bias, 28 as having some concerns or moderate risk, and 12 as high or serious risk, mainly because of non-random allocation, lack of blinding, and incomplete outcome data. Across studies, psychological interventions, particularly mindfulness-based support, Cognitive Behavioral Therapy (CBT), relaxation, and stress-management programs, showed the most consistent improvements in anxiety, depression, perceived stress, and infertility-related distress. Educational, counseling, and digital interventions mainly improved treatment knowledge, self-efficacy, adherence, satisfaction, and informed decision-making. Continuity-of-care and follow-up interventions supported early recognition of treatment-related concerns. Evidence for clinical pregnancy and live birth remained inconclusive due to heterogeneity in intervention type, timing, outcomes, and methodological quality.
Conclusion: Nursing and midwifery interventions improve psychological well-being, treatment knowledge, coping, self-efficacy, satisfaction, and quality of life in ART, IVF, and ICSI care. Their effects on clinical pregnancy and live birth remain uncertain; therefore, structured support should be integrated into infertility protocols, and high-quality trials should assess long-term clinical outcomes.
Keywords: Infertility, Assisted, Nursing Care, Reproductive Techniques, Patient-Centered Care

Pnm-16: Efficacy of Artificial Intelligence-Based Tools in Embryo Selection and Prediction of Pregnancy Outcomes in IVF Cycles: A Systematic Review and Diagnostic Meta-Analysis

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Background: Embryo selection remains one of the most critical and challenging steps in assisted reproductive technology (ART). Despite advances in morphological grading systems, a significant proportion of morphologically normal embryos fail to implant, leading to suboptimal live birth rates. Artificial intelligence (AI) — including machine learning (ML) and deep learning (DL) algorithms — has emerged as a promising adjunct to conventional embryologist evaluation, offering objective, reproducible, and high-throughput assessment of embryo viability. This systematic review and diagnostic meta-analysis aimed to quantitatively synthesize the diagnostic accuracy of AI-based tools in embryo selection and their impact on pregnancy outcomes in in-vitro fertilization (IVF) cycles.
Materials and Methods: A comprehensive systematic search was conducted across PubMed/MEDLINE, Scopus, Web of Science, and Cochrane Library from inception through March 2025, following PRISMA 2020 guidelines. Search terms included: "artificial intelligence," "machine learning," "deep learning," "embryo selection," "IVF," and "pregnancy outcomes." Original research articles evaluating AI diagnostic accuracy in embryo grading or implantation prediction were eligible. Studies reporting sensitivity, specificity, area under the curve (AUC), or live birth rate comparisons were prioritized. Quality was assessed using the QUADAS-2 tool. Pooled diagnostic metrics were calculated using a bivariate random-effects model, and heterogeneity was explored via I^2 statistics and meta-regression.
Results: A total of 28 studies involving over 15,400 embryos and 6,200 IVF cycles were included. AI-based systems dem-

onstrated a pooled sensitivity of 78% (95% CI: 72–83%) and specificity of 81% (95% CI: 76–86%) for predicting successful implantation. The pooled AUC across diagnostic meta-analysis was 0.84 (95% CI: 0.80–0.88), indicating good discriminative performance. DL models consistently outperformed support vector machine (SVM) and logistic regression models (AUC 0.87 vs. 0.79, P<0.01). Time-lapse-integrated AI tools showed superior performance compared to static image analysis. Subgroup analysis indicated that AI models trained on blastocyst-stage embryos yielded higher AUC values than those trained on cleavage-stage embryos (0.88 vs. 0.81). Heterogeneity was moderate to high (I^2=61–74%), partly explained by differences in AI architecture and outcome definitions.
Conclusion: AI-based embryo assessment tools demonstrate clinically meaningful diagnostic accuracy in predicting IVF implantation and pregnancy outcomes, potentially reducing subjectivity in embryo selection. Integration of time-lapse imaging with DL models appears most promising. However, multi-center prospective validation studies are needed before routine clinical adoption. Nursing and midwifery teams should be equipped to counsel patients on the evolving role of AI in personalized ART care.
Keywords: Artificial Intelligence, Machine Learning, Embryo Selection, IVF, Reproductive Medicine

Pnm-17: Investigating The Association Between Dairy Consumption and The Risk of Breast Cancer in Women: A Narrative Review Study

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Background: Breast cancer is recognized as one of the most common cancers among women both globally and in Iran. Laboratory data suggest that dairy products contain high amounts of vitamin D and calcium, which may be associated with breast cancer. Therefore, the present study was conducted with the aim of investigating the association between dairy consumption and the risk of breast cancer in women.
Materials and Methods: This study was conducted as a narrative review. A search was performed of sources published in Persian in the Magiran, SID, and Barkat databases, and in English in the PubMed, Scopus, Google Scholar, and Web of Science databases, using the keywords "Women," "Dairy products," "Breast cancer," and their Persian equivalents, covering the period from 2000 to 2025. Ultimately, out of 261 articles retrieved, 11 articles relevant to the objective were reviewed.
Results: Among the 11 included studies, 4 were review studies, 4 were case-control studies, and 3 were cohort studies. Three studies were conducted in Iran and 8 studies were conducted abroad. The results of the studies indicated that dairy consumption, particularly low-fat dairy, has a significant inverse association with breast cancer, meaning that as dairy consumption increases, the incidence of breast cancer decreases. This association is stronger in premenopausal women.
Conclusion: Given the significant inverse association between dairy consumption and the incidence of breast cancer, it is recommended that the role of dairy products in women's food baskets be strengthened. It is also suggested that nutritional education in this regard be provided to all women attending healthcare centers by healthcare service providers.
Keywords: Dairy Products, Breast Cancer, Women

Pnm-18: Prevalence of Anxiety and Depression and Their Associated Risk Factors in Women with Infertility: A Systematic Review and Meta-Analysis

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Background: Infertility is increasingly recognized not merely as a biomedical condition but as a profound psychosocial stressor with significant implications for mental health. Women with infertility face disproportionate emotional burdens, including elevated rates of anxiety and depression, which may in turn negatively influence treatment adherence, quality of life, and assisted reproductive technology (ART) outcomes. Despite a growing body of evidence, pooled estimates of the prevalence of anxiety and depression and their modifiable risk factors remain insufficiently characterized. This systematic review and meta-analysis aimed to determine the pooled prevalence of anxiety and depression among women with infertility and to identify key sociodemographic, clinical, and psychosocial risk factors associated with these conditions.
Materials and Methods: A systematic search was performed across eight international databases — PubMed, Web of Science, Embase, Cochrane Library, Sinomed, CNKI, VIP, and WanFang — from database inception through February 2025, in accordance with PRISMA 2020 reporting standards. Observational studies reporting prevalence of anxiety or depression — assessed via validated instruments (HADs, STAI, BDI, PHQ-9, GAD-7) — in women diagnosed with infertility were eligible. Two independent reviewers screened studies and extracted data. Pooled prevalence estimates were calculated using a random-effects model (DerSimonian-Laird method). Heterogeneity was assessed with I^2 and Cochran's Q statistics, and meta-regression was applied to explore sources of variability.
Results: A total of 42 studies from 24 countries, encompassing 18,600 women with infertility, were included. The pooled prevalence of anxiety was 41% (95% CI: 35–47%; I^2=82%) and depression was 42% (95% CI: 36–48%; I^2=79%). Significant risk factors for anxiety included: older age (OR=1.38, 95% CI: 1.10–1.73), longer duration of infertility (OR=1.68, 95% CI: 1.30–2.17), high treatment costs (OR=2.04, 95% CI: 1.78–2.34), and limited knowledge about ART (OR=1.70, 95% CI: 1.26–2.29). Depression was significantly associated with psychological distress (OR=1.63, 95% CI: 1.24–2.13) and female-factor infertility diagnosis (OR=1.40, 95% CI: 1.11–1.75). Women undergoing repeated ART failure showed significantly higher depression scores than treatment-naive individuals.
Conclusion: Over 40% of women with infertility experience clinically significant anxiety or depression, underscoring the urgent need for routine psychological screening integrated into infertility care pathways. Modifiable factors — including ART education, financial counseling, and psychosocial support — represent viable intervention targets. Nurses and midwives are ideally positioned to deliver structured psychological assessments, facilitate referral pathways, and implement evidence-

based psychosocial support interventions within the infertility care team.
Keywords: Infertility, Anxiety, Depression, Psychological Distress, ART

Pnm-19: A Review of Home-Based Insemination Technologies in Modern Infertility Management

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Background: Infertility is a growing global health concern affecting millions of couples worldwide. Although assisted reproductive techniques such as intrauterine insemination (IUI) and in-vitro fertilization (IVF) have improved fertility outcomes, many patients still face challenges including high treatment costs, psychological stress, limited accessibility, and frequent clinical visits. In recent years, home-based fertility technologies and insemination devices have gained increasing attention as patient-centered approaches aimed at improving privacy, comfort, and accessibility in infertility management. This review presents the design, methodology, and clinical evaluation framework of a modern at-home reproductive kit aimed at increasing fertility rates. The primary product has been developed and manufactured in Iran using localized technology. It has successfully obtained all necessary regulatory approvals, including licenses from the Ministry of Health and Medical Education (MOHME) and the Iranian Medical Device Directorate (IMED), ensuring compliance with national safety and quality standards.
Materials and Methods: A narrative review was conducted on currently available home-based insemination technologies and fertility-support devices. Relevant commercial products, published reports, and emerging fertility approaches were evaluated with focus on usability, accessibility, patient comfort, and potential application in infertility management.
Results: Review of currently available home-based insemination products demonstrated increasing global interest in patient-centered fertility solutions. Existing technologies mainly focus on improving accessibility, privacy, ease of use, and reducing the psychological burden associated with infertility treatment. Several commercially available devices were identified with different design approaches for intracervical insemination (ICI) and home fertility support. These innovations highlight the growing role of home-based reproductive technologies in modern infertility management.
Conclusion: Home-based insemination technologies represent a promising and emerging field in modern infertility management. These approaches may improve patient comfort, privacy, and accessibility while reducing some of the logistical and psychological challenges associated with conventional fertility treatments. Further clinical evaluation and technological development may help optimize the effectiveness and wider application of these patient-centered fertility solutions.
Keywords: Home-Based Insemination, Infertility Management, Patient-Centered Fertility Care, Patient-Centered Fertility Care

Pnm-20: Relationship Between Uterine Fibroid and Nutrition in Women of Reproductive Age: A Literature Review

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Background: Uterine fibroids (UF) are common noncancerous tumors that usually develop among reproductive-aged women. The incidence is about 30% to 70% according to different diagnostic methods. UF represents the principal cause for hysterectomies, and leads to specific symptoms including heavy menstrual bleeding, pelvic pressure, infertility and pregnancy loss. As the economic burden due to fibroids has increased, these benign tumors have become a significant health concern in women. The pathogenesis of UF is not well understood, but lifestyle factors including diet, physical activity and mental status have a potential effect on fibroids growth and development.
Materials and Methods: Searching was performed in some databases like PubMed, Scopus, and Web of Science. 24 full text articles in English from 2000 to 2026 were found which their topic was similar to our topic. In this way, we crossed the available data regarding the association between nutrition habits and dietary components and fibroma onset and growth.
Results: Many nutrients and dietary habits are associated with myoma development risk. These factors including low intakes of fruit, vegetables, and vitamin D. Intakes of total marine ω-3 polyunsaturated fatty acids were associated with elevated UF incidence. The protective role of dairy products, i.e., yogurt consumption has been indicated. The role of Vitamin A in the formation of fibroids is contradictory. Vitamin A-enriched diet, as well as synthetic retinoid analogs may be preventative or limit the growth of fibroids. Unambiguous conclusions cannot be drawn regarding Vitamin E and C supplementation.
Conclusion: The results of this study indicated that nutrition may be associated with the risk of fibroma, so encouraging people to modify their diet seems essential. Initial results are promising, but further randomized trials are needed to draw robust conclusions about the effects of diet and nutrients on UF.
Keywords: Fibroma, Diet, Nutrition

Pnm-21: Effect of Structured Educational Counseling on Anxiety and Treatment Knowledge Among Women Undergoing IVF/ICSI: A Quasi-Experimental Study

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Background: Women undergoing in-vitro fertilization (IVF) / Intracytoplasmic Sperm Injection (ICSI) often experience significant anxiety stemming from treatment complexity and limited treatment-related knowledge. Structured educational counseling may mitigate this anxiety by improving psychological preparedness and informed decision-making. This study assessed the effect of a structured nurse- or midwife-led counseling session on anxiety and treatment knowledge in women undergoing IVF/ICSI.
Materials and Methods: In this single-center quasi-experimental controlled pretest-posttest study, 92 women undergo-

ing IVF/ICSI were assigned to intervention or control groups in alternating recruitment weeks, with 46 participants in each group. The allocation was non-randomized and non-concealed due to the alternating-week design. The intervention group received routine care plus a 60–75-minute structured face-to-face educational counseling session delivered by a trained nurse or midwife, covering IVF/ICSI processes, medication use, self-care, possible complications, warning signs, and emotional coping. The control group received routine care only. Anxiety was assessed using the State Anxiety subscale of the State-Trait Anxiety Inventory (STAI-S), and treatment knowledge was measured using a validated IVF/ICSI knowledge questionnaire. Written informed consent was obtained from all participants. Data were analyzed using chi-square test, paired and independent t-tests, and ANCOVA, with $P<0.05$ considered significant. Ethical approval was obtained from Karaj Branch, Islamic Azad University and written informed consent was obtained from all participants.

Results: Ninety-two women completed the study, with 46 participants in each group. Baseline demographic and clinical characteristics, anxiety, and treatment knowledge scores were comparable between groups ($P>0.05$). After adjustment for baseline values, the counseling group showed a significantly greater reduction in anxiety than the control group (adjusted mean difference: -8.9 points; 95% CI: -11.4 to -6.4; $P<0.001$). Treatment knowledge also increased significantly more in the intervention group (adjusted mean difference: 5.8 points; 95% CI: 4.4 to 7.2; $P<0.001$). No intervention-related adverse effects were reported.

Conclusion: Structured educational counseling was associated with reduced anxiety and improved treatment knowledge among women undergoing IVF/ICSI. Despite the single-center, non-randomized design, these findings suggest that nurse- or midwife-led counseling may be a feasible supportive approach in infertility care. Further randomized trials are needed to assess its long-term effects on adherence, satisfaction, and clinical outcomes.

Keywords: Intracytoplasmic, Anxiety, Infertility, Counselin

Pnm-22: Comparative Efficacy of Postoperative Hormonal Suppression in Preventing Endometriosis Recurrence After Fertility-Preserving Surgery: A Systematic Review and Network Meta-Analysis

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Background: Endometriosis affects approximately 10% of women of reproductive age and carries a high postoperative recurrence rate, estimated between 20–50% within five years of conservative surgery. Despite widespread surgical management, the optimal postoperative medical regimen to suppress recurrence while preserving fertility remains controversial. Several hormonal agents — including dienogest, gonadotropin-releasing hormone agonists (GnRH-a), levonorgestrel-releasing intrauterine systems (LNG-IUS), combined oral contraceptives (COCs), and danazol — have been evaluated. This study aimed to systematically review and perform a network meta-analysis (NMA) to compare the relative efficacy and safety of these agents in preventing endometriosis recurrence and pain symptoms following fertility-preserving laparoscopic surgery.

Materials and Methods: A systematic search of PubMed/MEDLINE, Embase, Cochrane CENTRAL, and Web of Science was conducted through December 2024, adhering to PRISMA-NMA guidelines. Randomized controlled trials (RCTs) comparing at least one postoperative hormonal agent versus placebo, no treatment, or other agents in women aged 20–45 years following laparoscopic surgery for endometriosis were included. Primary outcomes were endometrioma recurrence rate and pain recurrence measured by VAS. Secondary outcomes included pregnancy rates, adverse events, and patient-reported quality of life. Risk of bias was assessed using the Cochrane RoB 2.0 tool. Bayesian NMA was performed to generate odds ratios (ORs) and surface under the cumulative ranking curve (SUCRA) values.

Results: Sixteen RCTs enrolling 1,605 participants evaluating 10 pharmacological agents were included. Dienogest demonstrated the highest probability of preventing endometrioma recurrence compared to placebo (OR: 0.21, 95% CI: 0.09–0.47; SUCRA=87%), followed by GnRH-a (OR: 0.28, 95% CI: 0.12–0.61; SUCRA=74%) and LNG-IUS (SUCRA=68%). COCs showed moderate efficacy (OR: 0.44, 95% CI: 0.22–0.87). Dienogest also significantly reduced pain recurrence scores versus placebo (mean difference: −2.3 VAS points, 95% CI: −3.1 to −1.5). No significant difference in postoperative pregnancy rates was observed across agents. Adverse event profiles differed: dienogest was associated with irregular vaginal bleeding, while GnRH-a carried a higher rate of menopausal symptoms and bone density concerns with long-term use.

Conclusion: Dienogest emerges as the most effective and well-tolerated postoperative hormonal agent for preventing endometriosis recurrence and managing pain following fertility-preserving surgery. Individualized treatment selection based on patient's reproductive goals, tolerability, and disease severity is paramount. Midwifery and nursing practitioners play a crucial role in long-term follow-up, adherence support, and early detection of recurrence signs in this patient population.

Keywords: Endometriosis, Postoperative Recurrence, Dienogest, Hormonal Suppression, Fertility-Preserving Surgery

Pnm-23: The Role of Adenomyosis in Infertility: Epidemiology, Pathophysiological Mechanisms, and Prevention of Modifiable Risk Factors — A Systematic Review

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Background: Adenomyosis, traditionally categorized as a structural uterine disorder, is gaining recognition as a prevalent factor in subfertility and reproductive failure. Despite its growing epidemiological burden, the causal relationship between its inflammatory microenvironment and poor implantation outcomes remains complex. This systematic review integrates current literature to clarify how adenomyosis acts as a common cause of infertility and defines proactive clinical pathways to mitigate its modifiable risk factors before irreversible tissue damage occurs.

Materials and Methods: A review was performed indepen-

dently by two people based on the PICO criteria and aligned to the research objective and based on the PRISMA checklist and using PubMed, CINAHL, Medline, Web of Science, SID databases Google Scholar search engine, and Boolean operators. The time limit between 2016 and 2026 was determined using the MESH keywords "Adenomyosis", "Pathologic Processes" and "Infertility". After checking the entry and exit criteria and critically evaluating the quality of the selected articles, a total of 8 articles were included in the study.

Results: The epidemiology of adenomyosis suggests a high correlation with otherwise unexplained infertility, with prevalence rates significantly impacting in-vitro fertilization (IVF) success. Our synthesis identifies that a persistent inflammatory state—driven by local overproduction of IL-6, TNF-α, and oxidative stress—directly degrades the endometrial-myometrial junction, thereby compromising embryo implantation sites. A critical finding is that the "prevention of progression" is more effective than late-stage intervention. By identifying modifiable factors such as chronic pelvic inflammation and specific metabolic markers, clinicians can implement targeted interventions. These strategies, ranging from anti-inflammatory lifestyle counseling to early-stage immunomodulation, are shown to limit myometrial infiltration and preserve uterine functionality. Consequently, early screening protocols serve as a preventive frontline in protecting the reproductive potential of symptomatic women.

Conclusion: Adenomyosis is a major, manageable obstacle in the landscape of infertility. Moving away from purely surgical perspectives toward the systematic management of modifiable risk factors offers a promising preventive framework. Prioritizing early diagnostic accuracy and inflammatory control is essential to enhancing cumulative pregnancy rates and reducing long-term reproductive loss.

Keywords: Adenomyosis, Infertility, Pathologic Processes

Pnm-24: Types of Uterine Fibroid and Nutrition in Women

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Background: Uterine fibroids are classified according to their anatomical location within the uterus into four major types: submucosal, intramural, subserosal, and pedunculated subserosal fibroids. Among dietary factors, macronutrients—including proteins, carbohydrates, fats, and dietary fiber—have attracted considerable attention as potential contributors to fibroid pathogenesis because of their roles in metabolism, hormonal regulation, and inflammatory processes.

Materials and Methods: This case–control study was conducted between November 2023 and March 2024 among 270 women of reproductive age who attended a gynecology clinic. The case group consisted of 90 women diagnosed with uterine fibroids, whereas the control group included 180 women without fibroids. All participants provided written informed consent prior to enrollment and completed demographic questionnaires as well as a validated Food Frequency Questionnaire (FFQ). The collected dietary data were analyzed using nutritional analysis software, such as Nutritionist IV and the Iran/USDA Food Composition Table, to estimate the participants' daily intake of total energy, macronutrients (proteins, carbohydrates, and fats), and micronutrients (vitamins and minerals).

Results: In the present study, the distribution of uterine fibroid types was assessed based on their frequency among the study population. The findings of this study, conducted in Iran, demonstrated no statistically significant association between different types of uterine fibroids and the intake of macronutrients, including proteins, carbohydrates, fats, and dietary fiber, or micronutrients, including vitamins and minerals. Nevertheless, the mean intake of protein, carbohydrates, and fiber was lower among women with submucosal fibroids, whereas the mean fat intake was higher among those with intramural fibroids compared with the other fibroid types; however, these differences did not reach statistical significance. Regarding vitamin intake, the mean consumption of vitamins C and K was lower in women with pedunculated subserosal fibroids, while vitamin A intake was lower in women with submucosal fibroids. In addition, the mean intake of vitamins E and D was lower among women with intramural fibroids compared with the other fibroid subtypes. Similarly, the mean intake of calcium, zinc, magnesium, manganese, selenium, and copper was lower in women with submucosal fibroids than in those with other fibroid types. In contrast, mean iron intake was lower among women with pedunculated subserosal fibroids compared with the remaining fibroid subtypes.

Conclusion: The findings of the present study demonstrated no significant association between the intake of macronutrients and micronutrients and the occurrence of different types of uterine fibroids in the Iranian population. However, despite these findings, it is possible that a larger sample size may yield more definitive results.

Keywords: Type of Fibroma, Diet, Macronutrient, Micronutrient

Pnm-25: Epigenetic Mechanisms in Ovarian Aging and Their Impact on Female Fertility

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Background: Ovarian aging is a key factor determining female reproductive lifespan and the progressive decline in fertility. Increasing evidence indicates that epigenetic mechanisms including DNA methylation, histone acetylation, and regulation by non coding RNAs play essential roles in maintaining ovarian function. Dysregulation of these processes has been linked to depletion of ovarian reserve, impaired follicular development, and reduced oocyte quality. A better understanding of these molecular mechanisms may improve current approaches for the diagnosis and management of ovarian aging–related infertility and premature ovarian failure (POF).

Materials and Methods: A systematic literature search was performed using Google Scholar and biomedical databases including PubMed/MEDLINE. Studies addressing epigenetic regulation in ovarian aging and female fertility were identified using relevant keywords related to DNA methylation, histone modifications, non coding RNAs, folliculogenesis, and POF. Eligible studies were screened and qualitatively synthesized to summarize key epigenetic mechanisms and their clinical relevance.

Results: Cumulative evidence shows that epigenetic dysregulation is a key driver of ovarian aging and POF. At the molecular

level, these epigenetic alterations interfere with pathways essential for follicular integrity and reproductive function. Such changes influence follicular activation, oocyte maturation, meiotic progression, and endocrine balance. During aging, aberrant DNA methylation, characterized by hypermethylation in primordial follicles and incomplete methylation during follicular development, is associated with reduced oocyte quality and accelerated follicular loss. Central epigenetic regulators including DNMT1, DNMT3A, DNMT3B, TET enzymes, MeCP2, and DCAF13 play vital roles in granulosa cell survival, estrogen synthesis, and meiotic regulation; their dysfunction contributes to follicular arrest and apoptosis. Likewise, abnormal histone acetylation disrupts chromatin accessibility and gene transcription essential for oocyte maturation. HDACs and sirtuins coordinate follicular quiescence, spindle stability, and reproductive lifespan. Altogether, these insights highlight the therapeutic potential of epigenetic based interventions, such as metformin or selective HDAC and sirtuin modulation, offering more targeted options beyond traditional hormone replacement therapy.
Conclusion: Epigenetic regulation represents a critical component of ovarian aging and fertility decline. Further large scale and clinically oriented studies are required to clarify the underlying molecular pathways and to support the development of precision based diagnostic and therapeutic strategies for ovarian aging and infertility.
Keywords: Ovarian Aging, Epigenetics, Premature Ovarian Insufficiency (POI), Folliculogenesis

Pnm-26: Artificial Intelligence and Multi-Omics Integration in The Genetic Diagnosis of Infertility: A Narrative Review

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Background: Infertility is a complex reproductive disorder influenced by genetic, epigenetic, hormonal, and environmental factors. With the rapid growth of genomic and multi-omics data, conventional diagnostic approaches are often insufficient to capture the full biological complexity of infertility. Artificial intelligence (AI) and computational biology offer promising tools to improve the identification of causative variants, interpret molecular signatures, and support personalized reproductive care.
Materials and Methods: This narrative review synthesizes recent studies on infertility genetics, including whole-exome sequencing, whole-genome sequencing, transcriptomics, and copy number variation analysis. We examined computational approaches such as machine learning, deep learning, variant prioritization pipelines, pathway-based analysis, and multi-omics data fusion. Studies focusing on reproductive phenotypes, embryo development, gametogenesis, and assisted reproductive technology outcomes were reviewed and compared based on methodological design, data sources, and validation strategies.
Results: Current evidence suggests that AI-assisted multi-omics frameworks can enhance the detection of pathogenic variants, improve phenotype–genotype association, and identify biologically meaningful pathways involved in male and female infertility. These approaches are particularly useful in cases with unexplained infertility, heterogeneous phenotypes, and small sample sizes. However, limitations remain, including limited external validation, poor interpretability, inconsistent phenotype annotation, and dataset bias across populations.
Conclusion: AI-driven multi-omics integration may substantially advance the genetic diagnosis and biological understanding of infertility. Future research should prioritize transparent algorithms, standardized reproductive phenotyping, and large multicenter validation studies to support clinical translation.
Keywords: Infertility, Genetics, Artificial Intelligence, Multi-Omics, Bioinformatics

Pnm-27: Digital Twins and Computational Modeling in Reproductive Genetics: A New Framework for Precision Infertility Research

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Background: Reproductive failure is increasingly recognized as a systems-level disorder involving genetic variation, endocrine regulation, developmental dynamics, and environmental interactions. Emerging computational technologies, including digital twins and predictive modeling, provide an innovative framework for simulating fertility-related biological processes and improving precision medicine in infertility.
Materials and Methods: This review analyzed literature on systems genetics, reproductive bioinformatics, digital twin modeling, and machine learning applications in infertility research. We focused on studies integrating genomic, clinical, hormonal, and embryological data to build predictive models of gamete quality, implantation success, embryo viability, and treatment response. Computational methods such as Bayesian modeling, graph neural networks, and dynamic simulation were evaluated in terms of data integration, predictive performance, and clinical applicability.
Results: The reviewed studies indicate that computational fertility models can capture complex interactions among genetic variants, reproductive phenotypes, and treatment outcomes more effectively than traditional rule-based approaches. Digital twin frameworks show particular promise for individualized forecasting in assisted reproduction. Nevertheless, progress is hindered by sparse longitudinal datasets, lack of standardized outcome measures, limited reproducibility, and unresolved ethical and privacy issues related to reproductive data.
Conclusion: Digital twins and computational systems biology may reshape infertility research by enabling individualized prediction and mechanistic modeling of reproductive dysfunction. Their clinical implementation will require robust validation, interoperable datasets, interpretable algorithms, and ethical governance.
Keywords: Infertility, Reproductive Genetics, Digital Twin, Computational Modeling, Precision Medicine

Pnm-28: Sexual Education for Couples and Its Impact on Healthy Fertility Promotion: A Systematic Review (2020–2025)

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Background: Sexual and reproductive health education for couples can significantly influence fertility awareness, healthy lifestyle choices, and timely decision-making. Despite the couple-centered nature of infertility, most educational programs have focused on individuals. Evaluating couple-based sexual education interventions is essential for promoting healthy fertility. This systematic review aimed to evaluate the effectiveness of sexual education programs targeting couples on fertility knowledge, reproductive behaviors, and promotion of healthy fertility.
Materials and Methods: A systematic search was conducted in PubMed, Scopus, Web of Science, Cochrane Library, and PsycINFO for randomized controlled trials, quasi-experimental studies, and high-quality reviews published between 2020 and 2025. Eligible studies included couple-based sexual education or fertility awareness interventions. Two independent reviewers performed study selection, data extraction, and quality assessment using the Cochrane Risk of Bias tool and GRADE approach
Results: Forty-one studies involving over 12,000 couples were included. Couple-centered sexual education programs significantly improved joint fertility awareness, including accurate knowledge of the fertile window, age-related fertility decline, and modifiable risk factors (obesity, smoking, alcohol, and sexually transmitted infections). Couples who participated in these programs showed higher rates of healthy lifestyle modifications, better communication regarding reproductive goals, increased adherence to preconception care, and earlier seeking of medical assistance for infertility. Interventions combining sexual education with fertility awareness methods resulted in higher natural conception rates in subfertile couples and reduced psychological distress. Digital couple-based platforms and group counseling sessions proved particularly effective. However, long-term sustainability of behavioral changes and cultural barriers in some populations were noted as limitations. Male partner involvement was associated with significantly better outcomes.
Conclusion: Sexual education programs specifically designed for couples are effective in promoting healthy fertility, improving reproductive knowledge, and encouraging positive behavioral changes. Integrating couple-based fertility education into routine preconception and infertility care can enhance natural conception chances and optimize assisted reproductive technology outcomes. Future programs should emphasize active male participation and long-term follow-up.
Keywords: Sexual Education, Couples Fertility Education, Fertility Awareness, Healthy Fertility, Reproductive Health Promotion

Pnm-29: Recent Advances in Infertility Diagnosis and Treatment: A Systematic Review of Achievements from 2020 to 2025

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Background: Infertility remains a global health challenge affecting millions of couples. Rapid technological and scientific progress in assisted reproductive technology (ART) has led to significant improvements in diagnosis, treatment success rates, and patient outcomes. An updated synthesis of the latest achievements is essential for evidence-based practice. This systematic review aimed to summarize the most recent advances in the diagnosis and management of infertility, focusing on innovations that have improved clinical outcomes between 2020 and 2025.
Materials and Methods: A systematic search was conducted in PubMed, Scopus, Web of Science, Cochrane Library, and EMBASE for original studies, randomized controlled trials, meta-analyses, and high-quality reviews published between 2020 and 2025. Studies evaluating novel diagnostic tools, ART techniques, and emerging therapies were included. Two independent reviewers screened articles, extracted data, and assessed quality using the AMSTAR-2 and GRADE tools.
Results: Eighty-seven studies met the inclusion criteria. Key achievements include the integration of artificial intelligence (AI) for embryo selection using time-lapse imaging, which has improved implantation and live birth rates compared to traditional morphological assessment. Preimplantation genetic testing for aneuploidy (PGT-A) and non-invasive methods have enhanced embryo selection, particularly in advanced maternal age. Personalized ovarian stimulation protocols guided by biomarkers (AMH and AFC) and pharmacogenomics have reduced ovarian hyperstimulation syndrome (OHSS) while optimizing response. Advances in male infertility include micro-TESE combined with intracytoplasmic sperm injection (ICSI) for non-obstructive azoospermia and improved sperm selection techniques. Emerging therapies such as in-vitro gametogenesis, stem cell-based ovarian rejuvenation, and simplified low-cost in-vitro fertilization (IVF) systems show promise for poor responders and low-resource settings. Cryopreservation techniques and uterine microbiome assessment have further contributed to better outcomes.
Conclusion: Significant progress in infertility care has been achieved through AI integration, genetic technologies, personalized approaches, and innovative laboratory methods. These advances have increased success rates and expanded treatment options. Continued research is needed to confirm long-term safety, cost-effectiveness, and accessibility, particularly in diverse populations.
Keywords: Infertility, Assisted Reproductive Technology, Artificial Intelligence, Preimplantation Genetic Testing, Personalized Medicine

Pnm-30: Neurodevelopmental Outcomes in Children Born After Assisted Reproductive Technology: A Systematic Review of Recent Evidence (2020–2025)

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Background: The rapid increase in the use of assisted reproductive technology (ART) has raised important questions about the

neurodevelopmental health of resulting children. While perinatal risks are well-documented, long-term neurodevelopmental outcomes require careful synthesis of recent high-quality evidence. This systematic review aimed to evaluate neurodevelopmental outcomes, including cognitive, motor, behavioral, and neurodevelopmental disorders, in children conceived through ART compared to spontaneously conceived children.

Materials and Methods: A systematic search was conducted in PubMed, Scopus, Web of Science, Cochrane Library, and EMBASE for cohort studies, meta-analyses, and high-quality reviews published between 2020 and 2025. Eligible studies assessed cognitive function, motor development, autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), cerebral palsy, and other neurodevelopmental outcomes in ART-conceived children. Two independent reviewers performed study selection, data extraction, and quality assessment using the Newcastle-Ottawa Scale and GRADE approach.

Results: Fifty-eight studies involving millions of children were included. After adjustment for confounders such as prematurity, multiplicity, and parental factors, most ART singletons showed comparable cognitive, language, and overall neurodevelopmental outcomes to spontaneously conceived children. A modest increase in risk was observed for cerebral palsy and certain motor delays, largely attributable to higher rates of preterm birth and low birth weight. Evidence regarding ASD and ADHD was mixed: some studies reported a small elevation in risk with intracytoplasmic sperm injection (ICSI), while large registry-based analyses and sibling comparisons found little to no increased risk after full adjustment. Frozen embryo transfer appeared neutral or slightly protective compared to fresh transfer. Overall, absolute risks remained low, and the majority of ART-conceived children demonstrated normal neurodevelopmental trajectories.

Conclusion: Current evidence is largely reassuring regarding neurodevelopmental outcomes in children born after ART. Observed differences are mainly explained by obstetric factors rather than the ART procedures themselves. Continued long-term monitoring, especially for ICSI-conceived children, and strategies to reduce multiple pregnancies remain important. Further studies in diverse populations are needed

Keywords: Assisted Reproductive Technology, Neurodevelopmental Outcomes, ART Children, Autism Spectrum Disorder, Cognitive Development

Pnm-31: Educational Interventions by Midwives to Improve Fertility Awareness in Women of Reproductive Age: A Systematic Review

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Background: Despite affecting an estimated 17.5% of adults worldwide, fertility awareness remains suboptimal among women of reproductive age. Limited knowledge regarding fertility decline, modifiable lifestyle factors, and reproductive planning may contribute to delayed childbearing decisions and unmet reproductive goals. Midwives are well placed to provide fertility education; however, the evidence on midwife-led interventions to improve fertility awareness has not been systematically reviewed.

Materials and Methods: A systematic literature search was undertaken across PubMed, Scopus, Web of Science, CINAHL, and the Cochrane Library for studies published from January 2010 to December 2024. Studies were eligible if they evaluated educational interventions delivered or co-led by midwives, included women of reproductive age, and reported outcomes related to fertility awareness. The methodological rigor of included studies was appraised using the Joanna Briggs Institute (JBI) critical appraisal tools. Findings were synthesized narratively following PRISMA recommendations.

Results: The synthesis is anticipated to demonstrate that midwife-led fertility awareness interventions are implemented across a variety of healthcare and digital settings and encompass a broad range of fertility-related educational content. These interventions are expected to address major determinants of fertility, including reproductive aging, lifestyle-related risk factors, and conception planning. Preliminary evidence indicates that such interventions may improve fertility knowledge and awareness, although their long-term effects on reproductive decision-making, health behaviors, and fertility outcomes remain uncertain. Recurring barriers to implementation are likely to include gaps in professional training, time limitations within routine care, and the absence of standardized fertility education frameworks.

Conclusion: This review is expected to provide a comprehensive overview of the current evidence on midwife-led fertility awareness education and identify key gaps in the existing literature. By synthesizing available findings, it may contribute to the development of standardized educational approaches, competency-based training programs, and more integrated models of fertility care. The review is also anticipated to inform policy and practice while highlighting priorities for future longitudinal research exploring the role of midwives in promoting fertility awareness across different healthcare settings.

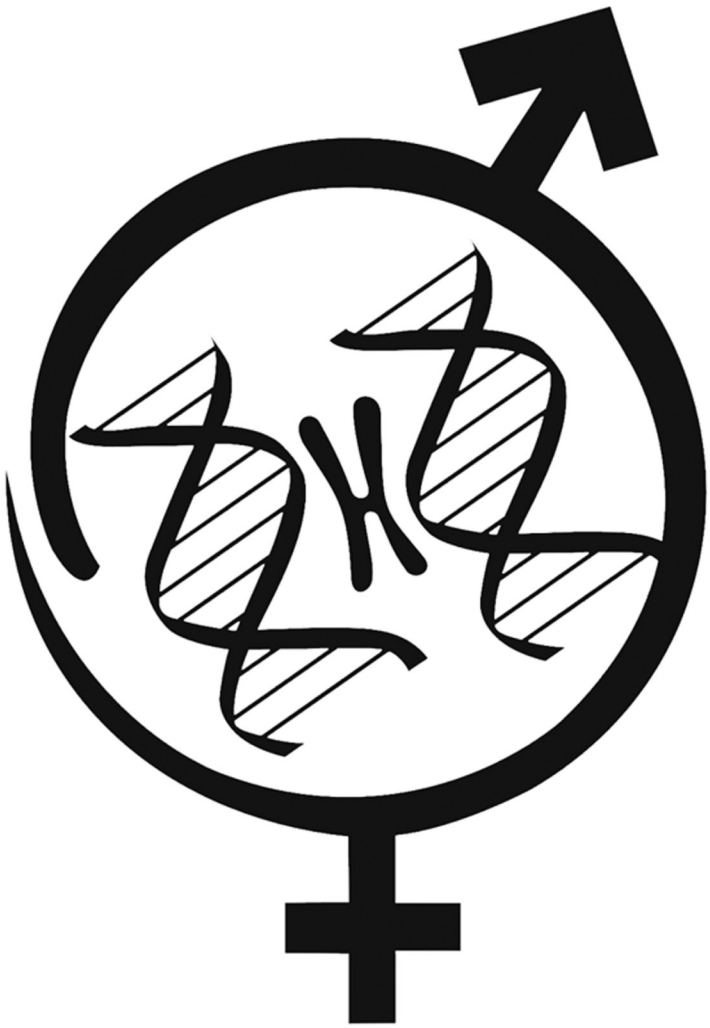
Keywords: Midwifery, Fertility Education, Fertility Awareness, Reproductive Health

Authors Index

Abstracts of

Royan International Hybrid Twin Congress

22nd Congress on Stem Cell Biology and Technology
2-3 September 2026



Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran

Abstracts of the 22th Congress on Stem Cell Biology and Technology (2-3 September 2026)

Contents:

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Reza Moghadasali, Ph.D.

Dear colleagues,
We're truly delighted and proud to welcome all professors, researchers, physicians, and hard-working students to the **22nd Royan International Congress on Stem Cell Biology and Technology**, taking place in **Tehran, Iran, from September 2 to 4, 2026**.
This event is a great chance to share knowledge, present the latest research, and build new collaborations in stem cells and regenerative medicine. We hope that through your discussions and interactions, we can make a real impact on advancing science, sparking innovation, and improving public health. Your presence and active participation are what make this event truly special.

Sincerely,
Reza Moghadasali, Ph.D.
Scientific Secretary,
22st Royan International Congress on
Stem Cell Biology and Technology

Invited Speakers

Is-1: Academic Resilience: Surviving and Thriving in Academia

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The profound transformations in higher education, the increasing complexity of academic roles, and the growing pressures associated with teaching, research, and administrative responsibilities have drawn considerable scholarly attention to the concept of academic resilience. In contemporary academic environments, faculty members, researchers, and postgraduate students face numerous challenges, including intense academic competition, publication pressures, limited resources and funding, job insecurity, and professional burnout, among others. Under such circumstances, academic resilience has emerged as a dynamic capacity that enables individuals to sustain performance, adapt effectively, and recover personal and professional functioning in the face of academic adversity and ongoing challenges.

The study and development of academic resilience are particularly important because this construct is closely associated not only with academic and professional success, but also with psychological well-being, job satisfaction, research productivity, sustained motivation, and the quality of scholarly engagement. Research suggests that individuals with higher levels of academic resilience are better equipped to cope with research setbacks, manage occupational stress, adapt to organizational changes, and maintain their professional commitment. Consequently, academic resilience can be regarded as a fundamental component of both individual and institutional sustainability and effectiveness in twenty-first-century universities.

This lecture is designed to explore both the theoretical and practical dimensions of academic resilience. The first part introduces the concept, its theoretical foundations, and its evolution within contemporary scholarship. The characteristics and key components of resilient individuals in academic settings will then be examined, together with the relationship between academic resilience and related constructs. The lecture will subsequently address major factors that undermine academic resilience, including chronic stress, burnout, promotion-related pressures, research constraints, job insecurity, and other individual and structural challenges. In addition, the factors and resources that contribute to the development and enhancement of academic resilience, such as self-efficacy, social support, organizational support, constructive professional relationships, and a supportive academic culture, will be discussed and analyzed.

In the final section, drawing upon the speaker's lived experiences and personal reflections from his academic, research, and professional journey, practical examples of coping with academic challenges will be presented. Furthermore, a range of evidence-based and actionable strategies for strengthening academic resilience among faculty members, researchers, and postgraduate students will be proposed. It is hoped that this lecture will not only deepen participants' theoretical understanding of academic resilience but also encourage reflection on effective approaches to fostering individual and organizational capacities within higher education institutions.

Keywords: Academic Resilience, Higher Education, Academic

Is-2: HER2-Specific TCR-T Cell Technology for Personalized Cancer Immunotherapy

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Background: T-cell receptor-engineered T-cell (TCR-T) therapy represents a promising approach for the treatment of solid tumors by enabling recognition of tumor-associated peptides presented by HLA molecules. HER2/neu is a well-characterized oncogenic driver expressed across multiple solid tumor types and therefore represents an attractive target for TCR-based immunotherapy. In this study, we focused on the HLA-A*02-restricted HER2/neu epitope KIFGSLAFL (HER2 369–377, E75). The aim was to develop and functionally validate a HER2/neu-specific TCR-T cell platform integrating antigen-specific T-cell enrichment, single-cell sequencing, computational TCR selection, and preclinical evaluation.

Materials and Methods: HER2/neu-specific CD8-positive T cells were generated from peripheral blood mononuclear cells of healthy donors using KIFGSLAFL-loaded dendritic cells and subsequently enriched using Flex-T technology. Single-cell RNA and TCR sequencing was performed using the BD Rhapsody platform to obtain paired TCR alpha and beta sequences together with transcriptional profiles. Candidate receptors were prioritized by integrating clonotype representation with ERGO-II-based prediction of TCR interaction with the KIF–HLA-A*02 complex. A lead TCR was selected, reconstructed in a third-generation lentiviral vector, and introduced into donor T cells. The resulting HER2-specific TCR-T cells were evaluated using *in vitro* cytotoxicity assays, secretome and transcriptomic analyses, followed by assessment of therapeutic activity in an *in vivo* xenograft model.

Results: The developed protocol resulted in more than a 215-fold enrichment of HER2/neu-specific T cells. Integration of paired TCR sequencing, clonal expansion, predicted TCR–peptide interaction, and transcriptional profiling enabled selection of a lead TCR associated with an expanded CD8-positive clonotype displaying a cytotoxic phenotype. Following lentiviral engineering, the highest transduction efficiency was achieved at MOI 1, reaching approximately 30%, while cell viability remained above 95%. HER2-specific TCR-T cells demonstrated pronounced antigen-dependent cytotoxic activity *in vitro*, reaching approximately 69–71% cytotoxicity against HER2-high SK-MEL-5 and SK-MEL-37 tumor cells, while substantially lower activity was observed against HCT-116 cells with moderate HER2 expression and HER2-negative MDA-MB-231 cells. Secretome analysis demonstrated increased secretion of IL-2, granzyme B, TNF-α, and IFN-γ, consistent with robust

antigen-specific activation and cytotoxic effector function. Single-cell transcriptomic analysis further revealed a distinct $CD4^+CD8^+$ T-cell population with increased expression of cytotoxic effector genes, including *GZMA, GZMB, GNLY, PRF1*, and *TNF-α*, confirming activation of a strong effector program. Importantly, in the SCID mouse xenograft model, treatment with HER2-specific TCR-T cells resulted in significant inhibition of tumor growth, with tumor volume reduction reaching up to 86%.

Conclusion: This study provides a complete preclinical proof-of-concept for a HER2/neu-specific TCR- T cell platform targeting the HLA-A*02-restricted KIFGSLAFL epitope. Integration of antigen- specific T-cell enrichment, single-cell TCR and transcriptomic profiling, computational receptor prioritization, and functional validation enabled selection and characterization of a therapeutically promising TCR. The resulting TCR-T cells demonstrated selective HER2- dependent antitumor activity *in vitro* and significant therapeutic efficacy *in vivo*, supporting further development of this approach for personalized TCR-T cell therapy of HER2-positive solid tumors.

Keywords: TCR-T Cell Therapy; HER2/neu; KIFGSLAFL; Cancer Immunotherapy; Single Cell Sequencing; Adoptive Cell Therapy

Is-3: Establishment of Novel Stem Cell and Pharmacological Therapies for Traumatic Spinal Cord Injury

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Spinal cord injury (SCI) is a leading cause of long-term neurological impairment due to extensive post-injury cell loss, disorganization of the neuroglial network, and disruption of spinal circuits. Transplantation of neural precursor cells (NPCs) has emerged as a promising approach since NPCs possess the ability to differentiate into the three main neural lineages. However, the outcomes of NPC engraftment for SCI remain modest due to 1) low long-term survival of transplanted NPCs, 2) restricted neurogenesis, and 3) limited functional integration of newly generated neurons into the host spinal network. Our group originally discovered that upregulation and deposition of chondroitin sulfate proteoglycans (CSPGs) inhibit regenerative capacity of transplanted NPCs. In addition, activation of serotonin receptor 5-HT1/2/7 increases neuronal maturity and synapse formation, and it enhances circuit re-assembly following SCI. Thus, we hypothesized that combining of NPC transplantation with inhibition of CSPGs signaling and activation of 5-HT1/2/7 receptors would optimize survival, neurogenesis, and circuit integration of NPC-derived neurons.

Using extensive *in vitro* and *in vivo* experiments, we demonstrated that pharmacological blockade of CSPG signaling substantially enhances the survival and neurogenesis of transplanted NPCs, significantly improving functional recovery in SCI rats. Although this approach increases neurogenesis in NPCs, a sizable number of NPCs still differentiate into astrocytes. In the second part of this thesis, we showed that NPC-derived astrocytes exhibit a pro-regenerative phenotype, supporting the NPC-mediated repair process and neuronal replacement. Lastly, our extensive animal studies reveal that suppressing CSPG signaling combined with activation of 5-HT1/2/7 receptors significantly enhances the integration of transplanted NPC-derived neurons into the host's local and supraspinal tracts. Altogether, this thesis provides new insights into the mechanisms of exogenous NPC-mediated repair in the injured spinal cord and establishes a novel, targeted, and effective cellular and pharmacological approach to enhance neuronal replacement and circuit reintegration following SCI.

Is-4: A Milli-Well Screening Platform for Studying Branching Morphogenesis of Stem Cell-Derived Ureteric Bud Organoids

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Background: Three-dimensional ureteric bud (UB) organoids have emerged as a powerful *in vitro* model for investigating kidney branching morphogenesis and have significant potential for applications in disease modelling, developmental biology, drug screening, and regenerative medicine. Nevertheless, their broader application is limited by the lack of robust and reproducible culture platforms.

Materials and Methods: A Milliwell-based platform was established for the generation and expansion of mouse pluripotent stem cell-derived ureteric bud organoids. Organoid morphology, phenotype, and molecular characteristics were evaluated using morphological image analysis, gene and protein expression analysis. The established platform was further applied to chemical compound testing.

Results: The Milliwell-based platform enabled the reproducible generation of branching organoids with consistent morphology and preservation of key UB-specific markers. As a proof of concept, the platform was successfully applied to investigate developmental processes and evaluate the effects of chemical compounds, demonstrating its utility for developmental biology and *in vitro* drug screening.

Conclusion: This optimized UB organoid platform provides a reliable and reproducible *in vitro* system for studying kidney branching morphogenesis and developmental mechanisms. Furthermore, its successful application highlights its potential as a versatile tool for kidney research, developmental toxicology, and future preclinical applications.

Is-5: Therapeutic Potential of Mesenchymal Stem Cell-Derived Extracellular Vesicles in Skin Regeneration and Atopic Dermatitis

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Background: Umbilical cord mesenchymal stem cell-derived extracellular vesicles (UC-MSC-EVs) are promising cell-free therapeutic agents for regenerative medicine. However, their comprehensive mechanisms in skin cells and therapeutic efficacy in atopic dermatitis (AD) remain poorly characterized. This study investigated the multifaceted effects of UC-MSC-EVs on skin regeneration, specifically focusing on anti-scarring, depigmenting, growth factor secretion, barrier-enhancing, and immunomodulatory properties, to define their therapeutic potential for skin disorders, particularly AD.
Materials and Methods: *In vitro* assays were performed using dermal fibroblasts to evaluate extracellular matrix remodelling and growth factor secretion, melanocytes to assess pigmentation, and a keratinocyte model to evaluate AD-related pathologies. The therapeutic efficacy and delivery of UC-MSC-EVs were further validated using an *in vivo* mouse model of AD.
Results: *In vitro*, UC-MSC-EVs promoted dermal fibroblast proliferation, matrix metalloproteinase expression, and growth factor secretion while suppressing melanin synthesis in melanoma cells. In AD-stimulated keratinocytes, the EVs restored epidermal structural protein expression and suppressed inflammatory mediator production. *In vivo*, topical application of UC-MSC-EVs significantly improved skin barrier function, reduced clinical AD severity, and normalized both histological features and serum PGE2 levels. Notably, while the EVs were efficiently internalized by skin cells *in vitro*, *in vivo* topical delivery showed predominant localization to the stratum corneum and hair follicles.
Conclusions: These findings demonstrate the multifaceted therapeutic potential of UC-MSC- EVs in skin rejuvenation, scar reduction, pigmentation control, and AD management, establishing them as a promising cell-free platform for treating inflammatory and degenerative skin disorders.

Is-6: Primed Mesenchymal Stem Cells Restore Bioenergetic Function in ND6-Mutant Fibroblasts of Mitochondrial Disease Patients

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Mitochondrial diseases caused by mitochondrial DNA (mtDNA) mutations impair oxidative phosphorylation and ATP production, with limited treatment options. This study investigated whether hypoxia and interferon-gamma (IFN-γ) priming enhances the therapeutic potential of mesenchymal stem cells (MSCs) in restoring ATP production in ND6-mutant fibroblasts. Human primary MSCs (n=3 donors) were cultured under normoxia, 3% hypoxia, 100 ng/mL IFN-γ, or combined hypoxia and IFN-γ. Primed MSCs were indirectly co-cultured with patient-derived ND6- mutant fibroblasts. MSC proliferation, ATP production, and mitochondrial transfer were evaluated. Combined hypoxia and IFN-γ priming significantly increased MSC proliferation (P=0.0018), while hypoxia alone and combined priming significantly enhanced ATP production (both P<0.001). Mitochondrial transfer was confirmed by Mito Tracker Green co-localization. Indirect co-culture with primed MSCs increased ATP levels in ND6-mutant fibroblasts by 40–60%. Hypoxia and IFN- γ priming enhances MSC proliferation, mitochondrial transfer, and ATP restoration, supporting primed MSCs as a promising cell-based therapy for mitochondrial diseases.
Keywords: MSC, ATP, Hypoxia, Mitochondrial Disease, IFN-γ

Is-7: CRISPR-Programming Early Human Embryogenesis

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Programmable CRISPR-based devices allow for engineering cell fate and developmental processes with unprecedented precision. Using minimal epigenome editing, we recently developed a reproducible mouse embryo model in which dCas9-based CRISPR activation rewires only two endogenous regulatory elements in embryonic stem cells to drive self-organization and pattern formation. This work demonstrated that modulation of a minimal gene regulatory network is sufficient to control collective developmental behaviors (Lodewijk and Kozuki et al, Cell Stem Cell, 2025, Guiltinan et al, Trends in Cell Biology, 2025). Building on this foundation, we have developed a multiplexable dCas12a-based CRISPR approach that enables efficient generation of human extraembryonic lineages from pluripotent stem cells. This system supports simultaneous activation of multiple developmental regulators, allowing the generation of programmable human embryo models with expanded control over lineage specification and gene expression. These CRISPR-programmed embryo models provide a tractable and ethically accessible framework for testing causal relationships that are otherwise impossible to interrogate in natural human embryos. Together, these advances establish CRISPR-programmed embryo models as engineered systems, in which gene regulatory networks can be designed and rewired to probe fundamental principles of early development. In my talk, I will highlight how programmable embryo models can be leveraged to modulate gene expression to uncover mechanistic rules governing early development, showcasing their application in understanding the role of extracellular matrix proteins in morphogenesis.

Is-8: Essential Skills Necessary for a Successful Young Scientist

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Academic Soft Skills is a very diverse and lengthy topic and these are highly essential for any academic to become successful in his/her career. Considering the fact that most of the audience will be research students, I will focus on the soft skills required at the level of Masters and PhD students. The essential fact to be realized is that soft skills are related to personality, field of research, work place environment, mentoring and personal motivation and conscious personal development of a student. These skills gradually develop like any other skill. As the student makes progress in career, new and better soft skills must also be acquired to adapt to additional demands of the research career and position. Most important soft skills include good written and oral communication, emotional intelligence,

team working, ability to intelligently manage untoward incidences, accepting new challenges and ability to accommodate and appreciate diversity. I will give practical examples in my presentation to give a better and practical view of academic soft skills to the students.

Is-9: Effect of Transduced Mesenchymal Stem Cells With IL-10 Gene as Cell Therapy for Control of Allergic Asthma

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Asthma is an important pulmonary disease associated with T helper lymphocyte (Th)2 dominant immune response, which can initiate allergic and inflammatory reactions. Interleukin (IL)-10 is the main immune suppressor cytokine, and mesenchymal stem cells (MSCs) have an immune-modulatory potential that can be transduced with the expression of the IL-10 gene to control pathophysiology of allergic asthma. Bone marrow's MSCs were isolated and transduced with the expression vector that contains the expressible IL-10 gene. Then, allergic asthma mouse model was produced and treated with manipulated MSCs. Methacholine challenge test; measurement of IL-4, IL-5, IL-8, IL-13, IL-25, and IL-33; and total and ovalbumin (OVA)-specific immunoglobulin (Ig)E levels were done. Hyperplasia of the goblet cell, secretion of mucus, and peribronchiolar and perivascular eosinophilic inflammation were evaluated in lung pathological sections. IL-25, IL-33, and total IgE levels; AHR; eosinophilic inflammation; hyperplasia of the goblet cell; and secretion of mucus could be controlled in M, MV, and MV-10 groups, and the control in the MV-10 group was strong compared to M and MV groups. MSCs have immune- modulatory capacity that can control allergic asthma pathophysiology, and this effect can be strengthened and reinforced by the expression of IL-10 gene.

Is-10: Engineering a Platelet-Rich Plasma-Based Multifunctional Injectable Hydrogel with Photothermal, Antibacterial, and Antioxidant Properties for Skin Regeneration

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Infected full-thickness skin wounds present a major clinical challenge due to persistent bacterial contamination, oxidative stress, and delayed tissue repair. This study developed an injectable multifunctional hydrogel (AGC–PRP–PDA) by incorporating platelet-rich plasma (PRP) and polydopamine nanoparticles (PDA NPs) into an alginate-gelatin matrix via one-pot synthesis and ionic crosslinking. The hydrogel exhibited excellent injectability, mechanical strength, and swelling properties, allowing conformal filling of irregular wounds. PDA NPs provided potent near-infrared (NIR) photothermal antibacterial activity against Staphylococcus aureus and robust antioxidant capacity to scavenge reactive oxygen species. PRP enabled sustained release of growth factors (VEGF, PDGF, TGF-β), promoting cell proliferation, collagen deposition, and angiogenesis. The system also demonstrated favorable hemocompatibility, cytocompatibility, and hemostatic effects. In vivo evaluation in a rat model of S. aureus-infected full- thickness wounds showed that the AGC–PRP–PDA hydrogel combined with NIR irradiation significantly accelerated healing, with enhanced re-epithelialization, granulation tissue formation, organized collagen matrix, and increased neovascularization compared to controls. In conclusion, this bio-photoactive hydrogel integrates antibacterial, antioxidant, hemostatic, and pro-regenerative functions within a single injectable platform. The synergistic combination of photothermal therapy and sustained growth factor delivery creates a favorable microenvironment for wound repair. This system offers a promising therapeutic strategy for managing complex infected wounds and advancing skin regenerative medicine.

Is-11: A Review of the Strategic Roadmap for European Regenerative Medicine and Lessons for Iran

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Despite two decades of investment in Regenerative Medicine (RM), however, only a handful of RM therapies have reached late-stage development or market approval. Venture funding directed toward the field has contracted markedly, and a number of major pharmaceutical companies have withdrawn from the space altogether. Overcoming this stagnation is generally understood to hinge on achieving a decisive inflection point — a clinical breakthrough robust enough to demonstrate both platform-level potential and commercial viability, thereby restoring confidence across the investment and industry ecosystem. Europe has introduced four strategic pillars to advance the field since June 2026: concentrating efforts in select high-potential hubs rather than dispersing resources thinly; embedding commercial and regulatory strategy into research from an early stage; investing in shared manufacturing infrastructure to lower production costs; and applying disciplined, portfolio-level prioritization to translate scientific discoveries into clinically and commercially viable candidates. Europe has started a coordinated, systems-level approach, rather than dispersed, project-by- project effort, toward restoring investor confidence and ultimately delivering meaningful patient impact in RM. While Iran's scientific output in this field is notable within the region, RM development faces not only the same global constraints such as high production costs and regulatory complexity but also country-specific challenges: persistent economic and political instability, an ongoing outflow of skilled scientific talent, a markedly narrower pool of risk-tolerant investment capital, and international isolation linked to sanctions that restricts access to global funding, partnerships, and supply chains. In practice, RM efforts in Iran remain fragmented across individual centers, with no unifying national strategic framework comparable to the coordinated approach in Europe. Addressing this fragmentation, beginning with steps to reduce international isolation and establish a harmonized national strategy, would appear to be a necessary first step in this regard. Keywords: Regenerative medicine; stem cell therapy; translational research; strategic roadmap; cell therapy manufacturing.

Is-12: Rewiring of Metabolism in HCC Spheroids Triggered by Tumor Microenvironment Components

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Metabolic reprogramming is a hallmark of hepatocellular carcinoma (HCC) and is shaped in large part by the tumor microenvironment (TME), which through hypoxia, stromal and immune cell interactions, and extracellular matrix remodeling drives shifts in glycolytic, lipid, and mitochondrial metabolism. Understanding these microenvironment-driven metabolic adaptations is essential for identifying new diagnostic and therapeutic strategies for HCC. A range of experimental models has been developed to recapitulate the TME, including 2D cocultures, 3D spheroids and organoids, scaffold-based systems, and microfluidic organ-on-chip platforms, each offering different trade-offs between physiological relevance and experimental control. However, most conventional metabolic assays used with these models are destructive or lack the spatial resolution needed to capture metabolic heterogeneity within complex, multicellular tumor architectures. Fluorescence lifetime imaging microscopy (FLIM), which enables label-free, real- time assessment of cellular metabolic states via NAD(P)H and FAD autofluorescence, offers a particularly promising approach for resolving spatially heterogeneous metabolic reprogramming within TME models and may provide new insight into HCC metabolic plasticity and potential therapeutic targets. Funding: The work was carried out with financial support from the Russian Science Foundation, grant number 25-45-20013 (Sechenov University); the Iranian National Science Foundation (INSF), grant number 4046298

Oral Presentation

Os-1: Novel Application of Endometrial Stem Cells A Suitable Approach for Parkinson's Disease Therapy in An Animal Model

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Background: Endometrial stem cells represent a new cell source for neurodegenerative diseases, which is abundant and can be easily isolated by a simple, safe, and painless procedure.
Materials and Methods: In this study, the effects of endometrial stem cells (intranasally) in the mouse model of Parkinson's disease (PD) was assessed using intrastriatal injection of 6-OHDA. After 30 days, endometrial stem cells were injected intranasally in three doses (10^4, 5×10^4 and 10^5 cells μl^{-1}). During four months after treatment, the behavioral tests were done. Then, the animals were sacrificed and the fresh section of the substantia nigra pars compacta (SNpc) was isolated for detection of stem cells-GFP labeled by fluorescence microscopy method. Also, to assessment of GFP, human neural Nestin, and tyrosine hydroxylase (TH) markers in the fixed brain tissues (SNpc) immunohistochemistry were done.
Results: The results indicated that behavioral parameters were significantly improved after cell therapy. Fluorescence microscopy assay in fresh tissue and GFP analysis in fixed tissue were showed that the stem cells-GFP labeled migrated to SNpc. The data from immunohistochemistry showed that the Nestin as a differential neuronal biomarker was expressed in SNpc. Furthermore, TH as a dopaminergic neuron marker significantly increased after cell therapy.
Conclusion: Finally, our study indicated that intranasal administration of endometrial stem cells improve the PD symptoms in the animal model of PD dose-dependent manner (optimum dose: 5×10^4) as a noninvasive method.
Keywords: Endometrial Stem Cells, Intranasal Delivery, Parkinson's Disease, Dopaminergic Neurons

Os-2: Development and Characterization of Bionanocomposite Films Based On Carboxymethyl Kappa-Carrageenan Reinforced with Curcumin Loaded Halloysite Nanotubes for Wound Dressing Applications

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Background: The composition of natural polysaccharides extracted from different types of algae and the preparation of biofilms from them have shown great potential for wound healing due to their biocompatibility and biodegradability. Poor mechanical properties are one of the main problems of these films.
Materials and Methods: In the current project, kappa-carrageenan (KG) was chemically modified and combined with sodium alginate and halloysite nanotubes, with different weight/weight concentrations were used to improve the physicochemical properties of the film. FT-IR confirmed the successful carboxymethylation of KG. These films were characterized using FT-IR, XRD, and scanning electron microscopy (SEM).
Results: Nanocomposite film (CMKG-SA-Col/HNT-Cur) showed the highest mechanical properties with tensile strength (11 MPa) and degree of swelling in laboratory conditions for 48 days (2395.41%) as the optimal sample. The loading of curcumin in Hallosite nanotubes caused slow release and reduced side effects, which increased antioxidant and antibacterial properties and increased cell viability of HFF fibroblast cells in the MTT test up to a concentration of 6% w/w in the wound site of the skin tissue.
Conclusion: The obtained results indicate the high application potential of this prepared film in wound dressing, controlled drug release and other industries.
Keywords: Sodium Alginate, Carboxymethyl Kappa-Carrageenan, Curcumin, Antibacterial

Os-3: Overcoming the Maturation Brake in Human Brain Organoids: Computational Targeting of YAP–TEAD Activity

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Background: Human brain organoids provide a tractable system for modeling early neurodevelopment, but persistent fetal-like transcriptional programs remain a barrier to studying mature neural circuits. Identifying regulatory programs maintaining these low-maturation states could enable strategies to improve maturation. Here, we investigated whether Yes-associated protein (YAP)–transcriptional enhanced associate domain (TEAD) signaling, a core effector of the Hippo pathway, represents a druggable maturation-brake axis.
Materials and Methods: Public single-cell RNA sequencing (scRNA-seq) datasets (GSE75140, GSE129519) were analyzed to compare organoid and fetal brain transcriptional states. Following differential expression analysis, candidate genes were prioritized by cross-referencing their profiles with established transcriptional signatures for neurodevelopmental maturation, progenitor maintenance, cellular stress, cell-cycle regulation, and YAP–TEAD and Wingless/Integrated (WNT)–T-cell factor (TCF) signaling. Machine-learning models (Random Forest, Extreme Gradient Boosting [XGBoost]) identified features associated with low-maturation states. Targets were refined via protein–protein interaction networks and independent validation. Computational perturbation simulated partial suppression of candidate programs, evaluating predicted effects on low-maturation scores. Subsequently, 2,676 ligand-bound TEAD3 structures were used for structure-based virtual screening. The top-ranked compound was evaluated by molecular docking, in-

teraction profiling, drug-likeness, and cytotoxicity predictions.
Results: YAP–TEAD activity was enriched in low-maturation/progenitor-like states, emerging as a stronger perturbation target than WNT–TCF or cell-cycle programs. Virtual screening prioritized PubChem CID 172643419 as the top TEAD3 candidate (MolDock: -154.111, AutoDock Vina: -10.73 kcal/mol). This compound outperformed co-crystallized reference ligands WCF/IAG933 (-143.241), KMU/CPD4 (-117.292), and KNE/CPD2 (-91.015). Interaction profiling revealed one hydrogen bond with Lys274 and stabilizing contacts with Thr395, Val415, and Glu417. The candidate showed zero Lipinski violations, no Pan Assay Interference Compounds (PAINS) or Brenk alerts, and no strong predicted cytotoxicity.
Conclusion: This study links single-cell organoid analysis to structure-based inhibitor prioritization, nominating YAP–TEAD activity as a computationally actionable maturation-brake axis. PubChem CID 172643419 is prioritized as a lead-like TEAD3 candidate for experimental validation.
Keywords: Brain Organoid, YAP–TEAD Signaling, Machine Learning, Neuronal Maturation, Virtual Screening

Os-4: Safety and Efficacy of Intradermal Injection of Human Umbilical Cord Blood Serum-Derived Extracellular Vesicles in Chronic Plaque Psoriasis: A Phase I Clinical Trial

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Background: Chronic plaque psoriasis is a persistent inflammatory skin disorder with limited response to conventional therapies and significant side effects. Human umbilical cord blood serum-derived extracellular vesicles offer a cell-free, immunomodulatory approach for tissue repair. However, clinical data on optimal dosing are lacking.
Materials and Methods: Extracellular vesicles were isolated from human umbilical cord blood serum by ultracentrifugation and characterized by dynamic light scattering, scanning electron microscopy, flow cytometry for CD9, CD63 and CD81, and BCA protein assay. In a split-body phase I trial, 33 patients received three monthly intradermal injections of extracellular vesicles in one target lesion and either placebo in 16 patients or topical clobetasol in 17 patients in the contralateral lesion. Follow-up lasted 4 months after the first injection. Outcomes included transepidermal water loss (TEWL), elasticity measured by cutometry, skin thickness assessed by high-frequency ultrasound, Target Lesion Assessment Scale (TLAS) score, histology with H&E staining, and immunohistochemistry for Ki-67 and interleukin-6 (IL-6).
Results: Isolated extracellular vesicles had a mean size of 225.3 nm (polydispersity index=0.335), a protein concentration of 19.33 mg/mL, and positive expression of CD9 (51.6%), CD63 (54.6%) and CD81 (48.4%). The 200 μg/cm² dose showed superior efficacy: significant reduction in TEWL by 21.0 g/m²/h versus placebo ($P < 0.001$), increased elasticity (R2 +0.28, $P < 0.001$), reduced total skin thickness by 240.7 μm (Spearman's $\rho=-0.70$, $P < 0.001$), and improvement of TLAS score from 7.8 to 1.5 ($P < 0.001$). Ki-67 and IL-6 expression decreased by 70% and 25% respectively ($P < 0.05$). No serious adverse events occurred. Four patients in the 50 μg group showed no response. The 200 μg dose was superior to both placebo and topical corticosteroid.
Conclusion: Three monthly intradermal injections of human umbilical cord blood serum-derived extracellular vesicles at 200 μg/cm² are safe and effective for resistant plaque psoriasis, significantly reducing keratinocyte hyperproliferation and local inflammation. This dose-finding study supports further phase II and phase III trials.
Keywords: Psoriasis, Umbilical Cord Blood Serum, Extracellular Vesicles, Phase 1 Clinical Trial

Os-5: Osteogenic Differentiation of Human Mesenchymal Stem Cells on Functionalized Decellularized Cellulose Scaffold Derived from Calotropis Procera Using Osteoinductive Peptides

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Background: Bone tissue engineering depends on biomaterial scaffolds that support cell attachment, proliferation, and differentiation into bone-forming cells. Plant-derived cellulose scaffolds are increasingly explored due to their natural abundance, biocompatibility, and porous structure; however, their limited osteoinductive capacity restricts their direct application. Functionalization with osteogenic peptides offers a promising strategy to enhance their biological performance and improve bone regeneration potential.
Materials and Methods: Decellularized cellulose scaffolds were prepared from Calotropis procera leaves through detergent and mild alkaline treatment, followed by washing, sterilization, and lyophilization to preserve porosity. Osteoinductive peptides were covalently immobilized onto the scaffold surface using EDC/NHS coupling chemistry. Scaffold morphology and surface properties were analyzed using SEM, FTIR spectroscopy, and contact angle measurement. Human adipose-derived mesenchymal stem cells (hMSCs) were seeded onto native and functionalized scaffolds and cultured under standard conditions. Cell viability and proliferation were assessed using the MTT assay, while osteogenic differentiation was evaluated using Alizarin Red staining for calcium deposition.
Results: SEM analysis confirmed a highly porous interconnected structure suitable for cell attachment. FTIR and contact angle results verified successful peptide functionalization and increased hydrophilicity. hMSCs cultured on peptide-functionalized scaffolds showed significantly improved adhesion, viability, and proliferation compared to non-functionalized scaffolds. Alizarin Red staining demonstrated enhanced mineralization and greater osteogenic differentiation in the modified group.
Conclusion: The findings indicate that peptide functionalization substantially enhances the bioactivity of Calotropis procera-derived cellulose scaffolds. Improved hydrophilicity and biofunctional surface cues likely contributed to better cell attachment and osteogenic induction. Overall, the combination of

a natural cellulose matrix and osteoinductive peptide modification provides a promising, sustainable, and effective platform for bone tissue engineering applications.
Keywords: Calotropis Procera, Cellulose Scaffold, Bone Tissue Engineering, Osteoinductive Peptide

Os-6: Boosting Wound Healing with The Secretome of Glibenclamide-Primed Adipose-Derived Mesenchymal Stem Cells

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Background: Chronic wounds remain a major clinical challenge due to impaired and prolonged healing processes. Mesenchymal stem cells (MSCs), particularly adipose-derived MSCs (Ad-MSCs), have demonstrated considerable regenerative potential, primarily through the secretion of bioactive paracrine factors. Glibenclamide, a sulfonylurea drug with immunomodulatory and anti-inflammatory effects, has been reported to influence MSC biological functions and secretory activity. Conditioned medium (CM) derived from MSCs represents a promising cell-free therapeutic approach that may overcome several limitations associated with cell-based therapies, including safety concerns, regulatory challenges, and manufacturing costs. Therefore, this study aimed to evaluate the wound-healing efficacy of CM obtained from glibenclamide-preconditioned Ad-MSCs (Gly-Ad-MSCs) in a full-thickness excisional wound mouse model.
Materials and Methods: Ad-MSCs were isolated from adipose tissue and characterized by flow cytometry and trilineage differentiation assays. Then, they were preconditioned with glibenclamide, and CM (Gly-Ad-MSCs) was collected after 48 hours. CM from untreated Ad-MSCs was also prepared. CM were concentrated using Amicon Ultra centrifugal filters, and used for subsequent *in vivo* experiments. Full-thickness excisional wounds were created on the dorsal skin of male Balb/c mice. Animals were randomly assigned to three groups (n=15/group): Gly-Ad-MSCs CM, Ad-MSCs CM, and PBS control. Treatments were administered by subcutaneous injection around the wound margins immediately after wound creation.
Results: Gly-Ad-MSCs CM significantly accelerated wound healing from day 7 onward compared with Ad-MSCs CM and PBS-treated. By day 14, the residual wound diameter was approximately 2 mm in the Gly-Ad-MSCs CM group compared with 5 mm in the PBS group. Histological evaluation revealed reduced inflammatory cell infiltration, enhanced re-epithelialization, greater collagen deposition, increased neovascularization, and a higher number of regenerated hair follicles in the Ad-MSCs CM treated wounds compared with both control groups. Gene expression analysis demonstrated significantly increased expression of angiopoietin-1 and TGF-β in the Gly-Ad-MSCs CM group relative to the PBS group ($P<0.05$).
Conclusion: Glibenclamide preconditioning appears to enhance the therapeutic efficacy of Gly-Ad-MSCs CM, resulting in accelerated wound repair and improved tissue regeneration. Gly-Ad-MSCs CM may represent a promising and scalable cell-free approach for the management of chronic wounds.
Keywords: Wound Healing, Glibenclamide, Adipose-Derived

Os-7: Evaluation of The Effect of Micropatterns on The Gene Transfer Efficiency to The Dental Pulp Stem Cells

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Background: Non-viral gene delivery has attracted considerable attention as a promising therapeutic approach due to its favorable clinical safety profile, ease of application, and reduced risk of immunogenic responses. However, low transfection efficiency remains a major limitation of these platforms. Most established strategies to enhance transfection efficiency have focused on chemical modification of gene delivery vectors or increasing the amount of transgene payload, approaches that frequently lead to elevated cytotoxicity. This highlights the need for alternative strategies to improve gene transfer efficiency while minimizing cytotoxic effects.
Materials and Methods: This study investigates how microscale patterning of biocompatible hydrogels modulates cellular behavior and non-viral transfection efficiency. To this end, smooth flat alginate hydrogels were fabricated using a 2% (w/v) alginate solution cross-linked with 100 mM calcium chloride via the "egg-box" model, providing an inherently non-adhesive substrate and forming a stable 3D network with high water content. Following surface activation, microscale extracellular matrix (ECM) patterns with grating and circular geometries were generated on the hydrogel surface via micro-contact printing. Physicochemical characterization of the hydrogels and patterns was performed using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy-dispersive X-ray analysis (EDAX). Next, human dental pulp stem cells (hDPSCs) were selectively cultured on the ECM-printed patterns. Cellular behavior was evaluated by nuclear staining using DAPI and actin cytoskeleton staining with phalloidin to assess cell morphology, nuclear orientation, and cytoskeletal organization. Gene transfection was then performed using an EGFP-encoding plasmid and the lipid-based transfection reagent Lipofectamine LTX. Transfection efficiency was assessed 48 hours after transfection qualitatively and quantitatively using fluorescence microscopy and flow cytometry, respectively.
Results: The results demonstrated that ECM surface patterns effectively guided cells and nuclear morphology and promoted directional organization of the actin cytoskeleton. The control group exhibited transfection efficiencies of 10–18%. In contrast, patterned substrates markedly improved gene delivery, with efficiencies reaching approximately 67% on grating patterns and 59% on circular patterns, corresponding to a three- to six-fold enhancement over non-patterned conditions.
Conclusion: Overall, these findings indicate that microscale surface topography engineering of hydrogel substrates can serve as a simple, safe, and cost-effective strategy to enhance non-viral gene transfection while modulating cellular behavior.
Keywords: Non-Vviral Gene Delivery, Dental Pulp Stem Cells, Micropatterning, Alginate Hydrogel

Os-8: Network Pharmacology-Based Analysis of The Anti-

Aging Effects of Quercetin and Melatonin Combination

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Background: Aging is a multifactorial biological process regulated by interconnected molecular pathways. Single-target therapies are often insufficient to modulate the systemic nature of aging, highlighting the need for multi-target approaches. Network pharmacology enables systems-level analysis of drug–target interactions and molecular networks to elucidate the mechanisms of combinatorial anti-aging therapies.
Materials and Methods: Structures of compounds were retrieved from the PubChem database. Potential targets were predicted using the PharmMapper and SwissTargetPrediction servers, and target identifiers were converted to official gene symbols using the UniProt database. Aging-related genes were collected from the CellAge, GenAge, LongevityMap, and Aging Atlas databases, with 1894 individual genes. Additionally, differentially expressed genes (DEGs) between young and aged samples across more than 14 tissues (3579) were obtained from GTEx project analyses. Overlapping genes between compound targets and aging-associated genes were identified using molbiotools.com. Protein-Protein Interaction (PPI) network analysis conducted by Cytoscape3.10.3, and functional enrichment analyses were performed using the DAVID database (both FDR and $P<0.05$), considered statistically significant.
Results: A total of 20 shared genes were identified through comparative analysis. Functional enrichment analysis revealed 59 significantly enriched KEGG pathways and 3 biological processes, including the insulin resistance pathway (hsa04931) and the insulin receptor signaling pathway (GO:0008286) respectively. PPI network and hub gene analysis highlighted MAPK1, AKT2, CDC42, GSK3B, and ABL1 as key nodes, suggesting their central roles in mediating the anti-aging effects of quercetin and melatonin.
Conclusion: Quercetin and melatonin, in combination, may modulate insulin-associated aging-related pathways, suggesting a potential regulatory role of insulin signaling in the anti-aging effects of these compounds. However, these findings are based on bioinformatics predictions and require further experimental validation to clarify their biological and therapeutic relevance.
Keywords: Quercetin, Melatonin, Anti-Aging Drugs, Network Pharmacology

Os-9: Development of A PTFE-Based Ultrasonic-Welded Encapsulation Macrodevice for Immunoprotected Islet Cell Therapy in Type 1 Diabetes

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Background: Type 1 diabetes is characterized by autoimmune destruction of pancreatic β-cells, requiring lifelong insulin replacement therapy. Islet encapsulation has emerged as a promising cell-based strategy for immunoprotection of transplanted cells; however, current devices are often limited by complex fabrication methods, inadequate mechanical stability, and fibrotic responses following implantation. In this study, Polytetrafluoroethylene (PTFE), a polymer known for its biocompatibility and use in FDA-approved medical applications, was used to fabricate a mechanically stable encapsulation device via ultrasonic welding for potential subcutaneous implantation in diabetes models.
Materials and Methods: Flat-sheet encapsulation devices were fabricated from PTFE membranes with a 0.45 μm pore size, allowing diffusion of glucose, oxygen, and small molecules while limiting immune cell infiltration. Device pouches were sealed using ultrasonic welding to achieve a reproducible and mechanically stable architecture. Mechanical properties of the welded regions were evaluated using ASTM tensile testing, while seal integrity was assessed with dye penetration testing to detect potential leakage or interfacial defects. As an initial immunoprotective barrier, MIN6 insulin-secreting cells were suspended in alginate hydrogel before loading into the device through a designated injection port. *In vitro* performance was evaluated over three days post-encapsulation using live/dead staining assays.
Results: Tensile testing demonstrated that ultrasonic welding generated uniform and mechanically stable seals, while the PTFE membrane exhibited a tensile strength of 9.22 MPa, exceeding the mechanical requirements for subcutaneous implantation. Dye penetration assays confirmed robust seal integrity with no detectable leakage. Encapsulated MIN6 cells maintained high viability (>80%) and preserved normal cellular morphology throughout the culture period, indicating favorable short-term cytocompatibility and adequate nutrient diffusion through the PTFE membrane.
Conclusion: This study demonstrates the successful development of a PTFE-based ultrasonically welded encapsulation device with favorable mechanical stability and promising short-term cytocompatibility. These findings support its potential application as a platform for subcutaneous transplantation of pluripotent stem cell-derived islets in type 1 diabetes models.
Keywords: Type 1 Diabetes, Cell Therapy, Encapsulation, Macrodevice

Os-10: Establishing An Accessible and Scalable Platform for Generating Human Blastoids from Naïve t2iLGö hESCs

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Background: Stem cell-based embryo models are alternative platforms for studying early embryonic development and implantation while mitigating ethical concerns and the limited accessibility of natural embryos. Various embryo models, including blastoids, have been developed to facilitate the study of embryogenesis; however, their broad application and scalability may be limited by reliance on high-cost specialized culture systems, such as Aggrewell plates, Gri3D plates, and hydrogel microwell plates. Here, we set up a more accessible culture system for generating blastoids using naïve human embryonic stem cells (hESCs).
Materials and Methods: To generate human blastoids, we first converted primed hESCs, Royan H6, to a naïve state using the t2iLGö cocktail, which includes MEK, protein kinase C, Tankyrase, and GSK3β inhibitors, along with hLIF. Next, t2iLGö cells were cultured at a density of 220 cells/mm² in non-treated culture dishes under 5% O_2 conditions using a medium containing ROCK inhibitor. The resulting aggregates were directed toward the trophectoderm lineage by inhibiting the MEK, TGF-β and Rho-kinase pathways for 48 hours, followed by continuous culture in medium containing ROCKi.
Results: Successful conversion of primed hESCs to a naïve state was confirmed by their dome-shaped colony morphology, elevated expression of naïve-specific markers such as KLF17, KLF4, NANOG, and TBX3 using qRT-PCR, and detection of STAT3 and TFCP2L1 via immunostaining. t2iLGö cells formed aggregates within 48 hours in non-treated dishes. After three days of induction, cystic structures averaging 100 µm in diameter were observed, featuring a distinct inner cell mass-like compartment surrounded by a TE-like outer layer. The similarities between t2iLGö-blastoids and their natural counterparts, blastocysts, were analyzed by examining the expression and localization of trophectoderm markers GATA3 and CDX2, as well as epiblast markers OCT4 and NANOG. Additionally, cultivating 10-day-old t2iLGö-blastoids under conditions for hESC derivation resulted in hESC-like outgrowths within 2 days, showing greater similarity in potential and structure to human blastocysts.
Conclusion: Our study provides a reproducible, scalable, and cost-effective method for generating human blastoids from hESCs in non-treated culture plates, offering a more accessible platform for embryo modeling research.
Keywords: Embryo Model, Blastoid, Human Blastocyst, Naïve Human Embryonic Stem Cell

Os-11: Umbilical Cord Serum Enhances Expansion and Cytotoxicity of CIK Cells

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Background: Cytokine-induced killer (CIK) cells represent a promising cell-based immunotherapy due to their potent antitumor activity and MHC-unrestricted cytotoxicity. *Ex vivo* expansion of CIK cells conventionally relies on fetal bovine serum (FBS)-supplemented media; however, animal-derived origin of FBS raises concerns regarding safety, reproducibility, regulatory compliance, and clinical applicability. To address this challenge, we evaluated umbilical cord serum (UCS) as a potential human-derived alternative for FBS in CIK cell culture.
Materials and Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors using Ficoll-Paque density-gradient centrifugation and cultured in RPMI-1640 medium supplemented with cytokine cocktail and either FBS or UCS. Following 10 days of *ex vivo* culture, cell morphology, viability, expansion rate, immunophenotype, and cytotoxic activity were assessed. To evaluate cytotoxic activity, expanded CIK cells were co-cultured with SKOV3 ovarian cancer cells at an effector-to-target (E) ratio of 10:1.
Results: CIK cells cultured in UCS-supplemented medium exhibited a higher proliferation rate at day 10 (150%) compared with the FBS group (120%). Although total cell counts were consistently higher in UCS cultures, the difference did not reach statistical significance. Furthermore, UCS cultures maintained sustained growth through day 10, whereas expansion in FBS cultures declined after day 7. Immunophenotypic analysis revealed distinct cellular compositions between culture conditions. In UCS cultures, $CD3^+CD56^-$, $CD3^+CD56^+$, and $CD3^-CD56^+$ populations accounted for 63%, 8%, and 6% of cells, respectively. In FBS cultures, the corresponding populations represented 53%, 19%, and 5%, respectively. In addition, NKG2D expression increased in UCS-cultured cells, whereas no significant expression was observed in the FBS group. Functional assessment against SKOV3 target cells (E: T=10:1) demonstrated enhanced cytotoxic activity in the UCS group, with approximately 20% killing activity compared with less than 10% in the FBS group.
Conclusion: UCS effectively supports the *ex vivo* expansion and functional activity of CIK cells and may represent a promising human-derived alternative to FBS. The use of UCS could improve the clinical translatability of CIK cell manufacturing by reducing reliance on animal-derived culture supplements.
Keywords: Cytokine-Induced Killer Cells, Umbilical Cord Serum, Fetal Bovine Serum, Cancer Immunotherapy

Poster Presentations

Ps-1: Integrative Analysis of TCGA-LIHC Reveals GADD45B as A Potential Downregulated Gene in Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is a common primary liver cancer with complex molecular alterations. Downregulated tumor-suppressive genes may clarify hepatocarcinogenesis and support biomarker discovery. This study identified significantly downregulated protein-coding genes in The Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) and prioritized candidates linked to apoptosis and Epithelial–Mesenchymal Transition (EMT).
Materials and Methods: RNA-seq expression data and corresponding clinical information for liver hepatocellular carcinoma were obtained from TCGA-LIHC through the Genomic Data Commons (GDC) portal. The final dataset included 421 samples, comprising 371 primary tumor tissues and 50 solid normal liver tissues. Differential gene expression analysis was performed in R using GDCRNATools. After removal of duplicate and non-relevant samples, normalization was conducted using gdcVoomNormalization, followed by Limma-based gdcDEAnalysis. Genes with $P < 0.05$ and $|\log2 \text{ fold-change}| \geq 1$ were considered differentially expressed. Downregulated genes were defined as protein-coding genes with log2 fold-change ≤ -2 and $P < 0.05$. HCC-associated genes were retrieved from NCBI Gene using "hepatocellular carcinoma", limited to protein-coding genes in Homo sapiens. Hallmark gene sets related to EMT and apoptosis were obtained from the Molecular Signatures Database/Gene Set Enrichment Analysis (MSigDB/GSEA). Venn diagram analysis identified overlapping genes among downregulated TCGA-LIHC genes, NCBI HCC-associated genes, and selected Hallmark gene sets. Candidate genes were then evaluated in relation to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with HCC.
Results: Differential expression analysis identified 2,123 genes with significantly altered expression in TCGA-LIHC, including 429 downregulated protein-coding genes. A total of 3,261 HCC-associated protein-coding genes were retrieved from *NCBI* Gene. The selected Hallmark gene sets included 200 EMT-related genes and 161 apoptosis-related genes. Integrative overlap analysis identified two common genes, *DCN* and *GADD45B*. To determine which gene was specifically implicated in HCC-related molecular pathways, the identified candidate genes were further examined using KEGG pathway analysis. This analysis revealed that GADD45B was involved in the p53 signaling pathway.
Conclusion: This integrative bioinformatics analysis identified GADD45B as a candidate downregulated gene linked to HCC-associated molecular pathways, particularly apoptosis and tumor progression. These findings suggest that GADD45B may represent a biologically relevant candidate for further investigation in hepatocellular carcinoma.
Keywords: Hepatocellular Carcinoma, TCGA-LIHC, Downregulated Genes, GADD45B

Ps-2: Intranasal Administration of Dental Pulp Stem Cell Secretome Improves Cognitive Deficits in A Rat Model of Sporadic Alzheimer's Disease

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Background: Cell-based therapies have shown promise for neurodegenerative diseases, particularly Alzheimer's disease (AD). The secretome collected from stem cells offers a less invasive and cost-effective alternative to direct cell transplantation. The intracerebroventricular (ICV) streptozotocin (STZ) model replicates key features of sporadic AD, including cognitive decline and metabolic dysfunction. This study aimed to evaluate the effect of intranasal administration of human dental pulp stem cell-derived secretome (DPSC-Sec) on cognitive function and the expression of SIRT1 and miR-134 in an STZ-induced rat model of AD.
Materials and Methods: Male Wistar rats (n=6/group) were divided into three groups: (1) PBS control, (2) STZ model (3 mg/kg, ICV), and (3) STZ + intranasal DPSC-Sec. On day 1, stereotaxic surgery was performed for ICV STZ injection. The secretome was collected from cultured human dental pulp stem cells after 48 hours of serum-free conditioning and concentrated 25-fold. On day 2, rats received a single intranasal dose of DPSC-Sec (10 μL/rat) following hyaluronidase pretreatment. Novel Object Recognition (NOR) testing was conducted on day 14 post-surgery. Rats were sacrificed on day 17, and hippocampal tissue was collected for real-time PCR analysis of *SIRT1* and *miR-134* (*β-actin* as housekeeping gene) and H&E staining. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with significance set at $P < 0.05$.
Results: STZ administration impaired NOR performance compared to controls ($P < 0.01$). Intranasal DPSC-Sec treatment improved recognition memory ($P < 0.05$ vs STZ). STZ downregulated SIRT1 and upregulated miR-134 expression in the hippocampus (both $P < 0.05$). DPSC-Sec treatment partially reversed these changes, restoring *SIRT1* and reducing *miR-134* levels (both $P < 0.05$ vs STZ). H&E staining revealed reduced neuronal damage in the treatment group.
Conclusion: A single intranasal dose of DPSC-derived secretome improved cognitive deficits and modulated key hippocampal genes (*SIRT1* and *miR-134*) in STZ-treated rats, suggesting potential as a non-invasive, cell-free therapeutic approach for sporadic AD.

Keywords: Alzheimer's Disease, Dental Pulp Stem Cells, Secretome, Intranasal Delivery

Ps-3: Rgd Immobilization on Carboxyl Surface-Modified Electrospun Poly-E-Caprolactone Vascular Scaffolds Reduces Blood Clotting and Platelet Adhesion Under Blood Flow within A Perfusion Bioreactor

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Background: Electrospun poly-ϵ-caprolactone (PCL) vascular scaffolds exposed to blood trigger platelet adhesion, coagulation activation, and thrombus formation. Endothelialization creates an antithrombotic interface. However, the hydrophobicity of PCL vascular grafts limits endothelialization. Previously, we showed that carboxyl (COOH) or amine (NH2) surface-modification followed by arginine-glycine-aspartate (RGD)-immobilization enhances endothelialization of electrospun PCL scaffolds. However, whether COOH or NH2 surface-modification followed by RGD-immobilization is most effective to reduce blood clotting and platelet adhesion under blood flow is unknown. We aimed to test whether COOH or NH2 surface-modification of electrospun PCL vascular scaffolds followed by RGD-immobilization most effectively reduces blood clotting and platelet adhesion under blood flow.
Materials and Methods: Hollow tubular PCL scaffolds (ø: 4 mm, length: 30 mm, thickness: 0.26 mm) were electrospun (30 kV; feed rate: 1.5ml/h; distance: 15 cm) using 13% (wt/wt) PCL in ¼ (v/v) acetic acid/formic acid. PCL scaffolds were surface-functionalized by COOH or NH2 functional groups using 3 M NaOH or 10% (wt/v) 1,6-hexamethydiamine. RGD was immobilized on surface-functionalized PCL scaffolds. Platelet adhesion and blood clotting index (BCI) were assessed to evaluate scaffold thrombogenicity. (Non)-endothelialized electrospun PCL scaffolds modified with COOH or NH_2 and immobilized with RGD were exposed to fresh human whole blood and platelet-rich plasma (PRP; Iranian Blood Transfusion Organization). Platelet adhesion was determined from the platelet count in PRP after blood exposure to the scaffolds. BCI was assessed from hemoglobin release following red blood cell lysis after scaffold contact.
Results: Without endothelialization, platelet adhesion was increased on PCL surface-modified with COOH (1.7-fold, $P < 0.0001$) or NH2 (1.6-fold, $P < 0.0001$) followed by RGD-immobilization compared to unmodified PCL. BCI was decreased on PCL surface-modified with COOH (0.3-fold; $P < 0.0001$) or NH2 (0.3-fold, $P < 0.0001$) followed by RGD-immobilization. Endothelialization of PCL decreased platelet adhesion (COOH: 0.2-fold; NH2: 0.3-fold, $P < 0.0001$) and increased BCI (COOH: 12.4-fold; NH2: 11.2-fold) in surface-modified PCL followed by RGD immobilization. COOH-modified and RGD-immobilized PCL scaffolds showed lowest platelet adhesion ($3.5 \pm 0.42\%$, mean ± SD, n=3) and highest BCI ($75.2 \pm 5.06\%$, n=3).
Conclusion: RGD immobilization on COOH surface-modified electrospun PCL scaffold was superior to NH2 surface-modified scaffolds in reducing blood clotting and platelet adhesion under blood flow, and is therefore more promising for in-vivo vascularization.
Keywords: Amine Functional Group, Anti-Thrombotic Effect, Carboxyl Functional Group, RGD

Ps-4: Alginate-Gelatin Hydrogel Microcapsulation for Generation Breast Cancer Tumoroids

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Background: In recent years, the development and fabrication of novel hydrogels for mimicking the 3D tumor niche are at the center of attention. Here, breast cancer tumoroids were generated using ionic cross-linking of alginate-gelatin hydrogel.
Materials and Methods: Three different cell lines, including HUVECs, HFFF-2, and MDA-MB-231 cells, were used for induction of tumoroids. Using hanging drop method, 5×10^4 cells (at a ration of 2: 1: 1) were resuspended in 25 µl methylcellulose and tumoroid system was generated. In another method, the same cell density was blended with alginate-gelatin solution (1×10^6 per 1 ml) and dropped into 100 mM $CaCl_2$ solution to solidify. The stability and integrity of tumoroids system induced by both systems under bright-field microscopy.
Results: Data indicated loosely cell-to-cell connection in tumoroids induced by hanging drop containing separate small-sized aggregates without whole mass integrity. Compared to tumoroids induced by hanging drop, microencapsulation of cells within the alginate-gelatin microspheres yielded unique multicellular units with suitable integrity in which cells were uniformly distributed inside the microspheres.
Conclusion: Ionic cross-linking of tumor cells with other cell types inside the alginate-gelatin hydrogel provide valuable platform for induction of complex and stable tumor system.
Keywords: Tumoroids, Dropping, Ionic Cross-Linking, Microencapsulation, Integrity

Ps-5: NK-92 Extracellular Vesicles: A Safer Alternative to NK-92 Cells with Reduced Cytokine Storm Risk

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Background: NK-92 cells are promising candidates for cancer immunotherapy due to their potent anti-tumor activity. However, their clinical application is limited by the risk of cytokine release syndrome (cytokine storm), a potentially life-threatening adverse event caused by excessive secretion of pro-inflammatory cytokines such as GM-CSF, IFN-γ, and IL-6. Extracellular Vesicles (EVs) derived from NK cells have recently emerged as cell-free therapeutic platforms that may retain anti-tumor effects while avoiding the drawbacks of live cell therapy. This study aimed to compare the cytokine secretion profile of NK-92-derived EVs versus intact NK-92 cells upon exposure to Raji and Jurkat target cells, with the hypothesis that EVs induce a significantly lower inflammatory response.
Materials and Methods: NK-92 cells were characterized by flow cytometry for $CD3^{-}/CD56^{+}$ phenotype. EVs were isolated from NK-92 culture supernatants by ultracentrifugation and characterized using Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM), and BCA protein assay. For NK-92-derived EVs, 60 μg/mL of total protein was used, while intact NK-92 cells were co-cultured with target cells at an effector-to-target (E:T) ratio of 1:1. These were then co-cultured with Raji (Burkitt's lymphoma) and Jurkat (T-cell leukemia) cell lines. After 24 hours, culture supernatants were collected and levels of GM-CSF, IFN-γ, and IL-6 were measured by ELISA.
Results: NTA and TEM confirmed successful EV isolation with typical size range (50–150 nm) and cup-shaped morphology. BCA allowed accurate protein normalization. ELISA Results demonstrated that intact NK-92 cells secreted high levels of GM-CSF, IFN-γ, and IL-6 in response to both Raji and Jurkat cells, indicating a robust pro-inflammatory response. In striking contrast, NK-92-derived EVs produced significantly lower levels of all three cytokines across both target cell lines ($P < 0.05$ for all comparisons). The reduction was consistent and substantial, suggesting that EVs trigger a much weaker cytokine response compared to parental cells.
Conclusion: The markedly reduced cytokine secretion profile of NK-92-derived EVs compared to intact NK-92 cells indicates a lower risk of cytokine storm, which is a major safety advantage for potential clinical applications. Therefore, NK-92-derived EVs may represent a safer cell-free alternative to live NK-92 cell therapy, potentially providing anti-tumor effects with minimized systemic inflammatory side effects. Further studies are warranted to evaluate their direct cytotoxic efficacy.
Keywords: NK-92 Cells, Extracellular Vesicles, Cytokine Storm

Ps-6: Comparison of The Oncolytic Effects of Herpes Simplex Virus Type 1 and Vaccinia Virus on The U87 Glioblastoma Cell Line

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Background: Glioblastoma (GBM) is the most aggressive primary malignant brain tumor in adults, marked by rapid proliferation, invasiveness, therapeutic resistance, and poor survival despite multimodal treatments like surgery, radiotherapy, and chemotherapy. Oncolytic virotherapy has emerged as a promising strategy, replicating selectively within tumor cells, directly lysing them, and stimulating antitumor immune responses. Herpes Simplex Virus Type 1 (HSV-1) and Vaccinia virus are among the most investigated oncolytic viruses, showing considerable potential in preclinical models. This study comparatively evaluates the antitumor and cytopathic effects of oncolytic HSV-1 and Oncolytic Vaccinia Virus Tiantan strain (Onco-VV-TT) in human U87 GBM cells, assessing them under both 2D monolayer and 3D spheroid culture conditions.
Materials and Methods: Human U87 GBM cells were cultured and infected with HSV-1 or Onco-VV-TT at defined MOIs to compare their oncolytic effects in-vitro. Cell viability was evaluated at 24 and 48 hours post-infection using the MTT assay. Apoptotic and necrotic cells were quantified by Annexin V/PI staining and flow cytometry. To better mimic the tumor microenvironment, multicellular spheroids were generated by the hanging-drop method to better mimic the tumor microenvironment. Viral cytopathic effects, including cell rounding, detachment, syncytia formation, and spheroid disintegration, were monitored by phase-contrast microscopy and compared across experimental groups.
Results: Both HSV-1 and Onco-VV-TT induced significant cytotoxic and oncolytic effects in U87 GBM cells over time. Viral treatment reduced tumor cell viability and increased apoptotic cell death compared to untreated controls. Furthermore, both viruses effectively disrupted the integrity and growth of 3D GBM spheroids, indicating substantial antitumor activity within tumor-like microarchitectures. Comparative observations suggested differential patterns of cytopathic activity and spheroid destruction between HSV-1 and Onco-VV-TT, highlighting their distinct mechanisms.
Conclusion: This study provides a comparative preclinical evaluation of HSV-1 and Onco-VV-TT as potential oncolytic agents against GBM. Both viruses demonstrated marked antitumor activity in conventional 2D cultures as well as in more physiologically relevant 3D spheroid models. The findings support the therapeutic potential of oncolytic virotherapy for GBM treatment and underscore the value of 3D tumor models for assessing viral efficacy in translational cancer research.
Keywords: Glioblastoma, Oncolytic Virotherapy, HSV-1, Onco-VV-TT, U87

Ps-7: Integrative Bioinformatics Analysis of Hypoxia-Treated Mesenchymal Stem Cells Identifies EGLN3 and VEGFA as Key Regulators with Implications for Cardiac Repair

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Background: Cardiovascular disease remains a leading cause of global mortality. Although mesenchymal stem cells (MSCs) hold substantial therapeutic potential for myocardial regeneration, the molecular mechanisms governing their survival and adaptation within the hypoxic post-infarct microenvironment are not yet fully understood. This study investigated the transcriptomic alterations in hypoxia-preconditioned MSCs through an integrative bioinformatics framework to identify critical regulatory genes and small-molecule candidates capa-

ble of enhancing cardiac repair.
Materials and Methods: The microarray dataset GSE20463, comprising human MSC samples cultured under normoxic (n=3) and hypoxic (n=3) conditions, was retrieved from the NCBI Gene Expression Omnibus (GEO). Differentially Expressed Genes (DEGs) were identified using the GEO2R tool with strict filtering criteria of an adjusted $P < 0.05$ and $|logFC| > 2$. Pathway enrichment analysis was subsequently performed using the Enrichr platform. To identify small molecules that can mimic the beneficial effects of hypoxia, drug repurposing screening was conducted through the LINCS L1000 database.
Results: The analysis revealed approximately 250 robustly altered DEGs in response to hypoxia. EGLN3 (logFC=4.21, P=1.2e-8) and VEGFA (logFC=3.52, P=5.6e-10) were identified as the most significantly up-regulated genes. As determined by functional annotation, these DEGs were primarily involved in the HIF-1 signaling pathway. In terms of therapeutic candidates, drug repurposing screening indicated that Phenformin and Verteporfin could effectively simulate the hypoxia-induced protective phenotype in MSCs.
Conclusion: These data demonstrate that EGLN3 and VEGFA serve as central molecular switches in the adaptation of MSCs to low-oxygen environments. Preconditioning MSCs under hypoxic conditions, or chemically stimulating these pathways using candidates like Phenformin, provides a promising strategy to optimize stem cell-based therapies for cardiac regeneration. These computational predictions offer a clear direction for subsequent experimental validation.
Keywords: Mesenchymal Stem Cell, Hypoxia, Cardiac Regeneration, Drug Repurposing, EGLN3

Ps-8: Tissue Engineering-Based Cell Therapy Approaches for Ovarian Reconstruction Following Chemotherapy

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Background: Chemotherapy-induced ovarian failure is a significant consequence of cancer treatment in women of reproductive age, leading to hormonal deficiency and irreversible infertility. While ovarian tissue cryopreservation exists, risks of malignant cell reintroduction limit its application. Tissue engineering-based cell therapy (TECT) offers a safer alternative by reconstructing functional ovarian tissue using decellularized extracellular matrix (dECM) scaffolds combined with autologous stem cells. This study investigated the potential of dECM-based scaffolds seeded with ovarian-derived mesenchymal stem cells (O-MSCs) to reconstruct ovarian tissue and restore fertility following chemotherapy-induced damage.
Materials and Methods: Ovarian dECM scaffolds were prepared through a detergent-based decellularization protocol and confirmed by DNA quantification and collagen/glycosaminoglycan (GAG) retention assays. O-MSCs were isolated, expanded, and seeded onto dECM scaffolds. Cell viability and proliferation were assessed using MTT assay and scanning electron microscopy (SEM). Constructs were transplanted into chemotherapy-damaged ovaries of rat models. Reproductive outcomes including estrous cyclicity, follicle stimulating hormone (FSH) and AMH serum levels, and pregnancy rates were evaluated over 12 weeks.
Results: Decellularized scaffolds retained native extracellular architecture with significantly reduced DNA content (<50 ng/mg dry weight). O-MSC-seeded constructs demonstrated high cell viability (>85%) at 7 days in-vitro. In-vivo transplantation restored regular estrous cycles in 70% of treated animals. Serum FSH levels decreased significantly while AMH increased, indicating improved ovarian reserve. Pregnancy was achieved in 60% of mated females in the treatment group versus 15% in controls ($P < 0.01$).
Conclusion: Tissue engineering-based cell therapy utilizing dECM scaffolds seeded with O-MSCs represents a feasible and effective strategy for ovarian reconstruction following chemotherapy. This approach offers a promising fertility preservation option for cancer survivors, warranting further investigation toward clinical translation.
Keywords: Tissue Engineering, Decellularized Scaffold, Cell Therapy

Ps-9: Combining Polymeric Scaffolds with Mesenchymal Stem Cells to Fix Damaged Ovarian Tissue

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Background: Major causes of female infertility worldwide are early ovarian insufficiency (POI) and ovarian damage from surgery or chemotherapy. Traditional therapies don only somewhat restore ovarian activity. Emerging as interesting ways to rebuild damaged ovarian tissue and restore endocrine and reproductive function are tissue engineering techniques mixing mesenchymal stem cells (MSCs) with polymeric scaffolds. The goal of this study was to ascertain how well MSC-seeded polymeric scaffolds could help to repair injured ovarian tissue and bring back follicular reserve in an experimental model.
Materials and Methods: Female Wistar rats got cyclophosphamide intraperitoneal injection to cause ovarian damage. Bone marrow provided MSCs that were identified using flow cytometry and differentiation tests. Electrospun poly (lactic-co-glycolic acid) (PLGA) scaffolds were created and MSCs were applied. The wounded ovaries received scaffold-MSC builds. Ovarian tissues were histologically examined using hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) at 4 and 8 weeks following transplantation. To assess functional recovery, follicle counts, serum anti-Müllerian hormone (AMH) levels, and oestrogen levels were quantified.
Results: MSC-seeded PLGA scaffolds greatly raised primordial and primary follicle counts above the control group ($P < 0.05$). At eight weeks, the treatment group's serum AMH and estradiol readings were noticeably higher. Histological study of scaffold-treated ovaries showed lower fibrosis and better vascularization. Reconstructed tissue IHC confirmed FSHR and CYP19A1 as ovarian markers.
Conclusion: a chemotherapy-induced ovarian damage model, MSCs and PLGA-based polymeric scaffolds work together to help repair ovarian tissue, stimulate folliculogenesis, and restore hormonal function. These Results show that MSC-scaffold structures could be used as a treatment for POI and for preserving fertility.
Keywords: Stem Cells, Polymeric Scaffold, Tissue Engineering

Ps-10: Evaluation of Doxorubicin-Loaded Deep Eutectic Solvent-Based Microemulsion Systems on The MDA-

MB-231 Cell Line

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Background: Breast cancer is one of the most prevalent malignancies among women, and its treatment, particularly in advanced stages, continues to face significant challenges, including severe side effects and limited therapeutic efficacy. Conventional administration of doxorubicin, one of the principal chemotherapeutic agents, is often associated with systemic toxicity and reduced clinical efficiency. Drug delivery systems have the potential to enhance therapeutic performance while minimizing side effects. In this regard, microemulsion-based delivery systems containing natural deep eutectic solvents (NaDES), like menthol–thymol (MT) and menthol-propionic acid (MP), may represent promising outcomes for improving drug efficacy and reducing systemic toxicity.
Materials and Methods: In this study, two microemulsion-based systems containing MT and MP were formulated. Pseudo-ternary phase diagrams were first constructed for both systems to identify the microemulsion regions. Suitable compositions within these regions were then selected for further characterization and drug loading. Droplet size and polydispersity index (PdI) of the microemulsions were determined using dynamic light scattering (DLS). Fourier-transform infrared (FTIR) spectroscopy was performed on both drug-free and drug-loaded microemulsion systems to investigate potential chemical interactions. Finally, the cytotoxic effects of doxorubicin-loaded formulations were evaluated in the MDA-MB-231 cell line using the trypan blue exclusion assay and compared with those of free doxorubicin.
Results: DLS analysis indicated that both microemulsion systems exhibited nanoscale droplet sizes with acceptable uniformity; PdI values of approximately 0.2 confirmed a homogeneous particle size distribution. FTIR spectra of both microemulsion systems and drug-loaded formulations showed no evidence of new covalent bond formation. The trypan blue exclusion assay demonstrated that the treatment with doxorubicin-loaded microemulsion systems reduced IC_{50} values compared with free doxorubicin at both 24- and 48-hour time points. Notably, the enhanced cytotoxic effect was more pronounced after 48 hours of treatment.
Conclusion: This study demonstrated that NaDES-based microemulsion systems can enhance the efficacy of doxorubicin in the MDA-MB-231 cell line, potentially enabling effective treatment at lower drug doses. Additionally, the developed systems offer advantages, including improved stability, simple preparation, lower cost, and the use of natural components, making them a promising platform for future drug delivery applications.
Keywords: Breast Cancer, Drug Delivery, Doxorubicin, Deep Eutectic Solvent, MDA-MB-231

Ps-11: Regulatory Challenges of Therapeutic Applications of Stem Cells and Genomic Data in Healthcare Systems

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Background: The growing use of stem cell and genomic technologies has opened new possibilities in regenerative medicine and the treatment of complex diseases. At the same time, the rapid expansion of these technologies has raised important concerns regarding clinical oversight, patient protection, and the use of genetic information in healthcare systems. In many cases, existing regulatory frameworks have not kept pace with recent biomedical advances, creating uncertainties in the distinction between approved therapies and experimental or commercial interventions.
Materials and Methods: This study was conducted using a descriptive-analytical approach based on library resources, international documents, and comparative review of selected regulatory models related to stem cell technologies and genomic data governance. The study focuses on identifying the main legal and regulatory challenges associated with therapeutic applications of emerging biomedical technologies.
Results: The findings show that one of the main regulatory concerns in stem cell medicine is the lack of clear criteria for distinguishing validated therapeutic procedures from research-based or commercially driven interventions. This situation may create challenges in protecting patients from unproven treatments and misleading therapeutic claims. In the field of genomic data, insufficient safeguards regarding the storage, access, and secondary use of genetic information may increase the risk of privacy violations and unauthorized use of biological data. The study also indicates that fragmented decision-making structures and the absence of specialized regulations have limited the development of an effective governance system for emerging health technologies. These findings highlight the need for a more coordinated and locally adapted model of health governance that reflects the legal structure and healthcare priorities of the country.
Conclusion: Advances in stem cell and genomic technologies require responsive regulatory frameworks and effective oversight within healthcare systems. Developing an indigenous and future-oriented model of health governance can help support biomedical innovation while protecting patients' rights, genetic data security, and public trust in emerging therapeutic technologies.
Keywords: Stem Cell Technologies, Genomic Data, Health Regulation, Health Governance, Patients' Rights

Ps-12: Beyond Autografts: Engineering High-Fidelity "Off-The-Shelf" Nerve Scaffolds through an Optimized Decellularization Paradigm

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Background: Peripheral nerve repair is currently bottlenecked by the limitations of autografts, including donor site morbidity and restricted availability. While acellular nerve grafts (ANGs) provide a biomimetic blueprint for regeneration, traditional decellularization protocols often sacrifice structural and mechanical integrity for cellular clearance. This study aimed to bridge this gap by re-engineering the classic Sondell method to develop a high-fidelity scaffold that preserves the "endoneurial highway" while ensuring maximum biocompatibility.
Materials and Methods: Sciatic nerves from Wistar rats (R-ANGs) and chickens (C-ANGs) were subjected to an Optimized Sondell Decellularization (OSD) protocol. This optimized paradigm integrated DNase-I enzymatic digestion, scavenging of residual detergents using ultrapure water (UPW), and UV-induced physical cross-linking. The engineered scaffolds were rigorously evaluated against the standard Sondell (SD) method using DNA quantification, MTT-based cytotoxicity assays with adipose-derived stem cells (ADSCs), tensile strength testing (Young's modulus), and multi-modal histological/ultrastructural analysis.
Results: The OSD paradigm redefined scaffold quality by achieving >90% DNA clearance while effectively eliminating the residual toxins that typically impede recellularization, resulting in significantly higher ADSC viability compared to SD grafts ($P < 0.001$). Biomechanically, the OSD protocol successfully restored the Young's modulus to native-like levels, ensuring the grafts can withstand the rigors of surgical suturing. Comprehensive imaging (FESEM and histology) confirmed perfectly preserved 3D architecture.
Conclusion: The OSD protocol offers a scalable and robust platform for generating "off-the-shelf" neural scaffolds. By balancing aggressive immunogenic clearance with biomechanical reinforcement, these grafts provide a superior alternative to current clinical standards, facilitating a pro-survival niche for functional nerve recovery in regenerative medicine.
Keywords: Neural Tissue Engineering, Decellularized Extracellular Matrix, Acellular Nerve Graft, Biomechanics, UV Cross-linking

Ps-13: A Hippo-Apoptosis Rheostat Index Links Tyrosine Kinase Inhibitor Response Biology to Microrna and Exosomal Regulatory Layers in Chronic Myeloid Leukemia

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Background: Chronic myeloid leukemia (CML) is a model of targeted therapy, but tyrosine kinase inhibitor (TKI) resistance and residual disease remain clinically relevant. Hippo signaling, YAP/TAZ output, apoptosis and microRNAs influence leukemic cell survival, yet their integrated behavior under TKI pressure is poorly captured by single-pathway readouts. We evaluated the Hippo-Apoptosis Rheostat Index (HARI) as a mechanistic transcriptomic summary of this balance.
Materials and Methods: Public CML mRNA, intracellular microRNA and exosomal microRNA datasets were re-analyzed. HARI was calculated from single-sample gene-set enrichment scores using four curated gene sets: Hippo tumor-suppressor arm, YAP/TAZ output, pro-apoptotic genes and anti-apoptotic genes. HARI was assessed in an imatinib-treated K562 model and three baseline CML cohorts with TKI response endpoints. Differentially expressed intracellular and exosomal microRNAs were integrated with HARI genes using target-evidence filtering, protein-protein interaction analysis and pathway enrichment.
Results: In K562 cells, imatinib shifted HARI toward a Hippo tumor-suppressor/pro-apoptotic-dominant state, although the difference was moderate and not statistically significant. Across clinical cohorts, higher baseline HARI showed directionally consistent but modest associations with favorable TKI outcomes in peripheral blood and peripheral blood mononuclear cell datasets, with area under the curve values of approximately 0.65 and 0.58. HARI showed little separation in CD34+ progenitor samples, with area under the curve approximately 0.56. Intracellular K562 analysis identified 21 deregulated microRNAs after imatinib and defined a 7-microRNA HARI-associated module. Exosomal analysis did not yield a robust module in the small discontinuation dataset, but a resistant-versus-sensitive cohort identified a distinct 7-microRNA module. These two modules did not overlap directly but converged on a 15-gene HARI hub including *YAP1, TEAD1, NF2, STK4, TAOK1, MAP4K* family genes, *BCL2L11, BIRC6 and API5,* enriched for Hippo signaling, apoptotic regulation and microRNAs in cancer.
Conclusion: HARI provides a compact, mechanism-oriented representation of Hippo-Apoptosis balance under TKI pressure in CML. Its clinical association with TKI outcome was modest and should not be interpreted as biomarker validation. However, convergence of transcriptomic, intracellular microRNA, exosomal microRNA and network layers supports HARI as a hypothesis-generating framework for functional studies of TKI persistence and rational combination strategies.
Keywords: Chronic Myeloid Leukemia, Tyrosine Kinase Inhibitor, Hippo Pathway, Apoptosis, Exosomal microRNA

Ps-14: Annotation-Refined Mirna-Target Integration Prioritizes EV-Compatible Regulatory Candidates in Type 2 Diabetes

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Background: Type 2 diabetes (T2D) involves interacting metabolic, inflammatory, vascular, endocrine and stress-response programs. MicroRNAs (miRNAs) may help coordinate these programs and are relevant to extracellular vesicle (EV)-focused regenerative research, but many public T2D miRNA reports remain difficult to interpret because mature-miRNA annotation and target-transcriptome support are inconsistent.
Materials and Methods: Public human T2D-control miRNA and mRNA datasets were curated separately. Cohorts were retained only when they supported defensible mature-miRNA or gene-level annotation. Differential-expression Results were summarized by meta-analysis, and strict mature-miRNA candidates were integrated with experimentally supported miRNA-target evidence, anti-correlated gene-level mRNA changes,

biological-axis annotation, contextual EV compatibility and metformin-related evidence.
Results: Annotation refinement retained two primary mature-miRNA cohorts, one sensitivity miRNA cohort and two gene-level mRNA cohorts. The primary mature-miRNA meta-analysis yielded three strict candidates: hsa-miR-1271-5p, hsa-let-7e-5p and hsa-miR-146b-3p. The gene-level transcriptomic backbone included 51,171 meta-analysed genes, and target-transcriptome integration identified 549 anti-correlated miRNA-mRNA pairs involving 429 unique genes. hsa-let-7e-5p showed the broadest target support, with 409 anti-correlated targets including 255 strict mRNA targets, whereas hsa-miR-1271-5p showed 135 anti-correlated targets including 91 strict mRNA targets. Both candidates received identical human evidence scores of 86.0 and mapped to diabetes-relevant inflammatory, insulin/metabolic, endocrine-stress, vascular/endothelial, small-GTPase and cellular stress-response axes. hsa-miR-146b-3p was retained as a lower-tier conditional inflammatory candidate because its mature-miRNA signal was consistent but its anti-correlated target context was narrow.
Conclusion: Annotation-aware mature-miRNA and target-transcriptome integration prioritized hsa-miR-1271-5p and hsa-let-7e-5p as co-priority human T2D candidates and hsa-miR-146b-3p as a conditional candidate. The findings provide a conservative candidate framework for future mechanistic and EV-focused regenerative studies, while requiring independent experimental validation.
Keywords: Type 2 Diabetes, Mature microRNA, Target Transcriptome, Extracellular Vesicles, Regenerative Medicine

Ps-15: Delayed microRNA Remodeling Follows Transient Hippo-YAP Transcript Responses to Imatinib in Chronic Myeloid Leukemia Cells

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Background: Chronic myeloid leukemia (CML) is a model of targeted cancer therapy, but early molecular responses to BCR-ABL1 inhibition remain incompletely defined. The Hippo-YAP pathway is relevant to stem/progenitor biology and malignancy, and microRNAs may modulate Hippo pathway output. We investigated whether imatinib induces time-dependent Hippo-YAP transcript remodeling followed by delayed microRNA changes in K562 CML cells.
Materials and Methods: K562 cells were treated with imatinib and assessed using MTT cell viability assay, phase-contrast morphology, acridine orange/ethidium bromide staining, intracellular reactive oxygen species measurement, and reverse-transcription quantitative PCR (RT-qPCR) profiling of core Hippo pathway genes and selected Hippo-associated microRNAs in three independent experiments. Candidate microRNA-Hippo interactions were prioritized using curated interaction databases and RNAhybrid support. To provide exploratory progenitor-level context, two public CD34+ CML transcriptomic datasets were re-analyzed: GSE12211, containing paired pre-treatment and day-7 imatinib samples, and GSE14671, containing baseline responder and non-responder samples.
Results: Imatinib reduced K562 viability with a half-maximal inhibitory concentration (IC_{50}) of 0.5 μM at 48 hours and increased apoptosis-compatible morphological changes. Intracellular reactive oxygen species levels decreased at later time points. At 12 hours, imatinib induced coordinated upregulation of all measured Hippo pathway transcripts, with LATS2 showing the strongest increase. By 48 h, this transcript surge had largely returned toward baseline. In contrast, five Hippo-associated microRNAs showed delayed upregulation at 48 h, including miR-135a-5p, miR-182-5p, miR-125b-2-3p, miR-103a-3p, and miR-590-3p. Several microRNA-target pairs showed temporally inverse patterns involving LATS1, LATS2, MOB1B, and SAV1, supporting a prioritized but hypothesis-generating microRNA-Hippo interaction map. In CD34+ CML datasets, composite Hippo kinase and YAP/TAZ-TEAD output scores showed heterogeneous patient-level trajectories after imatinib and did not stratify baseline imatinib responders from non-responders.
Conclusion: Imatinib exposure in K562 cells was associated with a transient Hippo-YAP transcript response followed by delayed Hippo-associated microRNA remodeling. The CD34+ analyses provided exploratory translational context but did not confirm a uniform primary-sample signature. These findings define a time-resolved, hypothesis-generating framework for future functional studies of microRNA-Hippo regulation in CML therapy response.
Keywords: Chronic Myeloid Leukemia, Imatinib, Hippo-YAP Pathway, microRNA, CD34+ Progenitors

Ps-16: Tracking The Presence of Recombinant PDX1 Lentivirus in Tissues and Organs of Chicken Embryo

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Background: The chicken embryo has long served as a valuable model for studying human developmental biology, disease pathogenesis, gene function during organogenesis, and regenerative medicine. This study aims to directly visualize the presence and expression of the *PDX1* gene in live chicken embryos following lentiviral vector transfer. The primary focus is on virus delivery at the blastoderm stage and tracking its biodistribution in growing embryos and various organs to establish a practical platform for future pancreatic development research.
Materials and Methods: Lentiviral particles containing the *PDX1* gene were produced using HEK293T-LentiX cells. Virus titer was determined by GFP expression using fluorescence microscopy and confirmed by flow cytometry. For in-vivo evaluation, lentiviral particles were injected into chicken embryos at the blastoderm stage (day zero). Treated embryos were harvested at days 1, 3, 5, and 21 and examined alive under a stereo fluorescence microscope to assess viral presence and distribution.
Results: High-titer lentiviral particles were successfully produced, and the titer was confirmed by flow cytometry. The most significant finding was the direct observation of GFP expression (indicating viral presence) in live chicken embryos at all examined stages. Blastoderm stage injection resulted in widespread and stable viral dissemination throughout the embryo. GFP expression was clearly detectable in various organs, including the brain, heart, liver, gastrointestinal tract, and re-

productive organs in live embryos. Fluorescence intensity and stability were maintained until developmental day 21, indicating successful and stable integration of the vector into the embryonic cell genome.
Conclusion: This study demonstrates that injecting lentiviral vectors carrying the *PDX1* gene into the sensitive blastoderm stage of chicken embryos is an efficient method for direct, live visualization of the virus in the embryo and its various organs. Successful observation of gene expression in multiple organs validates the high capability of this approach for developmental studies and gene therapy. Consequently, this method can be used to introduce desired genes into primordial germ cells under tissue-specific promoter control, ultimately enabling the production of genetically modified birds with beneficial biotechnological traits.
Keywords: Lentiviral Vector, *PDX1* Gene, Chicken Embryo, Genome Editing, Live Imaging

Ps-17: Isorhamnetin Modulates Fibrotic Markers, NOX/ROS Signaling, and Oxidative Stress in LX-2 Hepatic Stellate Cells: Implications for Liver Fibrosis Prevention

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Background: Liver fibrosis is one of the pathological processes in the liver, primarily associated with the activation of hepatic stellate cells (HSCs) and the excessive accumulation of scar tissue within the hepatic parenchyma. Isorhamnetin, a flavonoid compound known for its antioxidant properties, exerts protective effects against fibrotic liver injury by inhibiting the expression of cholesterol- dependent fibrogenic genes. This study investigated the effect of isorhamnetin on the expression levels of fibrosis-related genes and the intracellular levels of reactive oxygen species (ROS) in HSCs.
Materials and Methods: LX2 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) until reaching the desired confluency. The cells were then treated for 24 hours with 75 and 100 µM concentrations of isorhamnetin. To evaluate fibrotic status, the expression levels of fibrogenic genes were measured using RT- PCR, and intracellular ROS levels were assessed using the ELISA technique.
Results: The findings indicated that treatment of cells with cholesterol (100 µM) significantly increased the expression levels of α-SMA, α1 collagen, NOX1, and NOX2, as well as ROS levels compared with the control group. In contrast, treatment with isorhamnetin at both 75 and 100 µM resulted in a significant reduction in the expression of these genes and ROS levels relative to the fibrotic group.
Conclusion: Cholesterol promotes oxidative stress and extracellular matrix synthesis by increasing the expression of genes involved in the progression of liver fibrosis, including α-SMA, collagen1α, NOX1, and NOX2. Conversely, isorhamnetin inhibits the progression of liver fibrosis by downregulating these genes. Accordingly, isorhamnetin may be considered a hepatoprotective compound with potential utility in future therapeutic strategies.
Keywords: Isorhamnetin, Liver Fibrosis, Collagen α1, α-SMA, NOXs

Ps-18: Comparison of Antioxidant Enzymes and Growth Factors in Exosomes Derived from Adipose-Derived Stem Cells Under Basal Conditions and Curcumin Treatment: A Novel Approach to Therapeutic Exosome Engineering

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Background: Adipose-derived stem cell exosomes (ADSC-Exos) are considered effective tools in regenerative medicine due to their rich content of growth factors, microRNAs, and antioxidant enzymes. However, enhancing the efficacy of these vesicles through the treatment of parental cells with natural compounds such as curcumin represents a novel strategy. The aim of this study is to quantitatively compare the protein and enzyme contents of untreated ADSC-Exos and curcumin-treated ADSC-Exos (Cur-ADSC-Exos) in terms of antioxidant enzymes, growth factors, and inflammatory cytokines.
Materials and Methods: ADSCs were cultured in two groups: a control group and a treatment group exposed to an optimal concentration of 10 µM curcumin for 48 hours. Exosomes were isolated using a precipitation-based kit (AnaCell) and characterized by Western blotting, dynamic light scattering (DLS), and electron microscopy. Protein concentrations were measured by the Bradford method and exosome contents including oxidative stress enzymes, VEGF, and IL-1β were measured by ELISA kits.
Results: In the Cur-ADSC-Exos group compared to ADSC-Exos, a significant increase in antioxidant enzyme levels was observed. Specifically: SOD increased approximately 3-fold (from 1.7 to 5.6 U/mg), catalase increased 4-fold (from 1.1 to 4.1 U/mg), and GPx increased approximately 3.5-fold (from 0.16 to 0.57 U/mg). Additionally, VEGF in Cur-ADSC-Exos increased approximately 3-fold, reaching 470 pg/mg. The most notable change was an 8-fold reduction in IL-1β, reaching 1.1 pg/mL in ADSC-Exos.
Conclusion: Preconditioning of ADSCs with curcumin, through the activation of cellular signaling pathways, significantly shifts the exosome composition toward a super-antioxidant, anti-inflammatory, and pro-regenerative phenotype. Exosomes derived from this approach exhibit enhanced potential for regeneration including skin wound healing.
Keywords: Exosome, Regenerative Medicine, Inflammation, Oxidative Stress, Wound Healing

Ps-19: Honokiol Facilitates Metabolic Maturation of Neonatal Rat Cardiomyocytes via SIRT3

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Background: Reliable cardiac models depend not only on cardiomyocyte identity but also on the degree of maturation. Neonatal rat cardiomyocytes (NRCMs) are practical for *in-vitro* cardiac studies, yet their metabolism and contractile phenotype

remain immature. Because postnatal maturation is associated with a shift from glycolysis to mitochondrial oxidative phosphorylation, we evaluated whether Honokiol, a reported activator of SIRT3, could promote metabolic, structural and functional maturation of NRCMs.
Materials and Methods: NRCMs were isolated from one day old neonatal hearts and cultured in-vitro. To select an optimal concentration, cells were exposed to 2.5, 5, 10, 20 and 50 μM Honokiol, and viability was measured by MTS assay. Mitochondrial activity was assessed by JC-1 staining. Expression of *Atp5f1a* and *Ndufs7*, as oxidative phosphorylation-related genes, and *cTnI, α-MHC* and *Cx43*, as maturation associated markers, was analyzed by qRT-PCR. Cell morphology was examined by microscopy and circularity analysis in ImageJ. Cardiac troponin I expression and sarcomeric organization were evaluated by immunofluorescence. Beating activity and extracellular field potentials were recorded to assess functional maturation.
Results: Honokiol did not impact cell viability at the selected concentrations and increased mitochondrial membrane potential compared with untreated controls. Treated NRCMs showed higher expression of *Atp5f1a* and *Ndufs7*, together with increased expression of *cTnI*, *α-MHC* and *Cx43*. Immunofluorescence staining revealed slightly higher cTnI signal and more organized sarcomeric structures. Morphological analysis showed a reduction in circularity, indicating a shift from a rounded neonatal phenotype toward a more elongated cardiomyocyte morphology. Honokiol treated cells showed regular beating as controls.
Conclusion: Honokiol enhanced several key features of NRCM maturation, including mitochondrial activity, cardiac marker expression and morphology. These findings support SIRT3-related metabolic modulation as a feasible strategy for improving NRCM maturation *in-vitro*.
Keywords: Honokiol, Cardiomyocyte Maturation, SIRT3

Ps-20: Surgical Implantation of Rat Adipose-Derived Stem Cell Exosome-Loaded PVA/CH/PEC Hydrogel Nanocomposite Scaffold Attenuates p-SMAD3 Signaling in Liver Fibrosis

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Background: Exosomes offer several advantages over stem cells for tissue regeneration; however, they are rapidly cleared by the reticuloendothelial system. To address this limitation, biomedical scaffolds can act as protective carriers, prolonging their therapeutic effects. Moreover, encapsulating exosomes within biocompatible scaffolds enables targeted delivery to specific tissues and enhances the localized concentration of their therapeutic factors.
Materials and Methods: Hepatic fibrosis was induced by intraperitoneal injection of Carbon tetrachloride (CCl_4) (0.1 mL per 100 g body weight) twice weekly for 4 weeks. Rat adipose-derived stem cell exosomes (Exo- rADSCs) were isolated and cultured on polyvinyl alcohol–chitosan–pectin nanocomposite discs. Discs with a diameter of 1 cm were surgically implanted on the dorsal and ventral surfaces of the liver lobes. Discs without Exo- rADSCs were considered as the control group. The animals were then maintained under standard conditions for an additional 4 weeks without receiving CCl_4. All animals were sacrificed at the end of week eight.
Results: Serial administrations of CCl_4 resulted in the development of moderate fibrosis by week four, which persisted until week eight. Animals treated with Exo- rADSC discs exhibited more than an 80% reduction in collagen content, accompanied by a significant increase in serum albumin and total protein levels. Immunohistochemical staining for collagen type I demonstrated a marked decrease compared with animals that did not receive Exo- rADSC treatment, consistent with the reduced p- SMAD3 levels observed in the present study.
Conclusion: Treatment with Exo- rADSCs improved liver function and markedly reduced hepatic fibrosis. These pleiotropic and paracrine factors play reparative roles in the attenuation of liver fibrosis. These findings also highlight the use of Exo- rADSCs as a non- cellular therapeutic strategy and present them as an effective alternative to conventional cell- based therapies.
Keywords: Exosomes, PVA/CH/PEC Scaffold, Hepatic Fibrosis, p-SMAD3 Signaling Pathway, Carbon Tetrachloride (CCl_4)

Ps-21: Sustained p-Smad3 Signaling and Collagen α1 Deposition Underlie Persistent Liver Fibrosis Post-CCL4 Cessation in Male Albino Wistar Rats

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Background: The regression of liver fibrosis following the cessation of hepatic injury is a complex, dynamic process that remains incompletely characterized. This study aimed to elucidate the extent and kinetics of spontaneous fibrosis regression following the withdrawal of carbon tetrachloride (CCl4) in an experimental model of liver fibrosis.
Materials and Methods: Male Wistar rats underwent intraperitoneal injections of CCl4 (0.1 mL/100g body weight) twice weekly for 4 weeks to establish stage 3 hepatic fibrosis. Thereafter, CCl4 was discontinued for a 4-week period to evaluate spontaneous regression and intrinsic liver regeneration. All subjects were sacrificed at week 8 for histopathological and biochemical analyses.
Results: CCl4 withdrawal prompted a rapid normalization of serum transaminase levels (ALT and AST) within one week, signifying the cessation of active hepatocellular necroinflammation. In contrast, serum total protein and albumin concentrations remained significantly suppressed—falling even below the levels observed in the active fibrotic group—indicating a persistent impairment in hepatic synthetic capacity. While histological assessment revealed a marked resolution of bridging fibrosis, suggesting partial parenchymal remodeling, immunohistochemical analysis demonstrated that type I collagen deposition remained dense and stable, with no statistically significant reduction in the overall extracellular matrix (ECM) burden. Crucially, Western blot analysis identified the persistence of p-

Smad3 signaling despite the absence of the toxin, providing a mechanistic basis for the sustained collagen α1 accumulation and the failure of comprehensive fibrosis regression.
Conclusion: These findings demonstrate that in the early stages following the cessation of fibrogenic stimuli, biochemical normalization and structural remodeling can occur independently of effective collagen clearance or the restoration of hepatic synthetic function. This study highlights a significant temporal dissociation between enzymatic recovery, architectural restoration, and functional restoration during the early phase of regression in experimental liver fibrosis.
Keywords: p-Smad3 Signaling, Collagen α1, Liver Fibrosis, Spontaneous Fibrosis Regression, Carbon Tetrachloride

Ps-22: Drug Repurposing Using Biocomputational Methods Targeting SYK Protein in Treatment of Retinoblastoma

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Background: Retinoblastoma represents a highly malignant pediatric intraocular tumor that usually needs intensive treatment procedures such as chemotherapy. Conventional treatments may be accompanied by serious adverse effects, thereby stressing the necessity of targeted therapy development. It is known that the spleen tyrosine kinase (SYK) acts as an essential component responsible for tumor growth; thus, SYK protein inhibitors represent promising drugs for retinoblastoma treatment. The use of drug repurposing represents an effective way to design new drugs because it is cheaper and quicker than traditional approaches. This study is aimed at finding potential FDA-approved drugs capable of interacting with the SYK protein via in silico methods.
Materials and Methods: First, the 3D structure of the SYK target protein (with PDB ID: 6ZCS) was downloaded from the Protein Data Bank (PDB). Preparation of protein was done through AutoDock Tools by deleting the co-crystallized ligands and water molecules, and adding polar hydrogens and Kollman charges. Then, 2162 FDA approved drugs were downloaded as a screening library from e-Drug3D database, where ligand structures preparation was performed through Open Babel. The parameters for the binding pocket and grid box have been defined precisely by PrankWeb webserver. After that, docking analysis was performed via AutoDock Vina. Finally, the top candidates were validated through CB-Dock2 server.
Results: As a result of computational screening, the FDA approved drugs with high binding affinities were identified by showing stable interactions with SYK protein. The top five candidates demonstrating the lowest (most favorable) binding energies were Zavegepant (-11.94 kcal/mol), Lumacaftor (-11.53 kcal/mol), Entrectinib (-11.50 kcal/mol), Irinotecan (-11.17 kcal/mol), and Belumosudil (-11.05 kcal/mol).
Conclusion: Our computational investigation introduces Zavegepant, Lumacaftor, Entrectinib, Irinotecan, and Belumosudil as highly potent candidates for repurposing against SYK-driven retinoblastoma. These findings provide a rational foundation for future in-vitro and in-vivo experimental validations to confirm their clinical efficacy and precise molecular mechanisms in targeted pediatric cancer therapy.
Keywords: Retinoblastoma, Drug Repurposing, Spleen Tyrosine Kinase (SYK), Molecular Docking, FDA-Approved Drugs

Ps-23: Prime Editing in iPSCs for Pediatric B-ALL: A Systematic Review

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Background: Pediatric B-ALL is the most common childhood blood cancer. About 25% of cases carry the ETV6-RUNX1 fusion gene, which creates a preleukemic state. Prime editing (PE) is a new tool that fixes DNA without breaking it. When combined with patient-derived iPSCs, this approach could lead to personalized cell therapy. A systematic review was conducted to map the evidence on PE in iPSCs for pediatric B-ALL and to identify research gaps.
Materials and Methods: A literature search was performed in PubMed, Scopus, and Web of Science (Jan 2021–Apr 2026) using the Keywords "prime editing," "iPSCs," "ETV6-RUNX1," and "pediatric leukemia." Original studies using PE in human pluripotent or hematopoietic stem cells were included. Each study was assessed for three quality criteria: clarity of editing methods, reporting of negative controls, and confirmatory sequencing.
Results: From 142 initial records, 49 full-texts were screened after duplicate removal and title/abstract screening. Thirty-eight studies were excluded (no original data, n=15; non-human cells, n=12; no ALL focus, n=11). Eleven studies met the inclusion criteria. Quality assessment showed that all 11 had clear editing methods (100%), eight reported negative controls (73%), and all 11 used confirmatory sequencing (100%). Among these, three studies directly applied PE in iPSCs for B-ALL-relevant genes. One study corrected a PAX5 mutation in B-ALL patient iPSCs with 36% efficiency and demonstrated differentiation into CD19+ precursors (Jang et al., Mol Ther, 2024). Two other studies modeled an ETO2: GLIS2 fusion in iPSCs. Importantly, iPSC models carrying the ETV6-RUNX1 fusion have been established by other groups, but no published study has used PE to correct this specific fusion. Recent technological advances (e.g., vPE, Nature, 2025) offer improved precision, though their application to ETV6-RUNX1 remains unexplored.
Conclusion: PE has been successfully applied in iPSCs for certain B-ALL mutations, and iPSC models of ETV6-RUNX1 exist. However, direct PE-mediated correction of ETV6-RUNX1 in patient-derived iPSCs remains a key research gap. Future work should design pegRNAs targeting the fusion breakpoint and validate safety in preclinical models.
Keywords: Prime Editing, iPSCs, ETV6-RUNX1, Pediatric B-ALL, Systematic Review

Ps-24: Optogenetic Dissection of Liquid-Liquid Phase Separation in Cancer Hallmarks: A Systematic Review

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Background: Liquid-liquid phase separation (LLPS) drives formation of biomolecular condensates (e.g., MED1, BRD4, FUS) that regulate oncogenic transcription and stress respons-

es. Whether LLPS plays a causal or secondary role in cancer hallmarks remains unclear. Optogenetics allows light-inducible manipulation of condensate assembly, providing stronger causal inference than correlative methods.
Materials and Methods: A systematic review was conducted following PRISMA 2020 guidelines. PubMed, Scopus, and Web of Science were searched from inception to March 2026 for studies using optogenetic tools (e.g., Cry2/CIB1) to modulate LLPS in cancer models. Inclusion criteria: original research, optogenetic targeting of a condensate-forming protein, and a functional cancer hallmark readout. Two independent reviewers screened and extracted data (κ=0.89). Quality was assessed using ROBINS-I. Due to high heterogeneity (I^2>75%), a narrative synthesis was performed. No formal GRADE assessment was conducted; therefore, the strength of evidence for therapeutic recommendations is limited. Most studies used established cell lines rather than patient-derived models; blinding was reported in only two studies. Publication bias cannot be ruled out (Egger's test P=0.08). The small number of *in-vivo* studies (n=2) limits generalizability to "multiple cancer types."
Results: From 312 records, 48 studies met inclusion (32 in-vitro, 16 in-vivo). Condensate formation was confirmed microscopically in 87.5% (42/48). Optogenetic MED1 clustering in MCF-7 cells increased MYC expression by 2.5-4.0-fold (P < 0.01) and drove S-phase entry without growth factors. BRD4 condensate activation in U87MG increased temozolomide IC50 by 3.2-fold (P=0.003); FUS condensate dissolution resensitized resistant cells. YAP LLPS in lung cancer cells increased invasion by 70% (95% CI:45-95%, P=0.01). In two transgenic zebrafish, light-induced c-Myc condensates increased tumor volume (mean difference:4.1 mm^3, 95%CI: 2.3-5.9), though these findings are preliminary.
Conclusion: This systematic review suggests that optogenetically induced LLPS plays a potential causal role in driving proliferation and drug resistance in breast cancer and glioblastoma, with preliminary evidence for effects on metastasis and in-vivo tumor growth. However, the evidence base remains limited, particularly for in-vivo settings. Targeting condensate-specific interfaces (e.g., MED1 IDR) warrants further investigation, ideally in patient-derived models and with larger animal cohorts.
Keywords: Phase Separation, Optogenetics, Drug Resistance, Glioblastoma, Breast Cancer

Ps-25: Evaluation of Smoothened (Smo) Protein Expression in Pancreatic Cancer Cells (PANC-1) Following Treatment with hAMSCs-Derived Secretome

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Background: Pancreatic cancer remains one of the most lethal malignancies with limited effective therapies. Current conventional treatments often fail due to severe side effects on normal cells. Recently, stem cell-based therapies have attracted attention as targeted approaches, primarily because they are derived from human tissues and exhibit minimal adverse effects. The Hedgehog signaling pathway, particularly the Smoothened (Smo) protein, plays a key role in pancreatic cancer progression. This study aimed to investigate the effect of human Amniotic Mesenchymal Stem Cell (hAMSC) secretome on Smo expression in pancreatic cancer cells (PANC-1).
Materials and Methods: hAMSCs were obtained from Iranian Biological Resource Center (IBRC) and PANC-1 cells were purchased from Pasteur Institute (Tehran, Iran). PANC-1 cells were cultured in DMEM medium containing FBS (fetal bovine serum) and antibiotics. To evaluate the effects of hAMSCs secretome on cancer cells, hAMSCs and PANC-1 cells were co-cultured for 72 hours in Transwell plates where the cancer cells to be exposed to the stem cell secretome. Then, cells harvested and cell lysates were evaluated by Western blotting method. Data were analyzed compared to untreated control cells.
Results: Our findings indicate that treatment with secretome of hAMSCs significantly downregulated Smo protein expression in PANC-1 cells compared to untreated controls. This substantial reduction suggests a strong modulatory effect of secretome on the Hedgehog pathway.
Conclusion: These Results demonstrate that hAMSC-derived secretome effectively downregulates Smo expression in pancreatic cancer cells. Given the pivotal role of Smo in Hedgehog signaling, our findings propose that secretome may serve as a promising targeted therapeutic agent for pancreatic cancer, with the advantage of being human-derived and having low side effects. Further in-vivo studies are warranted.
Keywords: Pancreatic Cancer, Smoothened (Smo), Hedgehog Signaling, Secretome of Hamscs, Targeted Therapy

Ps-26: Targeting the Neurotrophic Signaling Axis in Primary Astrocytes: A Novel Neuroprotective Strategy Using Dimethyl Itaconate

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Background: Neuroinflammation is a central pathological feature of many neurodegenerative disorders and is closely associated with astrocyte dysfunction and impaired neurotrophic signaling. Astrocytes play a essential role in maintaining neuronal survival and homeostasis through the production of neurotrophic factors and regulation of their receptors. Among these, tropomyosin receptor kinase B (TrkB), the high affinity receptor for brain derived neurotrophic factor (BDNF), is a key mediator of pro survival and neuroprotective signaling in the central nervous system. The present study investigated whether dimethyl itaconate (DMI), a cell permeable derivative of itaconate with known anti inflammatory properties, modulates the neurotrophic receptor axis with particular emphasis on TrkB expression in primary astrocytes under inflammatory conditions.
Materials and Methods: Primary astrocytes were isolated from neonatal rat cortex and cultured under standard conditions. Neuroinflammation was induced by exposure to lipopolysaccharide (LPS; 1 μg/mL) for 6 hours. Following inflammatory stimulation, cells were treated with increasing concentrations of DMI (62.5, 125, and 250 μM) for 18 hours. Cell viability was assessed using the MTT assay. TrkB expression at the mRNA and protein levels was evaluated by quantitative real time PCR

and Western blot analysis, respectively.
Results: LPS exposure significantly reduced astrocyte viability and downregulated TrkB expression compared with untreated controls. Treatment with DMI markedly attenuated LPS induced cytotoxicity in a dose dependent manner. Importantly, DMI restored and significantly upregulated TrkB mRNA and protein levels in inflamed astrocytes, with the most pronounced effects observed at 125 and 250 μM. These findings suggest that DMI enhances astrocytic resilience to inflammatory stress, at least in part, through modulation of the TrkB mediated neurotrophic signaling pathway.
Conclusion: targeting the neurotrophic receptor axis—particularly TrkB—represents a promising neuroprotective strategy in the context of neuroinflammation. DMI may enhance astrocytic resilience by restoring neurotrophic signaling and improving cellular survival under inflammatory stress. Importantly, modulation of astrocyte mediated neurotrophic support may also contribute to creating a more favorable microenvironment for neural stem and progenitor cells, which is essential for effective neural repair and regeneration. Therefore, regulating astrocyte neurotrophic pathways using small metabolic derivatives such as DMI may represent a novel supportive approach in stem cell–based therapeutic strategies for neurodegenerative and inflammatory neurological disorders.
Keywords: Dimethyl Itaconate, Trkb, Astrocytes, Neuroinflammation, Neuroprotection

Ps-27: Study of *FGR* Gene Expression as Genetic Biomarker in Patients with Colorectal Cancer

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Background: Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. The inflammatory response pathway like Src family kinases (SFKs) has been widely implicated in tumor development in CRC. The FGR gene, a member of SFKs, encodes a cell signaling, cell adhesion regulation and migration protein. Currently the signaling pathways that drive inflammatory responses in CRC are not well understood. Therefore, in this study, we investigated the FGR expression as a genetic biomarker and a member of the inflammatory response pathway family.
Materials and Methods: The expression level of the FGR gene was measured by Realtime PCR technique on 30 samples of CRC patients who had referred to the tumor bank of Imam Khomeini Hospital in Tehran. Statistical analysis for Realtime PCR data was done by independent T-test and to check the correlation between genes expression, Pearson's test was performed. In order to evaluate the ability of the FGR gene to differentiate, Receiver Operating Characteristic (ROC) curve analysis was performed.
Results: According to the Results, FGR expression was significantly increased by an average of 4.5 ($P < 0.001$). Base on ROC curve Results, the cut-off point >10.47 has a very favorable diagnostic performance. At this threshold, the sensitivity was 96.67% (95%CI: 82.78%–99.92%) and the specificity was 73.33% (95%CI: 54.11% to 87.72%). Also, none of the clinical variables including age, tumor grade, stage, lymphatic invasion, vascular invasion, and EMBVI have a significant correlation with the Fold Change of FGR gene expression. The highest expression of this gene is in patients with lymphoid and vascular invasion, and stage II. And patients with lower average expression have perineural invasion and perineal invasion.
Conclusion: FGR expression is increased in CRC patients. The lack of significant correlation between FGR expression and clinical variables could indicate that FGR acts as an independent biomarker and may play a role in early stages of the disease or in specific pathways of carcinogenesis. These findings suggest that FGR expression in CRC may be independent of classical clinical features and its role can be further investigated at the molecular level and genetic settings.
Keywords: Colorectal Cancer, Biomarker, FGR

Ps-28: Primary Cilia Dynamics Play A Role in Maturation of Human Pluripotent Stem Cell-Derived Cardiomyocytes

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Background: Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) often display an immature, fetal-like phenotype, which limits their therapeutic application in cardiac regenerative medicine. Recent research underscores the role of the primary cilium, a specialized microtubule-based organelle, as a critical sensory hub that integrates extracellular signals to regulate cell fate and homeostasis. In many cell types, primary cilia undergo significant structural remodeling during differentiation; however, the spatiotemporal dynamics of these organelles—specifically fluctuations in ciliary length—during cardiac commitment and the subsequent maturation phase remain largely unexplored. Elucidating whether the primary cilium acts as a dynamic regulator of cardiac maturation is essential for understanding the transition from embryonic to adult-like cardiomyocyte characteristics. This study aims to characterize the morphological changes of the primary cilium during the maturation process of hPSC-CMs and to evaluate the impact of modulating ciliary length on this developmental progression.
Materials and Methods: hPSC-derived cardiomyocytes were monitored throughout the differentiation process to map the longitudinal changes in primary cilia. The high-resolution immunofluorescence imaging was employed to quantify variations in ciliary length across different stages of differentiation and maturation. To determine the functional significance of these structural changes, targeted pharmacological treatments were applied to systematically manipulate ciliary length in cardiac progenitor cells.
Results: The primary cilium showed distinct morphological remodeling, characterized by specific changes in length, during the maturation of hPSC-CMs. The pharmacological treatment resulted in more mature phenotype characterized by expression of cTNT in hPSC-CMs.
Conclusion: These Results suggests that ciliary length is a dynamic and regulated feature that reflects the maturation state of cardiac cells, establishing a foundational basis for utilizing ciliary structural changes as a potential marker or regulatory target in cardiac differentiation.
Keywords: Stem Cell, Cilia, Differentiation, Maturation, Cardiomyocyte

Ps-29: HUVEC-Derived Exosome Therapy Restores Skin Elasticity and Dermal Architecture in An Experimental Model of Aging

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Background: Skin aging is a complex biological process marked by reduced fibroblast activity, diminished collagen production, decreased elasticity, thinning of the dermis. These changes lead to wrinkles, skin fragility, and delays in tissue recovery. Extracellular vesicle-based cell-free regeneration therapies are becoming acceptable alternatives to stem cell-based therapies. Exosomes from human umbilical vein endothelial cells (HUVECs) may stimulate skin regeneration by bioactive paracrine signaling. However, the anti-aging effects of topically applied HUVEC-derived exosomes have not been thoroughly investigated in D-galactose-induced skin aging models.
Materials and Methods: HUVEC has been confirmed by flow cytometry utilizing the markers CD31 and CD34. Exosomes were extracted from HUVEC-conditioned medium by ultracentrifugation and analyzed by dynamic light scattering (DLS), zeta potential analysis, scanning electron microscopy (SEM), and flow cytometry for CD9, CD63, and CD81. Based on earlier in-vitro Results, the appropriate exosome concentration was embedded in a topical cream. D-galactose-induced skin aging was established in NMRI mice. The final in-vivo study was analyzed in five groups, including untreated control, aged control, vehicle treatment, glycolic acid 5%-treated, and HUVEC-Exo cream-treated groups. Biometric assessments including skin thickness, density, elasticity, and transepidermal water loss (TEWL) were performed. Histological examination was conducted using hematoxylin and eosin staining (H&E).
Results: HUVECs were positive for CD31 and negative for CD34, indicating their endothelial nature. The isolated HUVEC-derived exosomes had a nanoscale size range of 80-130 nm, negative zeta potential, and positive expression of the exosomal markers of CD9, CD63, and CD81. Topical use of HUVEC-Exo cream significantly decreased aging-associated skin changes compared to aged and vehicle-treated groups. A biometric study showed a significantly higher recovery of total and dermal thickness, increased skin density, increased overall elasticity, and decreased transepidermal water loss in the exosome-treated group. HUVEC-Exo cream exhibited a higher efficacy than glycolic acid 5% in restoring dermal thickness and dermal organization. Histological study further revealed the enhanced architecture of epidermis and dermis with greater cellular density in exosome-treated animals.
Conclusion: This study indicates that HUVEC exosomes may be a potential cell-free regenerative strategy for topical skin rejuvenation. HUVEC-Exo cream enhanced dermal matrix, skin elasticity, tissue density, and barrier function in a D-galactose-induced aging mouse model to support additional preclinical and clinical studies.
Keywords: Human Umbilical Vein Endothelial Cells (Huvecs), Exosomes, NMRI Mice, Skin Rejuvenation

Ps-30: Investigating The Role of Podocytes in The Formation of Kidney Organoids in The Presence of Human Umbilical Cord Blood Serum

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Background: Chronic kidney disease (CKD) represents an increasing global health burden, with limited options for regenerative therapy. Kidney organoids have emerged as promising models for studying kidney disorders. However, their application remains limited by dependence on fetal bovine serum (FBS), insufficient structural maturation, and poor clinical translatability. To address these limitations, we developed a novel protocol integrating podocytes, key glomerular filtration cells, with renal progenitor cells in a co-culture system. Furthermore, FBS was replaced with human umbilical cord blood serum (HUCBS), an ethical and clinically relevant supplement, to enhance organoid functionality and translational potential.
Materials and Methods: Primary mouse mesenchymal-like (MLC) and epithelial-like (ELC) cells were isolated from FVBN mouse kidneys. We established a three-dimensional four-cell co-culture system using MLCs, ELCs, human umbilical vein endothelial cells (HUVECs), and human podocytes. An optimized cell ratio of 10:5:2:10 (MLC/ELC/HUVEC/Podocyte) with a total of 420,000 cells per well yielded the most compact and uniform aggregates. In a key innovation, the culture system was maintained in RPMI-based medium supplemented with 15% HUCBS, completely eliminating FBS and recombinant growth factors.
Results: The optimized four-cell system cultured in HUCBS-supplemented medium generated significantly larger and more compact kidney organoids compared to podocyte-free controls. Quantitative real-time PCR (qRT-PCR) analysis at day 7 demonstrated significantly elevated expression of podocyte markers (NPHS1, PODXL, WT1), tubular markers (E-cadherin, Aquaporin-1), mesangial marker (PDGFR-β), endothelial marker (CD34), and collecting duct markers (GATA3, Aquaporin-2). Histological assessment using H&E staining confirmed high viability, dense cellular organization, and absence of central necrosis. Immunofluorescence further validated organized E-cadherin-positive epithelial structures.
Conclusion: This study presents a cost-effective and translational approach for generating structurally mature kidney organoids. Incorporating podocytes into a vascularized co-culture enhanced glomerular and tubular marker expression, while HUCBS served as an ethical human-derived alternative to FBS and expensive growth factors, reducing immunogenicity and production costs. This all-human-supplemented chimeric platform provides strong potential for drug screening, nephrotoxicity testing, and future autologous kidney organoid engineering.
Keywords: Kidney Organoid, Podocyte, Co-Culture, Human

Umbilical Cord Blood Serum (HUCBS), Tissue Engineering

Ps-31: Citicoline-Induced Modulation of Adult Hippocampal Neural Stem Cells in A Depression Model: The BDNF/SIRT1 Pathway as A Central Regulator of Neurogenesis and Cognitive Function

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Background: The hippocampus is one of the few neurogenic niches in the adult brain, where neural stem cells continuously generate new neurons throughout life. The learned helplessness (LH) paradigm is a well-established preclinical model of depression that induces behavioral deficits resembling human depressive and anxiety-like symptoms through exposure to inescapable stress. The neurobiological mechanisms underlying LH involve oxidative stress, neuroinflammation, impaired neurogenesis, and synaptic dysfunction, particularly within the hippocampus, a brain region essential for memory formation and emotional regulation.
Materials and Methods: LH was induced in mice by repeated exposure to inescapable footshocks over two consecutive days. Citicoline (75 mg/kg, i.p.) was administered to evaluate its neuroprotective effects. Following behavioral assessments, animals were anesthetized, and the hippocampus was rapidly dissected, frozen in liquid nitrogen, and stored at −70°C until analysis. Hippocampal expression levels of brain-derived neurotrophic factor (BDNF) and sirtuin-1 (Sirt-1) proteins were determined by Western blotting, and band intensities were quantified relative to a housekeeping protein.
Results: Citicoline administration significantly increased hippocampal BDNF and Sirt-1 protein expression compared with the LH group ($P < 0.05$ and $P < 0.005$, respectively).
Conclusion: Citicoline enhanced the expression of neurogenesis-related signaling molecules, including BDNF and Sirt-1, in the hippocampus of LH-induced mice. These findings suggest that activation of the BDNF/Sirt-1 pathway may contribute to the neuroprotective and pro-neurogenic effects of citicoline in depression-like conditions.
Keywords: Adult Neural Stem Cells, Hippocampal Neurogenesis, Citicoline, Brain-Derived Neurotrophic Factor (BDNF), Sirtuin-1 (Sirt-1)

Ps-32: Gene Expression Analysis Reveals RASSF6 as A Potential Biomarker in Papillary Thyroid Carcinoma

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Background: Papillary thyroid carcinoma (PTC) is a prevalent type of thyroid malignancy. The PTC progression is influenced by environmental and genetic factors. Although many genes have been studied in association with PTC, the exact genetic mechanism of PTC remains poorly clarified. This study was aimed to identify genes associated with the prognosis of PTC.
Materials and Methods: The GSE33630 dataset was analyzed, including 49 PTC tissue samples and 45 samples of adjacent normal tissue to PTC tumors. Data quality was evaluated using Log2 transformation and Principal Component Analysis (PCA). Differentially expressed genes (DEGs) analysis was performed using the Limma package. Genes with a log fold change (LogFC) greater than 1.2 and an adjusted $P < 0.05$ were defined as DEGs. RNA-seq data was analyzed to examine survival, clinical stage, and nodal metastases. Ten PTC tissue samples and ten adjacent normal tissue samples were obtained after thyroidectomy. Total RNA extraction, cDNA synthesis, and RT-qPCR will be performed. The relative expression levels will be investigated using REST2009 software.
Results: Ras Association Domain Family Member 6 (RASSF6) was identified as one of the most significantly down-regulated genes in the microarray analysis, with a LogFC of -2.7 and an adjusted P of 1.93E-22. High RASSF6 expression was associated with a lower probability of patient survival compared to low RASSF6 expression levels (P=0.0083). Evaluation of RASSF6 expression across clinical stages showed median values of 0.151 in Stage II and 0.16 in Stage IV tumors. Their expression was strongly correlated with increased tumor aggressiveness (P=2.65E-02). The median expression level of RASSF6 was 0.249 in tumors without regional lymph node infiltration (N0) and 0.217 in tumors with axillary lymph node metastasis (N1), representing a statistically significant difference (P=2.66E-03). No significant difference in RASSF6 expression was observed between male and female patients.
Conclusion: RASSF6 expression level was significantly decreased in PTC compared to adjacent normal tissues. The increased expression of RASSF6 was associated with reduced survival probability and increased tumor aggressiveness, while decreased expression was observed in metastatic tumors.
Keywords: RASSF6, PTC, Microarray, Expression Profiling

Ps-33: Association of WDR72 Expression with Survival and Metastatic Potential in Papillary Thyroid Carcinoma

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Background: Papillary thyroid carcinoma (PTC) represents the most common form of thyroid malignancy and is classified as a well-differentiated type. The development of PTC is influenced by environmental and genetic factors. Although numerous genes associated with PTC have been investigated, the precise molecular mechanisms underlying its initiation remain

incompletely elucidated. The present study aims to identify genes implicated in the onset and progression of PTC.
Materials and Methods: The GSE33630 dataset, consisting of 49 PTC and 45 paired normal tissue samples, was analyzed. The validation of data quality required the application of Log2 transformation and quantile normalisation. Quality control was validated by principal component analysis. A differential expression analysis was performed using the Limma program. An analysis was conducted using RNA-seq data to examine survivability, stage, and nodal metastasis. Ten PTC tissue samples and ten adjacent normal tissue samples were obtained after thyroidectomy. Total RNA extraction, cDNA synthesis, and RT-qPCR will be performed. The relative expressions will be examined using REST2009 software.
Results: The analysis of microarray data revealed many genes exhibiting differential expression. One of the differentially expressed genes was WD Repeat Domain 72 (WDR72), with a logFC of -2.76 and an adjusted P of 2.97E-19. Increased WDR72 expression, in contrast to low expression, was significantly correlated with reduced patient survival probability (P of 0.014). The expression of WDR72 was evaluated at different biological stages, showing a median expression level of 11.931 in Stage 2 and 4.709 in Stage 4. Our investigation revealed that WDR72 expression in Stages 2 and 4 is significantly associated with increased aggression (P of 2.39E-05). The median expression level of WDR72 in tumors without regional lymph node infiltration (N0) was 8.731, but it was 4.69 in tumors with axillary lymph node metastases (N1). Analyses revealed a statistically significant disparity in WDR72 expression between N0 and N1 tumors (P of 3.08E-06).
Conclusion: The expression of the WDR72 gene was significantly reduced in PTC compared to the adjacent normal tissues. The reduced expression of WDR72 was associated with improved patient survival and increased metastasis.
Keywords: WDR72, Differentially Expressed Genes, Papillary Thyroid Cancer

Ps-34: Investigating Bovine Type II Collagen Scaffolds Including Berberine and Bone Marrow Derived MSCs in Osteoarthritis Rat Model

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Background: Osteoarthritis (OA) is a widespread disease that frequently leads to pain and disability, caused by degeneration of cartilage that cushions the joints. Tissue engineering approaches have been proposed to ameliorate lesions to the articular surface leading to OA, however, directing cellular differentiation within scaffolds to generate tissue with appropriate structural, compositional, and mechanical characteristics remains a significant challenge. This study aims to assess the efficacy of using Berberine-loaded type II collagen scaffolds combined with rat bone marrow-derived mesenchymal stem cells (RBMSCs) in repairing cartilage defects.
Materials and Methods: Rat BM-MSCs will be isolated, expanded, characterized, and tested in-vitro to determine the nontoxic effective dose of berberine and its chondrogenic potential using MTS assay, pellet culture, and histological staining. Bovine type II collagen will be extracted from knee cartilage, crosslinked with BDDE, and loaded with berberine. Knee OA was induced in adult rats by intra-articular injection of collagenase and papain/cysteine into the knee joint, followed by confirmation of cartilage degeneration using X-ray imaging. Rat models were assigned to seven experimental groups: intact control, Col II-MSCs, Col II-BBR, Col II-BBR-MSCs, sham Col II, untreated OA, and hyaluronic acid control. Cartilage repair will be assessed macroscopically by X-ray imaging and pathological scoring, and microscopically by histological evaluation and quantitative gene-expression analysis of chondrogenic markers, including Col II, Aggrecan, SOX9, and Col X, using real-time PCR.
Results: The regenerated OA tissue demonstrated higher expression of genes associated with cartilage degradation than native OA tissue. Berberine treatment increased the expression of key chondrogenic markers, particularly Sox9 and Col2, indicating enhanced chondrogenic commitment and cartilage matrix formation. Aggrecan expression showed a more delayed increase, with stronger expression at day 14 than at day 7, suggesting progressive extracellular matrix maturation. However, increased Col2 expression was also observed in treated groups, indicating possible hypertrophic differentiation that requires further validation at the protein and histological levels. Histopathological evaluation further confirmed improved cartilage regeneration in the treated groups, as evidenced by enhanced cartilage matrix deposition.
Conclusion: A berberine-loaded type II collagen scaffold combined with BM-MSCs represents a promising regenerative construct for osteoarthritic cartilage repair. Berberine promotes chondrogenic differentiation, as shown by increased SOX9, COL2, and Aggrecan, particularly by day 14. The Results of this study suggested that berberine ameliorates cartilage repair and could be used as a therapeutic in patients with OA.
Keywords: Type II Collagen, Berberine, Collagen Scaffolds, Osteoarthritis, Mesenchymal Stem Cells, Rat Model

Ps-35: Tailoring Morphology in PGS-Chitosan Scaffolds for Skin Tissue Repair

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Background: Skin injuries caused by burns, diabetes, and chronic wounds remain major clinical challenges due to delayed healing and scar formation. Tissue engineering has emerged as a promising approach for wound repair through the development of biomimetic scaffolds that support tissue regeneration. Among different biomaterials, poly(glycerol sebacate) (PGS) has gained attention because of its biodegradability and elastomeric properties similar to soft tissues. Additionally, chitosan and its derivatives, including sulfated chitosan (SCS) and carboxymethyl chitosan (CMCS), possess beneficial properties such as biocompatibility, antibacterial activity, and angiogenic potential. In this study, a 3D PGS scaffold modified with chitosan derivatives was designed for full-thickness and diabetic wound healing.
Materials and Methods: In this study, a three-dimensional poly(glycerol sebacate) (PGS) scaffold with dual porosity was fabricated using NaCl particles and an extrusion-based 3D printing technique. The fabricated scaffold was subsequently surface-modified with chitosan and its derivatives. Surface characterization and confirmation of the modification were performed using X-ray photoelectron spectroscopy (XPS) and attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR).
Results: The study groups included unmodified PGS scaffolds, chitosan-modified PGS (PGS-CS), sulfated chitosan-modified PGS (PGS-SCS), and carboxymethyl chitosan-modified PGS (PGS-CMCS). ATR-FTIR analysis, SEM imaging, mechanical testing, swelling behavior, porosity, and degradation assays were performed for the unmodified PGS, PGS-CS, and PGS-SCS groups. The obtained findings confirmed successful surface modification and demonstrated physicochemical and structural properties consistent with the expected outcomes and previously reported studies in tissue engineering applications.
Conclusion: The obtained Results demonstrated that surface modification of PGS scaffolds with chitosan-based biomaterials successfully improved the physicochemical and structural characteristics of the scaffolds. These findings suggest that modified PGS scaffolds, particularly the sulfated chitosan-coated group, may provide promising candidates for future skin tissue engineering applications.
Keywords: Tissue Engineering, 3D Printing, Angiogenesis, Surface Modification

Ps-36: Antibacterial Activity of Lactobacillus-Derived Extracellular Vesicles and Cell-Free Supernatants against Clinical Pathogens

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Background: Probiotic bacterial extracellular vesicles (EVs) are nanoscale, lipid-enclosed particles that carry proteins, nucleic acids, and metabolites, enabling them to modulate host physiology. Compared to live probiotics, EVs offer a safer postbiotic alternative without risks of bacteremia or horizontal gene transfer. These vesicles contain antimicrobial peptides and bacteriocins and can fuse with bacterial targets to deliver their cargo directly. In this study, we investigated the antibacterial activity of cell-free supernatants (CFS) and EVs derived from Lactobacillus strains.
Materials and Methods: CFS were prepared from Lactobacillus fermentum and Lactobacillus reuteri cultures. Antibacterial activity was evaluated against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and methicillin-resistant Staphylococcus aureus (MRSA) using broth microdilution following CLSI M07-A11 guidelines. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. EVs were isolated from L. plantarum cultures by commercial kit and similarly tested for antimicrobial activity.
Results: L. fermentum CFS showed notable antibacterial potency, with MICs of 5, 10, 25, and 25 mg/mL against S. aureus, E. coli, P. aeruginosa, and MRSA, respectively; corresponding MBCs were 10, 10, 25, and 25 mg/mL. In contrast, L. reuteri CFS had higher MIC/MBC values (25, 20, 25, 25 mg/mL for both). Thus, L. fermentum CFS was generally more active against these pathogens. The isolated L. plantarum EVs also demonstrated antibacterial effects: both S. aureus and *E. coli* were inhibited at 1000 µg/mL (MIC), and *E. coli* was killed at 1000 µg/mL (MBC). No activity against P. aeruginosa or MRSA was observed up to 1000 µg/mL. In summary, probiotic CFS and EVs retained measurable inhibitory activity against Gram-positive and Gram-negative pathogens, although effective concentrations were higher for EVs than for crude supernatants.
Conclusion: These data confirm that secreted factors from probiotic Lactobacillus strains have broad-spectrum antibacterial properties. Although only antibacterial effects were investigated in this study, probiotics EVs have been reported to exert anticancer, immunomodulatory, and regenerative activities, including enhancement of cancer-cell chemosensitivity, induction of apoptosis, and regulation of mesenchymal stromal cell–associated immune responses. Therefore, leveraging probiotic EVs could support stem cell and regenerative medicine strategies, such as improving tissue repair, delivering immunomodulatory factors, or protecting stem cells from infection and inflammation.
Keywords: Lactobacillus-Derived Extracellular Vesicles, Cell-Free Supernatants, Antibacterial Activity, Minimum Inhibitory Concentration (MIC), Gram-Positive and Gram-Negative Pathogens

Ps-37: Feasibility Study of Concentrating γ-Retroviral Vectors Produced in PG 13 Cell Line for Efficient CAR T Cell Production

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Background: Efficient CAR-T cell production requires high-titer viral vectors with preserved infectivity. Stable γ-retroviral

producer cell lines provide scalable vector production; however, low-titers are often insufficient for efficient transduction. This study aimed to optimize a simple and cost-effective centrifugation-based concentration strategy.
Materials and Methods: γ-retroviral vectors encoding GFP labeled constructs were produced using a stable PG-13 producer cell line. Viral supernatants were concentrated using different centrifugation conditions (time/g). Viral infectivity was evaluated by HEK-293T transduction and flow cytometric analysis of GFP expression. Viral titers and recovery rates were calculated.
Results: Initial concentration using 20,000×g for 3 hours resulted in severe viral loss and low recovery (6.7%). Optimization using 9,000×g for 3h significantly improved recovery and infectivity compared with high-speed centrifugation. Concentrated viral preparations reached titers up to 6.4×10^6 TU/mL with approximately 53% recovery. Longer centrifugation durations decreased residual virus levels in the supernatant; however, overall recovery remained relatively unchanged, suggesting concurrent loss of viral infectivity during prolonged centrifugation. Since considerable loss of functional viral particles was still observed at 9,000×g, lower centrifugal force conditions (6000×g) were subsequently investigated to minimize mechanical damage while maintaining concentration efficiency.
Conclusion: These findings suggest that low-speed centrifugation may provide an accessible and effective strategy for concentrating GALV-pseudotyped γ-retroviral vectors while preserving viral infectivity for CAR-T cell production applications.
Keywords: CAR-T cell, γ-retrovirus, Centrifugation, Viral Concentration

Ps-38: Incorporatng Quercetn into Silk Fibroin/Bacterial Nanocellulose Composite Scaffolds Enhances Their Biological Performance for Bone Tssue Engineering Applicatons

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Background: Polymeric composite scaffolds composed of natural biomaterials have aVracted increasing attention due to their biocompatibility, structural similarity to extracellular matrix, and favorable physicochemical properties. The present study aimed to fabricate and evaluate three-dimensional composite scaffolds based on bacterial nanocellulose (BC) and silk fibroin (SF) loaded with Quercetin (5%) to improve the biological performance of the scaffold for bone tissue engineering purposes.
Materials and Methods: Quercetin was incorporated at a concentration of 5% into BC/SF composite scaffolds during scaffold fabrication. The morphology of the scaffolds was examined using scanning electron microscopy (SEM). Physicochemical characteristics were evaluated through porosity analysis, mechanical testing including compressive strength and elastic modulus, and Fourier Transform Infrared Spectroscopy (FTIR) to verify interactions between scaffold components. Human adipose-derived stem cells (hASCs) were cultured on the prepared scaffolds to assess their biological performance. Cell phenotype was confirmed by flow cytometry. Cellular metabolic activity was measured using the MTT assay, while osteogenic potential was evaluated through mineralization staining and alkaline phosphatase (ALP) enzyme activity assays.
Results: SEM observations demonstrated that the composite scaffolds possessed an interconnected porous structure suitable for cell infiltration and tissue growth. Incorporation of BC and 5% Quercetin into the SF matrix slightly modified the scaffold porosity, yielding pores with an average size of approximately 200–210 μm while improving structural stability. Mechanical evaluation indicated enhanced compressive strength and elasticity compared with scaffolds lacking Quercetin. FTIR analysis confirmed the successful incorporation of BC and Quercetin within the SF network. Flow cytometry verified the characteristic surface markers of hASCs. Biological assays showed that the BC/SF scaffolds containing 5% Quercetin supported greater cell attachment and metabolic activity compared with the control scaffolds. In addition, increased mineral deposition and elevated ALP activity suggested improved osteogenic differentiation of hASCs on Quercetin -containing scaffolds.
Conclusion: The developed BC/SF composite scaffolds enriched with 5% Quercetin exhibited favorable physicochemical properties and promoted osteogenic activity of stem cells. These Results indicate that this biocomposite scaffold could serve as a promising platform for bone tissue regeneration.
Keywords: Bone Tissue Engineering, Curcumin, Biocomposite Scaffold, Osteogenic Activity

Ps-39: Immune Profile of Early-Onset Colorectal Cancer: A Systematic Review

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Background: Early-onset colorectal cancer (EOCRC), usually defined as colorectal cancer diagnosed before the age of 50, has been increasing in recent years and is expected to continue rising until 2030. Compared with late-onset colorectal cancer (LOCRC), EOCRC often shows more aggressive features, including poorly differentiated histology, and is associated with poorer survival outcomes. However, its immune characteristics are still not fully understood. This study aimed to systematically review the available evidence on the immune profile of EOCRC.
Materials and Methods: A systematic review was conducted using the Web of Science and Scopus databases, and 23 studies were included. Eligible studies were original articles published between 2020 and 2026 that investigated the immune profile of EOCRC in humans. EOCRC was defined as colorectal cancer diagnosed before age 50 years, and in some studies before age 40 years. Studies using laboratory experiments, bioinformatics, or machine learning approaches were also included. Exclusion criteria included review articles, case reports, animal studies, and publications outside the 2020 –2026 time frame.
Results: Studies showed that immune cell infiltration was generally increased in tumor tissues of EOCRC, although some studies reported lower infiltration in more invasive tumors. An immunosuppressive tumor microenvironment was also observed, mainly characterized by increased expression of pro-

grammed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1). However, immune suppression was also reported with reduced PD-1 expression. Transforming growth factor beta (TGF-β) pathways were also involved in immune suppression. In peripheral blood, an activated immune state was linked to immunosenescence. Also, higher natural killer (NK) cell activity and expression of innate immune related genes were associated with poorer survival outcomes. Overall, EO-CRC showed a complex immune landscape.
Conclusion: The Results showed that the immune phenotype of EOCRC is different from that of LOCRC and shows significant heterogeneity, which may indicate that other factors, including microbiota and genetic mutations, play a role in shaping the immune microenvironment. Therefore, identifying different populations of innate and adaptive immune cells could support the development of more targeted and effective therapies.
Keywords: Early-Onset Colorectal Cancer, Colorectal Cancer, Immune Profile, Immunity

Ps-40: Evaluation of The Controversial Role of KDM6A on Epithelial-Mesenchymal Transition in Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is a common and lethal malignant neoplasm worldwide. Lysine demethylase 6A (KDM6A) is a histone H3 lysine 27 demethylase, which plays an essential role in cell differentiation, apoptosis, EMT (epithelial-mesenchymal transition) and tumor progression; Moreover this enzyme serves as a tumor suppressor or oncogene, depending on the cancer types. Several genes and signaling pathways are altered in HCC, which can alter the phenotype and feature of the cancer cells. However, whether KDM6A participates in the progression of HCC remains unclear. In the present study we evaluated the effect of KDM6A down-regulation by specific siRNA on cell migration and EMT evaluation in Huh-7 cells.
Materials and Methods: In this study, assessments were performed on the Huh-7 cells. The specific siRNA was used to knock-down the target gene and q-PCR was performed to validate loss of function. Moreover, in the mRNA expression level, CDH1/2 genes expression were quantified, and at the functional level scratch assay was performed.
Results: Down-regulation of the KDM6A gene led to significant morphological alterations, enhanced expression of the mesenchymal marker, N-cadherin, and reduced level of the epithelial marker, E-cadherin; Moreover, the treated cells exhibited a boosted migration rate compared to the control group.
Conclusion: In summary, our findings indicate that the loss of KDM6A function in HCC may enhance migration and promote the EMT. Further investigation could provide more evidence of its tumor-suppressor role, potentially establishing this gene as a molecular target for future therapeutics.

Keywords: KDM6A, Liver Cancer, Epigenetic Modifier, Molecular Targeted Therapy, EMT

Ps-41: Overcoming Therapy Resistance in Triple-Negative Breast Cancer: Computational Targeting of The ALDH1A1 Axis

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Background: Breast cancer remains a leading cause of global cancer mortality, with cancer stem cells (CSCs) driving therapy resistance and clinical recurrence. Aldehyde dehydrogenase 1A1 (ALDH1A1) functions as a crucial molecular driver in this pathology, particularly within triple-negative breast cancer (TNBC). This study investigates the druggability of ALDH1A1 as a therapeutic strategy to overcome CSC-mediated resistance.
Materials and Methods: A focused library of 7,159 ligands was constructed by retrieving structural analogs of established ALDH1A1 inhibitors and substrates from the PubChem and SwissSimilarity databases. Following filtration via Lipinski's Rule of Five, 3D conformations were generated using Frog2. The crystallographic target (PDB ID: 7UM9) was retrieved from the Protein Data Bank (PDB) and prepared in PyMOL. Subsequently, structure-based virtual screening targeting the catalytic pocket was executed via Molegro Virtual Docker (MVD). To mitigate algorithmic bias, the top 10 candidates (MolDock score < -190) were subjected to cross-docking utilizing AutoDock Vina, with the co-crystallized NQR ligand serving as a benchmarking reference. The premier consensus candidate was then evaluated for drug-likeness, pharmacokinetic properties, and toxicity profiles using ADMETlab 3.0 and ProTox-3.0.
Results: Compound PubChem CID 19792152 was identified as the optimal consensus ALDH1A1 inhibitor (MolDock: -197.136, Vina: -10.1 kcal/mol), exhibiting superior binding affinity compared to the reference NQR (MolDock: -191.307, Vina: -8.714 kcal/mol). Pose analysis indicated the formation of four critical hydrogen bonds: two with Ser247, and individual interactions with Lys353 and Gln350. The candidate displayed an ideal absorption, distribution, metabolism, and excretion (ADME) profile, maintaining zero Lipinski violations, exceptional membrane permeability in parallel artificial membrane permeability assays (PAMPA: 0.999), and favorable synthetic accessibility (SAscore: 2.0). Toxicological assessments revealed an absence of pan assay interference compounds (PAINS), ALARM NMR, and BMS alerts, alongside a predicted low cytotoxicity against A549 and HEK293 cell lines. However, computational profiling indicated a potential risk for genotoxicity and organ-specific toxicities.
Conclusion: This computational framework integrates rigorous virtual screening and structure-based prioritization to establish ALDH1A1 inhibition as a viable strategy against CSC-mediated resistance. PubChem CID 19792152 is nominated as a highly promising lead-like candidate. Subsequent structure-activity relationship (SAR) optimization and empirical validation, encompassing biophysical binding and cell-based assays, are warranted to translate its therapeutic potential.
Keywords: ALDH1A1, Cancer Stem Cells, Triple-Negative Breast Cancer, Molecular Docking, Computational Drug Design

Ps-42: An Update to Approved Cell, Gene, and Tissue Therapy Products

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Background: Cellular and gene therapy products (CGTPs) are distinct therapeutic products intended for human use to provide cures, prevention, palliative care, or diagnostic functions. These therapeutic approaches are revolutionizing medical science by targeting previously incurable diseases, including inherited genetic disorders, blood-related diseases, malignancies, and neurodegenerative disorders.
Materials and Methods: A systematic review was conducted according to PRISMA guidelines. We searched regulatory agency databases (FDA, EMA, PMDA), clinical trial registries (ClinicalTrials.gov), relevant company websites, and peer-reviewed articles (PubMed, Scopus) from January 2010 to November 2025. Inclusion criteria: approved or investigational CGTPs for human use with publicly available regulatory or manufacturer data. Exclusion criteria: non-human studies, withdrawn products, and duplicate reports. Two independent reviewers extracted data on product characteristics, indications, and approval status. Descriptive synthesis was performed.
Results: A total of 121 CGTPs met the inclusion criteria. These were categorized as follows: Gene therapy products: 45 products (37.2%) Cell therapy products: 44 products (36.4%) Tissue engineering products: 32 products (26.4%) For each product, we extracted and synthesized data regarding product description, manufacturer, indications, approval dates, and corresponding regulatory agency. Notably, the latest analysis by the Alliance for Regenerative Medicine reports 2,125 ongoing or completed clinical trials for CGTPs globally as of November 2025. The majority of approved products target hematologic malignancies, genetic disorders, and musculoskeletal conditions.
Conclusion: The landscape of CGTPs is rapidly expanding, with 121 distinct products (45 gene therapy, 44 cell therapy, 32 tissue engineering) now available or in late-stage development. These products are shifting clinical practice toward curative and regenerative approaches. However, standardization of regulatory reporting and long-term safety data remain needed.
Keywords: Cancer, Immunotherapy, Solid Tumor, Car-T Cell, Extracellular Vesicles

Ps-43: Targeting Matrix Metalloproteinases to Treat Diabetic Foot Ulcers

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Background: Today, diabetes is recognized as one of the greatest concerns of the global community. One of the most challenging of its complications are wounds that develop in the lower extremities, and they are among the most important causes of hospitalization in diabetic patients. Impaired healing of these wounds can provide the basis for their chronification and infection, and in acute cases, complications resulting from infection can lead to amputation and ultimately disability and death.
Materials and Methods: We found 56 articles from the PubMed search database using the Keywords Diabetes, DFU, and MMPs. The time range of the articles was from 2010 to 2024. After reviewing the titles, introductions, and Results, we selected 47 articles and 9 articles were excluded due to duplication and lack of relevance to the subject.
Results: Oxidative stress and chronic inflammation resulting from hyperglycemia, increased production of Advanced Glycation End Products (AGEs) and the subsequent increased activity of the AGE/RAGE axis, and the creation of a reinforcing cycle between cytokines and macrophages can lead to increased expression of certain Matrix metalloproteinases (MMPs) such as (MMP-9), which under normal conditions is responsible for the degradation of Extracellular Matrix (ECM) in the inflammatory phase of wound healing. Furthermore, in the event of wound infection and the activity of bacterial metalloproteinases, their synergistic effect with the human type causes further destruction of the healing area and will lead to slow or arrested wound healing. Inhibition of MMPs is carried out using small inhibitory molecules, interfering RNAs, and antibodies. Today, the use of mesenchymal stem cells for wound healing has also become a promising strategy; animal studies have shown that this method can lead to a significant reduction in MMP-9 expression.
Conclusion: The use of MMP inhibitory compounds that restore the MMPs/TIMPs balance can lead to the improvement of chronic wounds. Although more studies are needed, today MMP-9 measurement can be used to determine the status of wound healing and establish a personalized treatment protocol for the patient.
Keywords: Diabetis, DFU, MMPs

Ps-44: Computational Targeting of The DUSP6–ERK Feedback Axis to Support Higher Signal-Processing-Associated States in Human Cerebral Organoids

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Background: Human cerebral organoids are increasingly explored as biological platforms for studying neural activity and signal processing. However, identifying molecular regulators that may support higher processing-associated states remains a major challenge. Connected cerebral organoids provide a relevant model to investigate transcriptional programs associated with increased network complexity. Extracellular signal-regulated kinase (ERK) signaling is involved in activity-dependent neural responses, while dual-specificity phosphatase 6 (DUSP6), also known as mitogen-activated protein kinase phosphatase 3 (MKP3), negatively regulates ERK activity. Here, we investigated whether DUSP6 could represent a druggable regulatory target linked to higher signal-processing-associated organoid states.
Materials and Methods: Public single-cell RNA sequencing (scRNA-seq) data from GSE190729 were analyzed across single, fused, and reciprocally connected cerebral organoid condi-

tions. Following quality control, normalization, clustering, and cell-state annotation, differential expression analysis and pathway-level scoring were used to identify molecular programs associated with connected organoid states. Candidate targets were prioritized through machine-learning-based feature selection, protein–protein interaction (PPI) network analysis, functional relevance to ERK signaling, and druggability assessment. DUSP6 was selected as a protein-level ERK-feedback target. Structure-based virtual screening was then performed using the experimentally resolved human DUSP6/MKP3 catalytic-domain structure from Protein Data Bank (PDB) entry 1MKP. Since BCI/NSC150117 is a reported allosteric DUSP6 inhibitor, docking was focused on the BCI-like allosteric pocket near the general acid loop and helix α7. A total of 1,188 BCI analogs were screened, and the top compound was evaluated by docking, interaction profiling, drug-likeness, and toxicity prediction.
Results: PubChem CID 16573196 was identified as the top BCI-like analog, showing a stronger MolDock score than the reference inhibitor BCI/NSC150117 (-128.676 versus -115.364). AutoDock Vina further supported favorable binding with a score of -7.313 kcal/mol. Interaction profiling showed contacts with key residues within the BCI-like allosteric region, including Trp264, Asn335, Phe336, Asp262, Arg299, Ser261, Gln266, and Gln339. The compound showed zero Lipinski violations and no Pan Assay Interference Compounds (PAINS) or Brenk alerts. Toxicity prediction suggested acceptable acute toxicity, while indicating liabilities that require further optimization.
Conclusion: This study links connected organoid transcriptomic analysis with allosteric structure-based screening, nominating DUSP6 as a computationally actionable ERK-feedback target. PubChem CID 16573196 is prioritized as a promising BCI-like allosteric DUSP6 hit for future experimental validation aimed at modulating signal-processing-associated states in cerebral organoids.
Keywords: Brain Organoid, DUSP6, ERK Signaling, Signal Processing, Virtual Screening

Ps-45: Micropatterned PDMS Soft Substrate Promotes The Maturation of Neonatal Rat Cardiomyocytes

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Background: Cardiomyocytes derived from stem cells or neonatal sources represent a promising platform for cardiac regeneration and disease modeling. However, their structural and functional immaturity remains a major limitation, as they fail to fully recapitulate the adult cardiomyocytes phenotype. Accumulating evidence indicates that physical properties of culture substrate, including stiffness and topography, play critical role in promoting cardiac maturation through mechanotransductive signaling pathways. This study aimed to establish a cost- and time-efficient protocol using a soft micropatterned polydimethylsiloxane (PDMS) substrate to enhance the maturation of neonatal rat cardiomyocytes (NRCMs) for cardiovascular research applications.
Materials and Methods: NRCMs were isolated from 1-day-old pups and cultured for 7 days under three substrate conditions: (i) standard tissue culture polystyrene (TCPS, control), (ii) soft non-patterned PDMS (30:1), and (iii) soft PDMS containing aligned micropatterns (30:1, 20 µm width, 5µm depth). Following culture, maturation was evaluated at morphological, molecular, and functional levels. Immunofluorescence staining for cTnI and F-actin was performed to assess sarcomere organization, circularity, and aspect ratio. The expression levels of maturation-related genes (*cTnI*, *α-MHC,* and *Cx43*) and mechanotransduction-associated genes (*RhoA* and *ROCK*) were analyzed by qRT-PCR. Functional maturation was assessed by measuring spontaneous beating activity and field potential characteristics using multielectrode array (MEA) recordings.
Results: NRCMs cultured on micropatterned soft PDMS exhibited significantly improved morphological maturation compared with TCPS and non-patterned PDMS controls. These cells displayed a more elongated morphology, reflected by reduced circularity and increased aspect ratio, together with enhanced sarcomere organization and aligned F-actin fibers. Gene expression analysis demonstrated significant upregulating of *cTnI* as well as an slight increase in *α-MHC* and *Cx43* in the micropatterned PDMS group relative to controls. Additionally, increased expression of RhoA but not ROCK indicated possible activation of mechanotransductive signaling pathways. Functionally, cardiomyocytes cultured on the micropatterned substrate exhibited more synchronous and stable spontaneous beating activity, along with improved field potential characteristics.
Conclusion: These findings demonstrate that a soft PDMS substrate incorporating aligned micropatterns effectively promotes the structural, molecular, and functional maturation of NRCMs. This cost- and time-efficient platform provides a reliable strategy for generating more mature cardiomyocytes *in-vitro*, with potential applications in cardiac disease modeling, drug screening, and developmental studies in cardiovascular regenerative medicine.
Keywords: PDMS, NRCMS, Cardiomyocytes, Micropattern, Maturation

Ps-46: Curcumin-Enhanced Bacterial Cellulose/Silk Fibroin Scaffolds for Bone Regeneration

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Background: Large bone defects remain a major clinical challenge, and current grafting approaches are often limited by donor site morbidity, limited availability, and insufficient integration with host tissue. Bacterial cellulose/silk fibroin (BC/SF) scaffolds are attractive biomaterials for bone tissue engineering due to their biocompatibility and structural support; however, their inherent bioactivity is limited. Curcumin, a natural polyphenolic compound derived from Curcuma longa, possesses antioxidant, anti-inflammatory, and osteogenic properties, making it a promising candidate for scaffold functionalization. Nevertheless, the effect of curcumin loading concentration on the physicochemical and biological performance of BC/SF scaffolds has not been fully clarified.
Materials and Methods: The present study aimed to fabricate BC/SF scaffolds loaded with curcumin at 1%, 2%, and 4 wt%

and to evaluate their potential for bone regeneration. Scaffold morphology was examined by field emission scanning electron microscopy (FE-SEM), while chemical interactions were analyzed using Fourier transform infrared spectroscopy (FTIR). In addition, swelling behavior, degradation rate, porosity, and compressive mechanical properties were assessed. MG63 osteoblast-like cells were used to investigate cell viability by MTT assay, alkaline phosphatase (ALP) activity, calcium mineralization by Alizarin Red S staining, and osteogenic gene expression including RUNX2, ALP, and osteocalcin (OCN).
Results: Curcumin incorporation led to a concentration-dependent reduction in pore size, porosity, swelling, and degradation rate, while significantly improving compressive strength and elastic modulus. Among the tested groups, the scaffold containing 4 wt% curcumin showed the best biological performance, including significantly higher cell viability, ALP activity, mineral deposition, and osteogenic gene expression compared with pristine BC/SF scaffolds and lower curcumin concentrations ($P < 0.05$).
Conclusion: In Conclusion, curcumin significantly enhanced both the structural and osteogenic properties of BC/SF scaffolds. The 4 wt% curcumin-loaded scaffold demonstrated the most favorable balance between mechanical integrity and biological activity, suggesting its potential as a promising biomaterial for bone tissue engineering applications.
Keywords: Curcumin, Bacterial Cellulose/Silk Fibroin, Osteogenesis, Bone Tissue Engineering

Ps-47: Regeneration Potential of Calcitonin Incorporated into Alginate/Gelatin Scaffolds

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Background: This study aimed to fabricate and evaluate porous scaffolds based on alginate and gelatin for bone tissue engineering applications. The scaffolds were further functionalized with calcitonin (CT) using a freeze-drying method to enhance their biological performance. The main Objective was to investigate the ability of these scaffolds to support cell attachment, proliferation, and osteogenic differentiation of human adipose-derived stem cells (hASCs).
Materials and Methods: The physicochemical properties of the prepared scaffolds were characterized by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). In addition, porosity, compressive strength, swelling behavior, and degradation rate were evaluated to determine the structural suitability of the scaffolds for bone regeneration. Their biological performance was assessed by MTT assay, alkaline phosphatase (ALP) activity, Alizarin Red S staining, and expression analysis of osteogenic genes.
Results: The incorporation of calcitonin into the alginate/gelatin (Alg/Gel) scaffold influenced the scaffold architecture and physicochemical properties, resulting in reduced pore size and swelling ratio, while maintaining an interconnected porous structure favorable for cell infiltration. MTT Results confirmed good biocompatibility of all scaffold groups, with calcitonin-loaded Alg/Gel scaffolds showing significantly improved cell viability after 72 hours. Hemocompatibility assessment also demonstrated minimal hemolytic activity in the engineered scaffolds. Importantly, osteogenic analyses showed that calcitonin-loaded Alg/Gel scaffolds significantly enhanced ALP activity and calcium mineral deposition compared with scaffolds without calcitonin. Moreover, the expression levels of osteogenic markers, including RUNX2, ALP, and osteocalcin (OCN), were markedly upregulated in hASCs cultured on calcitonin-incorporated scaffolds.
Conclusion: These findings indicate that calcitonin incorporation improves the osteoinductive properties of alginate/gelatin scaffolds and supports their application as promising biomaterials for bone regeneration and bone tissue engineering.
Keywords: Osteogenesis, Bone Regeneration, Calcitonin, Alginate, Gelatin

Ps-48: Targeting Esophageal Cancer in Mouse Models by Co-Administration of Enoxacin and miR-106a Inhibitors

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Background: Esophageal squamous cell carcinoma (ESCC) remains treatment-resistant. The small molecule enoxacin boosts tumor-suppressive miRNAs via stimulating DICER-TRBP2 complex, and miR-106a-5p inhibition may enhance ESCC therapy, as evidenced by our *in-vitro* findings. This study explores their combined potential in ESCC inhibition *in-vivo*.
Materials and Methods: Two xenograft models were established using KYSE-30 cells in nude mice. In the first model, cells were pretreated with enoxacin (62 and 124 μM), miR-106a-5p antagomir (100 nM), or combination therapy prior to subcutaneous injection. In the second model, untreated KYSE-30 cells were first injected into mice, followed by systemic administration of enoxacin (5 and 10 mg/kg, intraperitoneal) and intratumoral injection of miR-106a-5p antagomir (1 μg/tumor). Tumor growth was monitored by measuring tumor volume and weight. Histopathological analyses were performed using hematoxylin and eosin staining, while Ki67 and KLF4 protein expression analyses were assessed by immunofluorescence staining. Quantitative RT-PCR was used to analyze the expression of genes associated with proliferation, migration, and cancer stemness.
Results: Combination treatment significantly inhibited tumor growth in both xenograft models compared with single treatments. Remarkably, the combination of 62 μM enoxacin with miR-106a-5p antagomir produced anti-tumoral effects comparable to those observed with high-dose enoxacin alone (124 μM). Histological evaluation revealed reduced mitotic activity, lower cellular density, decreased nuclear pleomorphism, and increased apoptotic features in the combination group. Molecular analyses demonstrated significant modulation of several cancer-related genes, including *c-MYC, CCND1, SNAIL1, CD44, CD133, LIN28, OCT4, SOX2, KLF4*, and *NANOG*. Furthermore, immunofluorescence staining confirmed reduced Ki67-positive cells and decreased KLF4 expression following combination therapy.

Conclusion: Our findings demonstrate that simultaneous targeting of miR-106a-5p and miRNA maturation pathways enhances the anti-tumoral efficacy of enoxacin in ESCC xenograft models. This combinational strategy may represent a promising therapeutic approach for esophageal cancer and provides a foundation for future translational and clinical investigations.
Keywords: ESCC, Enoxacin, miR-106a-5p, Antagomir, Combination Therapy

Ps-49: Combination of Enoxacin and miR-106a Inhibitor Potently Suppresses The Survival And Migration Of Esophageal Cancer Cells In-Vitro

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Background: Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive malignancies with poor prognosis and limited therapeutic options. Previous studies from our group demonstrated that enoxacin, a fluoroquinolone small molecule known to enhance global microRNA maturation through stimulation of the DICER-TRBP2 complex, suppresses ESCC cell growth both *in-vitro* and *in-vivo*. Since enoxacin inadvertently upregulates certain pro-tumor miRNAs, we asked whether targeting a typical pro-tumor miRNA upregulated by enoxacin—i.e. miR-106a-5p—could enhance the inhibitory effects of enoxacin on ESCC cell growth and migration.
Materials and Methods: KYSE-30 cells were transfected with a miR-106a-5p antagomir using Lipofectamine 3000. Transfection efficiency was optimized and validated using FITC-labeled oligonucleotides and fluorescence microscopy. The effects of enoxacin (62 and 124 μM), antagomir (100 nM), and combination treatment were evaluated using MTS assay, crystal violet staining, live/dead staining, and wound-healing migration assay. Quantitative RT-PCR was performed to assess miR-106a-5p expression and the expression of validated target genes including *BMPR2, MEF2C, ID1, CD28*, and *KLF9*.
Results: Optimization experiments demonstrated that 0.05 μL Lipofectamine achieved approximately 75% transfection efficiency without significant cytotoxicity. Although antagomir treatment did not significantly reduce miR-106a-5p levels, increased expression of its downstream target genes confirmed functional inhibition of the miRNA. Inhibition of miR-106a-5p alone had no significant effect on KYSE-30 cell viability but substantially reduced cell migration. Combination treatment with enoxacin and antagomir produced a markedly stronger inhibitory effect on cell viability and migration compared with either treatment alone. Notably, combined treatment with 62 μM enoxacin and 100 nM antagomir exhibited anti-growth activity comparable to 124 μM enoxacin alone, suggesting a synergistic interaction between the two treatment approaches.
Conclusion: Combined targeting of miR-106a-5p and miRNA maturation pathways significantly enhances the anti-cancer effects of enoxacin in ESCC cells. These findings support the therapeutic potential of combinational miRNA-based strategies for esophageal cancer treatment.

Keywords: ESCC, Enoxacin, miR-106a-5p, Antagomir, Combination Therapy

Ps-50: Isolation, Purification, and Wound Healing Activity of Human Umbilical Cord Wharton's Jelly Collagen in A Rat Animal Model

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Background: Collagen is a key ECM protein essential for tissue function. Its biocompatibility makes collagen biomaterials widely used in regenerative medicine. However, animal-derived collagen carries diseases and ethical risks. Wharton's jelly (WJ) from umbilical cord tissue is rich in collagen and offers a non-invasive, ethical alternative. This study extracted collagen from human WJ as a promising biomaterial for tissue engineering.
Materials and Methods: Umbilical cord samples were collected from healthy mothers after signing the informed consent. Placental segments (~15 cm) were separated, washed, and dissected to separate Wharton's jelly, which was stored at -20°C. For collagen extraction, the ground tissue was treated with NaOH to remove non-collagenous proteins, followed by acid extraction and pepsin-assisted digestion. Then, the extracted protein was analyzed for purity, the type of isolated collagen, toxicity, and wound healing in a rat wound model, using BCA, Western blot (WB), hydroxyproline, SDS-PAGE, MTS, and cell migration tests, as well as histological techniques.
Results: WB Results showed distinct protein bands in all extract samples, corresponding to collagen I, III, IV, and V. Hydroxyproline assay indicated collagen content equivalent to 3.5% of tissue dry weight. Up to 1mg/mL, extracted collagen showed no cytotoxicity on human fibroblast cells (HDFs) and enhanced fibroblast viability beyond 100%. It also induced migration of HDFs significantly compared to the control group, confirming bioactivity and chemoattractant properties. On day 14, histopathological evaluation revealed acceptable epidermal thickness, dermal collagen density, inflammatory cell infiltration, and angiogenesis compared to commercial collagen.
Conclusion: Human Wharton's jelly represents a promising alternative source of collagen for regenerative medicine and tissue engineering applications. Compared with animal-derived collagen, human-derived collagen may provide reduced immunogenicity and improved clinical compatibility. The present study establishes a preliminary platform for the extraction of collagen proteins from Wharton's jelly and highlights its potential for future applications in biomaterial fabrication, wound healing, scaffold engineering, and regenerative therapies.
Keywords: Wharton's Jelly, Collagen Extraction, Umbilical Cord, Skin Wound Model

Ps-51: Assessment of Properties of Collagen Proteins Extracted from Humans Wharton Jelly in-Vitro and In-Vivo

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Background: Collagen, a key extracellular matrix component, supports wound healing by enhancing cell migration, proliferation, and tissue remodeling. Improving healing speed remains challenging in regenerative medicine. Wharton's jelly has recently gained attention as a rich, biocompatible collagen source with promising therapeutic potential.
Materials and Methods: Collagen, a principal component of the extracellular matrix with a vital role in wound healing, has emerged from Wharton's jelly as a promising biocompatible source; accordingly, the present study aims to evaluate its extracted from using *in-vitro* and *in-vivo* approaches, including the culture of human fibroblasts on collagen-based hydrogels. Cell migration will be assessed using the scratch (wound healing) assay by measuring wound closure over 0, 12, 24, and 48 hours. Cell viability will be evaluated using the Live/Dead assay with Calcein-AM and Ethidium Homodimer-1 staining at days 1, 3, and 5. Additionally, metabolic activity and proliferation will be quantified using the MTS assay by measuring optical density at 490–500 nm.
Results: It is anticipated that collagen derived from Wharton's jelly will enhance fibroblast viability, proliferation, and migration in-vitro, and significantly improve wound closure, angiogenesis, and tissue organization in-vivo compared to controls.
Conclusion: This study aims to demonstrate the therapeutic potential of Wharton's jelly-derived collagen as an effective biomaterial for wound healing applications, providing a basis for its future use in regenerative medicine and clinical treatments.
Keywords: Wharton's Jelly, Collagen, Wound Healing, Fibroblast, Hydrogel

Ps-52: The Role of GCLC Gene Expression in Colorectal Cancer

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Background: Colorectal cancer (CRC) is the fourth leading cause of cancer-related death in the world. There are many prevention methods, one of which is timely screening. According to studies, measuring biomarkers is considered as one of the preventive methods that can be used non-invasively. Therefore, identifying biomarkers involved in CRC is very important. The GCLC gene is a prognostic factor in kidney and lung.
Materials and Methods: The expression of the GCLC gene was examined using Real Time PCR in 30 patients with CRC. To evaluate the diagnostic power of GCLC gene expression in differentiating CRC patients from healthy individuals, ROC curve analysis was performed.
Results: The average Fold change of GCLC gene expression in the control group was 1 and in the cancer group was 4.74, indicating a significant increase of more than 4 times compared to the normal value ($P < 0.001$). The Results of ROC curve analysis showed that the area under the curve (AUC) was equal to one, indicating the high accuracy of the test in diagnosing the disease. The sensitivity of the model was calculated to be 96.67% (95% CI=82.78% to 99.92%) and its specificity was 100% (95% CI=88.43% to 100%). The optimal cutoff point for GCLC gene expression was reported to be greater than 10.05. The significance level of the test was equal to 0.0001, indicating a statistically significant difference between the diseased and healthy groups.
Conclusion: GCLC plays a role as a driver gene in CRC and its increased expression is associated with advanced stages of the disease. The Results of ROC curve analysis indicate that GCLC gene expression can be used as an effective biomarker in the diagnosis of CRC.
Keywords: Colorectal Cancer, Biomarker, GCLC

Ps-53: Identification of Novel Candidate Genes Regulating Dopaminergic Neuron Differentiation from Stem Cells for Parkinson's Disease Therapy

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Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons. Current treatments primarily offer symptomatic relief, highlighting the need for disease-modifying therapies. Stem cell-based approaches hold promise for regenerative medicine by differentiating stem cells into replacement neurons. This study aimed to identify and characterize key genes involved in stem cell differentiation into dopaminergic neurons to advance cell therapy for PD.
Materials and Methods: Bioinformatic analysis was performed on 23,398 genes to identify those with significant differential expression. The STRING database and Cytoscape software were used for protein-protein interaction network construction and analysis to pinpoint hub genes within the differentiation pathway. A literature review was conducted to compare identified genes with existing knowledge.
Results: The analysis identified 3,657 differentially expressed genes. Network analysis revealed 10 pivotal hub genes. Six of these genes were previously documented, while four novel candidate genes were discovered, potentially critical for dopaminergic neuron differentiation.
Conclusion: This research identified key genes, including four novel candidates, crucial for stem cell differentiation into dopaminergic neurons. These findings enhance the molecular understanding of this process and offer potential targets for future Parkinson's disease cell therapies. Experimental validation of the novel genes is recommended to explore their full therapeutic potential.
Keywords: Parkinson's Disease, Neuronal Differentiation, Cell Therapy, Gene, Bioinformatics

Ps-54: Effects of Conditioned Media Derived from Human Wharton's Jelly Mesenchymal Stem Cells on The Expression of Genes and Micro-RNAs Regulating Inflammatory and Apoptotic Signaling Pathways in Human Granulosa Cells of Normal and Polycystic Ovaries

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Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age, affecting more than 10% of this population. It is characterized by insulin resistance, oxidative stress, inflammation, and dysregulated apoptosis. Mesenchymal stem cell-derived conditioned medium (CM) contains a variety of growth factors, cytokines, and bioactive molecules that can regulate cellular processes and exert therapeutic effects in different disease models. This study aimed to investigate the potential modulatory effects of CM derived from human umbilical cord Wharton's jelly mesenchymal stem cells (h-WJMSCs-CM) on inflammatory and apoptotic signaling pathways in human granulosa cells of normal and polycystic ovaries.
Materials and Methods: Human granulosa cells were obtained during oocyte retrieval from 50 healthy women undergoing IVF/ICSI treatment and 50 women diagnosed with PCOS. Following purification and characterization using flow cytometry, granulosa cells from normal and polycystic ovaries were divided into two experimental groups and cultured in 6-well plates for 48 hours. Cells were treated either with standard culture medium (control) or with a non-toxic concentration of h-WJMSCs-CM determined by the MTT assay. Quantitative real-time PCR (qRT-PCR) was performed to assess the relative expression levels of selected genes and microRNAs involved in inflammatory and apoptotic signaling pathways.
Results: Granulosa cells derived from PCOS patients showed decreased expression of protective genes associated with the regulation of inflammation and apoptosis compared with cells from healthy individuals. These alterations were accompanied by dysregulated expression of specific microRNAs, including miR-222-3p, miR-132-3p, and miR-34a-5p, which are linked to the regulation of target genes such as PGC1-α, FOXA1, and LDHA. Treatment with h-WJMSCs-CM (200 μg/mL) significantly improved this imbalance by modulating the expression of these microRNAs and restoring the expression of their associated genes toward levels observed in healthy granulosa cells, suggesting a reduction in inflammatory and apoptotic signaling.
Conclusion: These findings indicate that h WJMSCs CM may exert protective effects on granulosa cells by regulating inflammation and apoptosis related molecular pathways in PCOS. CM derived from Wharton's jelly mesenchymal stem cells may therefore represent a promising therapeutic approach and contribute to improved understanding of the molecular mechanisms underlying PCOS.
Keywords: Granulosa Cells, Polycystic Ovary, h-WJMSCs-CM, Inflammation, miRNA

Ps-55: Inflammatory Priming of BM-MSCs Using IL-1β and Its Effect on TGF-β Secretion

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Background: Bone marrow-derived mesenchymal stem/stromal cells (BM-MSCs) are widely studied in regenerative medicine due to their immunomodulatory, anti-inflammatory, and tissue repair properties. Their therapeutic effects are mainly mediated through paracrine signaling and secretion of bioactive molecules rather than direct differentiation. Inflammatory priming has been shown to enhance and modulate MSC function, leading to changes in their secretory and immunoregulatory profiles. Among inflammatory mediators, interleukin-1β (IL-1β) plays an important role in regulating MSC behavior and has been reported to influence cytokine secretion and tissue remodeling-related pathways. This study aimed to evaluate the effect of IL-1β preconditioning on BM-MSC viability and its impact on TGF-β secretion.
Materials and Methods: BM-MSCs were cultured under standard conditions and preconditioned with IL-1β for 24 hours. Untreated cells served as the control group. To determine a non-cytotoxic concentration, cells were exposed to IL-1β at 3, 5, 7, and 10 ng/mL, and cell viability was assessed using the MTT assay. Based on the results, 5 ng/mL was selected for further experiments. TGF-β secretion levels were evaluated using ELISA as an indicator of regenerative and tissue remodeling-related activity.
Results: BM-MSCs maintained acceptable viability following 24-hour IL-1β treatment. Among the tested concentrations, 5 ng/mL was identified as the optimal non-cytotoxic dose. Morphological assessment showed no significant changes compared with the control group. ELISA Results suggested a trend toward modulation of TGF-β secretion following IL-1β preconditioning, indicating that inflammatory priming may influence MSC secretory behavior. Further quantitative analysis is required to confirm these findings.
Conclusion: IL-1β preconditioning may modulate the functional behavior of BM-MSCs while preserving viability. These findings support the concept that inflammatory priming can reshape the MSC secretome and enhance regenerative-related signaling pathways. Further studies are needed to clarify the underlying mechanisms and functional significance.
Keywords: Mesenchymal Stem Cells (MSCs), IL-1β, Inflammatory Priming, TGF-B, Regenerative Medicine

Ps-56: Adipose-Derived Mesenchymal Stem Cells Enhanced The Endoplasmic Reticulum Stress Response in Ischemia–Reperfusion–Induced Acute Renal Failure

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Background: Adipose-derived mesenchymal stem cells (ADMSCs) are emerging as a promising therapeutic strategy for renal failure. This research investigated their potential to allevi-

ate the pathological progression of acute kidney injury (AKI) through the regulation of endoplasmic reticulum (ER) stress mechanisms.

Materials and Methods: This study included 18 male rats allocated into three groups (n=6 per group): Sham, ischemia–reperfusion injury (IRI), and IRI treated with AD-MSCs. Renal IRI was induced by clamping both the renal artery and vein for 45 minutes, whereas animals in the Sham group underwent an identical surgical procedure without vascular occlusion. In the IRI + AD-MSC group, a single intraperitoneal dose of 1×10^6 AD-MSCs was administered immediately following clamp removal. Serum and kidney tissue samples were harvested 48 hours after reperfusion.

Results: The AD-MSC treatment group exhibited significantly lower levels of serum urea nitrogen (BUN) and creatinine ($P < 0.001$), alongside reduced renal tissue damage. Furthermore, the IRI+AD-MSC group demonstrated a significant reduction in ER stress protein levels compared to the IRI group ($P < 0.01$).

Conclusion: The findings of this study suggest that AD-MSC therapy attenuates AKI, potentially through the regulation of the ER stress response.

Keywords: Ischemia, Reperfusion, Mesenchymal Stem Cells, ER Stress Response

Ps-57: Investigating PDGF-Modified PLA/PVA Nanofibers for Tissue Engineering Applications

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Background: Wound healing is a complex physiological process regulated by a sequence of biological and mechanical signals. Any disruption in this process may delay healing and lead to chronic inflammation. Platelet-derived growth factors (PDGFs) play an important role in wound healing by promoting fibroblast proliferation and migration, collagen deposition, and granulation tissue formation. Polylactic acid (PLA) nanofibers are widely used as tissue engineering scaffolds because of their biocompatibility, biodegradability, and favorable mechanical properties. However, their hydrophobicity and limited biological activity can reduce cell adhesion and proliferation. To overcome these limitations, PLA is combined with polyvinyl alcohol (PVA), which improves hydrophilicity, moisture absorption, and cell adhesion. In addition, PDGF acts as a potent mitogenic and chemotactic factor that stimulates cell proliferation, angiogenesis, and tissue regeneration.

Materials and Methods: Preparation of PDGF-loaded PLA/PVA hybrid nanofibers Fabrication of nanofibers using the electrospinning method Loading PDGF into the nanofibrous scaffold Design of a recombinant vector containing the optimized PDGF-BB coding sequence with microbial expression Investigation of nanofiber morphology Evaluation of recombinant PDGF expression and biological activity Assessment of the effect of PLA/PVA combination and PDGF incorporation on scaffold bioactivity.

Results: The combination of PLA and PVA improved: Surface hydrophilicity Moisture absorption Cell adhesion and growth PDGF-containing nanofibers were successfully fabricated by electrospinning. Recombinant microbial expression of PDGF-BB was successfully achieved. Incorporation of PDGF significantly enhanced the bioactivity of the nanofibrous scaffolds. PLA/PVA/PDGF scaffolds demonstrated high potential for stimulating cell proliferation, angiogenesis, and tissue regeneration.

Conclusion: The incorporation of PDGF into PLA/PVA nanofibrous scaffolds significantly enhances their bioactivity and regenerative potential. Due to their favorable physical and biological properties, these hybrid scaffolds show strong potential for applications in tissue engineering, wound healing, and regenerative medicine.

Keywords: Electrospun, Nanofibers, PDGF-BB, Wound Healing

Ps-58: Optimization of EGCG Treatment Conditions in Hepatocellular Carcinoma Cell Lines for Further Mechanistic Studies

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Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer mortality worldwide and remains difficult to treat in advanced stages. Epigallocatechin gallate (EGCG), the major polyphenol in green tea, possesses anticancer, epigenetic, and pro-apoptotic properties and may regulate tumor cell survival. This study aimed to optimize EGCG treatment conditions in HCC cell lines for future mechanistic studies of apoptosis-related pathways, particularly regulation of the C-FLIP/Ku70 complex.

Materials and Methods: Cytotoxic effects of free EGCG were evaluated in HepG2 and Huh7 HCC cell lines and normal human fibroblasts using MTS viability assays. Cells were treated with increasing EGCG concentrations for different exposure times to determine effective dose ranges and inhibitory concentrations. Propidium iodide (PI) staining was used to confirm cell death.

Results: EGCG induced dose- and time-dependent cytotoxicity in both HCC cell lines. HepG2 cells were more sensitive, showing measurable IC_{50} values under optimized conditions. Huh7 cells were relatively resistant and exhibited only partial reductions in viability at IC_{30} levels. Low-dose EGCG produced limited immediate effects, whereas prolonged exposure and higher concentrations significantly reduced viability. Differences between cell lines may be related to variations in differentiation status, metabolic activity, and oxidative stress defense mechanisms.

Conclusion: These findings establish optimized EGCG treatment parameters for future mechanistic investigations in HCC. The results also support the potential of EGCG to enhance apoptotic sensitivity through modulation of survival pathways, possibly involving dissociation of the C-FLIP/Ku70 complex and restoration of apoptosis susceptibility.

Keywords: Hepatocellular Carcinoma (HCC), Epigallocatechin Gallate (EGCG), Apoptosis, C-FLIP/Ku70 Complex

Ps-59: Development of Sustainable Bioengineered Skeletal Muscle Structures Using Plant-Derived Bioactive Compounds and Natural Scaffolds

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Background: The increasing global demand for protein, together with environmental and ethical concerns associated with conventional livestock production, has accelerated the development of cultured meat and engineered muscle tissues as sustainable alternatives. In parallel, regenerative medicine continues to face major challenges in the repair and reconstruction of skeletal muscle injuries and degenerative disorders. Advances in tissue engineering suggest that scaffold composition, cell source selection, and optimization of culture conditions play critical roles in successful muscle regeneration. This study aimed to develop a sustainable and biologically efficient strategy for engineered skeletal muscle production using natural scaffolds and plant-derived substitutes for animal growth factors.
Materials and Methods: A combined bioinformatics and experimental approach was employed. In the computational phase, genomic and proteomic databases were analyzed to identify plant-derived compounds with potential functional similarity to myogenic growth factors. Structural modeling and molecular interaction analyses were performed to evaluate their possible involvement in muscle differentiation signaling pathways. In the experimental phase, natural edible scaffolds were prepared through decellularization techniques and characterized using physicochemical analyses, including fourier-transform infrared (FTIR), X-ray diffraction (XRD), atomic force microscope (AFM), hydrophilicity, and biodegradation assays. Mesenchymal stem cells and C_2C_{12} myoblasts were cultured on the scaffolds under optimized differentiation conditions. Cell viability, adhesion, proliferation, and myogenic differentiation were assessed using MTT assay, fluorescence staining, and quantitative PCR.
Results: Bioinformatics analyses identified several plant-derived bioactive compounds with predicted interactions related to myogenic signaling pathways. The developed natural scaffolds demonstrated suitable structural integrity, biocompatibility, and hydrophilic properties for muscle cell attachment and growth. Cell culture experiments showed improved cellular viability, alignment, and differentiation on optimized scaffolds under reduced animal-derived culture conditions. Preliminary findings indicated that plant-derived compounds may partially support skeletal muscle differentiation and tissue organization.
Conclusion: The findings of this study demonstrate the potential of integrating sustainable natural scaffolds with plant-derived bioactive compounds for engineered skeletal muscle production. This approach may reduce dependence on animal-derived materials while providing a promising platform for applications in cultured meat technology and regenerative medicine.
Keywords: Cultured meat, Tissue Engineering, Bioinspired Scaffolds, Stem Cells

Ps-60: Inflammatory Priming of Mesenchymal Stem Cells with TNF-α and Modulation of Secretory Function

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Background: Mesenchymal stem cells (MSCs) are widely recognized for their regenerative and immunomodulatory properties, primarily mediated through paracrine signaling and secretion of bioactive molecules rather than direct differentiation. Increasing evidence suggests that the functional properties of MSCs can be significantly influenced by their microenvironment, particularly inflammatory stimuli. In this context, inflammatory priming has emerged as a potential strategy to enhance MSC therapeutic efficacy. Tumor necrosis factor-alpha (TNF-α) is a key pro-inflammatory cytokine that may modulate MSC behavior and alter their secretory profile, including transforming growth factor-beta (TGF-β), which plays an important role in tissue repair and immunoregulation. The present study aimed to investigate the regenerative profile of MSCs following TNF-α preconditioning.
Materials and Methods: BM-MSCs were cultured under standard in-vitro conditions and exposed to TNF-α for 24 hours with untreated cells as controls. Cells were treated with 3, 5, 7, and 10 ng/mL to determine a non-cytotoxic range. Viability was assessed by MTT assay, and 5 ng/mL was selected for further analysis. TGF-β levels were measured by ELISA.
Results: MTT assay showed no significant cytotoxicity across doses. 5 ng/mL provided optimal balance between viability and metabolic activity. Morphology remained spindle-shaped with preserved adherence compared to controls. ELISA indicated a trend toward modulation of TGF-β secretion following TNF-α exposure, suggesting altered paracrine regenerative activity though not fully quantified.
Conclusion: TNF-α preconditioning may modulate MSC secretory function without affecting viability. Findings suggest inflammatory priming influences regenerative paracrine signaling, particularly TGF-β, and may be useful for optimizing MSC-based therapies. Further mechanistic studies are required.
Keywords: Mesenchymal Stem Cells (MSCs), Tumor Necrosis Factor-Alpha (TNF-α), Inflammatory Priming, Secretome, Transforming Growth Factor-Beta (TGF-β)

Ps-61: Third-Generation Anti-PSCA CAR-T Dysfunction: Computational Prioritization of STAT3

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Background: Chimeric antigen receptor T (CAR-T) cell therapy has reshaped cancer immunotherapy, yet durable activity against solid tumors remains limited by post-transfer dysfunction, impaired persistence, and exhaustion-associated signaling. In the source immunoproteomic study, prostate stem cell antigen (PSCA)-specific CAR-T cells sharing the same antigen-binding domain but carrying different intracellular designs showed divergent signaling behavior. Notably, the third-generation PSCA-8t28BBZ receptor, despite incorporating both CD28 and 4-1BB co-stimulatory domains, showed weaker signaling and antitumor activity than the second-generation construct. This suggests that additional signaling modules may not necessarily improve CAR-T function. Here, we re-analyzed the third-generation anti-PSCA CAR-T transcriptional profile before and after adoptive transfer to prioritize a druggable regulatory node associated with post-transfer dysfunction.
Materials and Methods: The public Gene Expression Omnibus (GEO) dataset GSE102823 was analyzed by comparing PSCA3_pre and PSCA3_post samples. Differentially expressed genes (DEGs) were identified using GEO2R and interpreted through functional enrichment, pathway analysis, and gene ontology annotation. Candidate regulators were prioritized through protein-protein interaction (PPI) network topology and biological relevance to T-cell signaling, cytokine regulation, inhibitory signaling, and exhaustion-associated remodeling. Signal transducer and activator of transcription 3 (STAT3) was selected for structure-based inhibitor assessment. A reference STAT3 inhibitor, PubChem CID 1324494, and 858 related analogs were screened by molecular docking, interaction profiling, drug-likeness assessment, and absorption, distribution, metabolism, excretion, and toxicity prediction.
Results: Post-transfer comparison revealed marked transcriptional remodeling, enriched in immune-regulatory, cytokine-associated, and T-cell signaling signatures. Network analysis prioritized STAT3 as the main hub candidate. Among 858 screened analogs, PubChem CID 2058979 was prioritized as a traceable lead-like candidate, showing a stronger MolDock score than the reference inhibitor PubChem CID 1324494 (-130.555 versus -123.258). The compound showed no Lipinski violation and no Pan Assay Interference Compounds (PAINS) or Brenk alerts, while cytotoxicity prediction did not indicate a strong liability.
Conclusion: This study links post-transfer transcriptomic remodeling of third-generation anti-PSCA CAR-T cells to network-guided STAT3 prioritization and structure-based inhibitor screening. PubChem CID 2058979 is proposed as an early STAT3-directed lead-like candidate for further refinement and experimental validation.
Keywords: CAR T-Cell, STAT3, Exhaustion, PSCA, Virtual Screening

Ps-62: Cracking The Code of Pluripotency: Unveiling Novel Molecular Regulators of Spermatogonial Stem Cell Reprogramming via Integrative Multi-Omics

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Background: Spermatogonial stem cells (SSCs) are unipotent germline cells with emerging pluripotent potential under specific *in-vitro* conditions. This study aims to investigate the capacity of SSCs for reprogramming and to elucidate the molecular mechanisms and regulatory networks involved in this transition.
Materials and Methods: SSCs were isolated from Oct4-GFP C57BL/6 transgenic mice using enzymatic digestion and maintained in defined culture media. ES-like colonies were characterized via immunocytochemistry and teratoma formation assays. Comprehensive transcriptomic analyses were performed using bulk and single-cell RNA sequencing datasets (GSE43850, GSE38776, GSE149512). Bioinformatic workflows included the construction of protein-protein interaction (PPI) networks using STRING and Cytoscape, as well as Weighted Gene Co-expression Network Analysis (WGCNA) to identify hub regulatory genes.
Results: ES-like cells derived from SSCs exhibited robust expression of pluripotency markers (OCT4, DAZL, and VASA). Transcriptomic comparisons confirmed that the derived cells possess gene expression profiles highly similar to embryonic stem cells (ESCs). WGCNA identified critical co-expression modules and hub regulatory RNA-binding genes, specifically Ctdsp1, Rest, and Stra8, which appear to act as key drivers of the reprogramming process. Furthermore, teratoma assays confirmed the acquisition of pluripotency, and single-cell RNA-seq validated the expression of key markers across the reprogrammed population.
Conclusion: Our findings demonstrate that SSCs can be successfully reprogrammed into ES-like cells. The integration of transcriptomic and network-based analyses provides novel insights into the molecular drivers of this reprogramming process. This study highlights the potential utility of reprogrammed SSCs in regenerative medicine and male fertility preservation strategies.
Keywords: Spermatogonial Stem Cells (SSCs), Pluripotency, Reprogramming, WGCNA, Regenerative Medicine

Ps-63: HNF4α Translational Upregulation by SINEUP Enhances Hepatic Metabolic Maturation in Huh7-Based 3D Liver Microtissues

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Background: The development of a physiological liver platform remains a major challenge in drug screening and toxicol-

ogy. Hepatocellular carcinoma (HCC)-derived cell lines, such as Huh7, are widely used due to their availability and cost-effectiveness. However, impaired metabolic capacity limits their utility for drug screening. In this study, we evaluated whether SINEUP-mediated upregulation of hepatocyte nuclear factor 4α (HNF4α) translation can improve metabolic function in a 3D bioengineered liver microtissue.

Materials and Methods: Liver extracellular matrix (ECM)-derived microparticles (MPs) were incorporated into multicellular liver microtissues composed of Huh7 cells, HUVECs, and WJ-MSCs using AggreWell™ technology. Microtissues from the experimental group were generated using Huh7 cells manipulated with long non-coding RNA SINEUP targeting HNF4α. And then, the experimental and control groups were compared in different aspects.

Results: Enhanced expression of HNF4α promoted hepatocytere-differentiation, as demonstrated by increased expression of mature hepatic markers (HNF4α, ALB, CDH1) and suppression of fetal liver markers (AFP, CDH2). In addition, metabolic reprogramming was observed, including suppression of glycolysis-related genes, activation of gluconeogenesis key gene, and increased expression of cytochrome P450 enzymes. Functional assays confirmed improved albumin (ALB) and fibrinogen (FG) secretion, increased urea synthesis, glycogen storage, decreased AFP secretion, and lactate production.

Conclusion: Our findings indicate that SINEUP-mediatedupregulation of HNF4α partially restores hepatocyte-like metabolic identity in cancer-derived hepatic microtissues. This strategy provides a promising platform for the development of more predictive in-vitro liver models for use in drug screening.

Keywords: HNF4α, SINEUP Technology, 3D Liver Models, Metabolic Reprogramming, Drug Screening

Ps-64: QRT-PCR Evaluation of Cell-Free RNA and Urinary Sediment Biomarkers in Prostate Cancer (PCa) Using PCa Cell Lines and Human PCa Urine Samples

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Background: Prostate cancer (PCa) is the second most common cancer in Iran and worldwide. PCa early detection significantly improves patient survival. Conventional diagnostic methods such as digital rectal examination (DRE), MRI, and biopsy are associated with limitations including pain, infection, and hematuria. Therefore, molecular biomarker panels have gained attention as non-invasive alternatives for diagnosis and monitoring. In this study, qRT-PCR was used to detect validated PCa biomarkers, including HOXC6, DLX1, SPDEF, ERG, PSA, and PCA3, in PC3, and LNCaP cell lines and human urine samples from Iranian PCa patients.

Materials and Methods: Two PCa cell lines (PC3 and LNCaP) were cultured, and the expression of HOXC6, DLX1, and PSA was analyzed using two-step qRT-PCR. LNCaP was then used to optimize the one-step qRT-PCR protocol. The expression of SPDEF, ERG, and PCA3 as cell-free RNAs in the LNCaP supernatant was evaluated by both one-step and two-step methods. In the clinical phase, urine samples from PCa patients, healthy individuals, and benign prostatic hyperplasia (BPH) patients were collected. Biomarker expression was analyzed in urinary sediment (HOXC6, DLX1, and PSA) and in the cell-free urine supernatant (SPDEF, ERG, PCA3, and PSA).

Results: The results showed that the androgen-dependent LNCaP cell line, with higher expression of HOXC6, DLX1, and PSA than the PC3 cell line, was more suitable for optimizing one-step qRT-PCR. Although both one-step and two-step methods confirmed amplification specificity, the performance of one-step qRT-PCR was strongly affected by kit type and buffer composition and showed limited reproducibility in low-RNA samples such as LNCaP supernatant. In contrast, the two-step qRT-PCR method provided more stable and reliable detection of cell-free RNAs, including SPDEF, ERG, PCA3, and PSA. In clinical urine samples, despite the low concentration and instability of urinary RNA, biomarker expression was successfully quantified using the two-step approach, indicating higher levels of these markers in patients compared to healthy controls.

Conclusion: One-step qRT-PCR was efficient in detecting RNAs extracted from cultured cells, but the two-step method was more reliable in quantifying RNAs from cell culture supernatants and clinical urine samples. We observed that HOXC6 and DLX1 in urinary sediment and PCA3 in cell free urine supernatant were significantly more abundant in both PCa and BPH samples. This non invasive strategy may reduce unnecessary prostate biopsies and improve diagnostic accuracy.

Keywords: Prostate Cancer, Urinary Biomarkers, qRT-PCR, Cell Free RNA, Early Detection

Ps-65: Improved Biological Characteristics of AD-MSCs and Their Small-Extracellular Vesicles Under Combined Low-Serum and Low-Oxygen Conditions

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Background: Small extracellular vesicles from adipose-derived mesenchymal stem cells (Ad-MSCs small EVs) are promising for targeted drug delivery. The study aimed to enhance their yield and bioactivity using low-oxygen and low-serum culture conditions.

Materials and Methods: Human adipose-derived MSCs were isolated, characterized by flow cytometry, and cultured under control, low-oxygen, low-serum, and combination of both (low-oxygen/low-serum) conditions. The effects of various culture conditions were evaluated on total cell count, viability, migration, and stemness potential of Ad-MSCs. Ad-MSCs small EVs were extracted and assessed for morphological (shape/size/topography/distribution), structural (aggregation/homogeneity/colloidal behavior/surface charge/stability), and biocompatibility (cytotoxicity/systemic toxicity/irritation/pyrogenicity/microbial contamination), according to the United States Pharmacopeia 2024 guidelines, ISO10993-5, -10, -11, and -12.

Results: Ad-MSCs maintained their stemness, morphology,

and stemness potential under all culture conditions. While low-oxygen and low-serum conditions alone did not affect total cell count and viability, combination of both (low-serum/low-oxygen) condition significantly reduced cell proliferation. The highest migration of Ad-MSCs and the greatest expressions of CD90, CD73, and CD105 biomarkers were detected in the low-oxygen/low-serum group ($p \leq 0.05$). Small-EVs remained round under all conditions but varied in size, homogeneity, aggregation, and stability. The low-oxygen/low-serum condition yielded more homogeneous EVs (60–100 nm) with the lowest Z-average (104.6 nm), best PDI (0.18), and highest stability, as indicated by a markedly negative surface charge (-61.8 mV) ($p \leq 0.05$). All EVs extracted under various culture conditions demonstrated acceptable biocompatibility, showing no acute or subacute systemic cytotoxicity, no irritation, no pyrogenicity, and no microbial contamination on Wistar rats, New Zealand rabbits, according to ISO10993 ($P > 0.05$).

Conclusion: Low-oxygen and low-serum preconditioning synergistically boost small EVs biogenesis and bioactivity, enabling next-generation cell-free therapies, targeted drug delivery, and precision diagnostics.

Keywords: Adipose-Derived Mesenchymal Stem Cells, Low-Serum, Low-Oxygen, Extracellular Vesicles, Biocompatibility

Ps-66: Serum-Deprived Condition Modulates The Architecture and Biocompatibility Profiles of Human Adipose-Derived small-Extracellular Vesicles

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Background: This study aimed to investigate the effect of serum deprivation on the biological properties of adipose-derived mesenchymal stem cells (AD-MSCs) and their extracellular vesicles (sEVs).

Materials and Methods: AD-MSCs were isolated from human adipose tissue and propagated under serum deprivation (1% exosome-depleted FBS) and control (10% exosome-depleted FBS) conditions. The effect of culture conditions on cell density, migration, and stemness potential of AD-MSCs was assessed. sEVs were subsequently isolated from treated AD-MSCs and characterized for morphological (FESEM), biochemical (FTIR/BCA), and structural (DLS/Zeta potential) characteristics. Additionally, biocompatibility profiles (MTT, and in-vivo irritation/sensitization/acute systemic toxicity) of sEVs were detected, according to ISO10993 guidelines.

Results: No significant differences were detected in cell number, viability, and stemness potential of AD-MSCs; however, serum starvation significantly enhanced AD-MSC migration. FESEM indicated higher homogeneity of serum-starved sEVs, showing a distinct size peak at 47–49nm, whereas control sEVs displayed a broader distribution with an average size of 340–350 nm. The control sEVs exhibited polydispersity (80% at 223.7 ± 45.7 nm, 20% at 50.8 ± 5.7 nm), whereas serum-starved sEVs were monodispersed around 34.1 ± 3.1nm. Moreover, serum-starved sEVs demonstrated a significantly higher average zeta potential and electrophoretic mobility (-4.9 mV, -0.000070 cm^2/Vs) compared to control sEVs (-9.0 mV, -0.000038 cm^2/Vs) ($p \leq 0.05$). Higher protein content was detected in the serum starvation group (4.34699 mg/mL vs. 2.1754 mg/mL; $p \leq 0.05$). sEVs extracted under both culture conditions exhibited acceptable biocompatibility, showing no evidence of acute systemic cytotoxicity, irritation, sensitization, or microbial contamination ($P > 0.05$).

Conclusion: Serum starvation significantly improved the morphological, biochemical, and structural properties of sEVs without any adverse effects on their biocompatibility and safety profile.

Keywords: Extracellular Vesicles, Biocompatibility, Mesenchymal Stem Cell, Serum Starvation, Characterization

Ps-67: Standardized Extraction of Glycyrrhizin from Glycyrrhiza Glabra for Regenerative Biomedical Applications

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Background: Glycyrrhizin, a triterpenoid saponin derived from Glycyrrhiza glabra, is a natural bioactive compound with well-documented anti-inflammatory, antiviral, and cytoprotective properties, making it relevant for biomedical and regenerative research applications. Due to its potential role in modulating cellular stress and inflammatory responses, improving its extraction efficiency is important for providing consistent material for downstream experimental studies.

Materials and Methods: In this study, a systematic optimization of extraction parameters was performed to enhance glycyrrhizin recovery from Glycyrrhiza glabra. The effects of solvent composition, extraction technique, temperature, time, and fermentation pretreatment were evaluated. Ethanol–water and methanol–water mixtures at different ratios (30:70, 70:30, and 80:20 v/v) were applied using conventional maceration and ultrasound-assisted extraction methods. Extraction conditions included time ranges from 30 to 360 minutes or overnight processing, temperatures from ambient to 60°C, and fermentation periods of 1 and 3 days prior to extraction.

Results: The results demonstrated that extraction efficiency was significantly influenced by solvent polarity and process conditions. The highest glycyrrhizin yield was obtained using a methanol/water (70:30 v/v) system under repeated 30-minute extraction cycles at 60°C. Prolonged extraction time and non-optimal solvent ratios resulted in reduced yield, indicating the importance of controlled extraction conditions. Fermentation pretreatment showed condition-dependent effects, suggesting an interaction between biological processing and extraction efficiency.

Conclusion: Overall, this study presents an optimized and reproducible extraction strategy for glycyrrhizin from Glycyrrhiza glabra, which may support future biomedical and regenerative research requiring standardized natural bioactive compounds for experimental and translational studies.

Keywords: Glycyrrhizin, Extraction Optimization, Ultrasound-Assisted Extraction, Regenerative Biotechnology

Ps-68: CRISPR/Cas9-Mediated Disruption of HLA-I Expression in Human Embryonic Stem Cells

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Background: Human embryonic stem cells are valuable candidates for regenerative medicine due to their self-renewal capacity and differentiation potential. However, their allogeneic application is limited by immune rejection, largely mediated by human leukocyte antigen class I (HLA-I) expression. Beta-2 microglobulin (B2M) is an essential component required for stable HLA-I surface presentation; therefore, targeting B2M may provide an effective strategy to reduce HLA-I expression and generate hypoimmunogenic stem cell lines.

Materials and Methods: Human embryonic stem cells were subjected to CRISPR/Cas9-mediated targeting of the B2M gene using the pX459-EF1a vector. Following genome editing, targeted mutations in B2M were confirmed by Sanger sequencing. Changes in B2M transcript expression were assessed by RT-PCR, while HLA-I surface expression was evaluated by flow cytometry. Non-edited cells were used as controls for comparison.

Results: CRISPR/Cas9-mediated editing successfully introduced targeted mutations in the B2M gene. The edited cells showed reduced B2M transcript expression compared with non-edited controls. Flow cytometry analysis also demonstrated decreased HLA-I surface expression in edited cells, indicating impaired HLA-I presentation following B2M targeting.

Conclusion: CRISPR/Cas9-mediated targeting of B2M can effectively reduce HLA-I expression in human embryonic stem cells. This strategy may provide a useful platform for developing hypoimmunogenic stem cell lines and supports further investigation toward universal stem cell-based therapeutic applications.

Keywords: CRISPR/Cas9, Human Embryonic Stem Cells, HLA-I

Ps-69: A comparative Evaluation of The Safety and Efficacy of Acellular Dermal Matrix Transplantation Combined with Adipose-Derived Mesenchymal Stem Cells versus Standalone Adipose-Derived Mesenchymal Stem Cell Injection in The Treatment of Diabetic Foot Ulcers Refractory to Standard Therapies

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Background: Diabetic foot ulceration (DFU) is a major complication of diabetes that significantly affects patients' quality of life and often results in amputation of the affected limb. There is no definitive curative treatment for this condition. This study is designed as a randomized controlled clinical trial to compare the effects of three different therapeutic approaches on wound healing in patients with DFU who are resistant to standard treatments.

Materials and Methods: The study population consists of 15 patients with DFU who have not responded to conventional therapies. Patients are assigned to three treatment groups: Group 1 receives injections of adipose-derived stem cells (ASC); Group 2 receives ASC in combination with an acellular dermal matrix graft (ADM+ASC); and Group 3 receives routine wound care with standard dressings from the center (control). Treatment is administered at the first, third, and sixth weeks. Follow-up for treatment outcomes continues for three months after the intervention. The primary outcomes of this study include wound area and vascular flow, which are measured using a digital camera and Doppler ultrasonography, respectively. Doppler ultrasonography is performed prior to treatment and at three months post-treatment. Histopathological assessment of inflammation at the graft site with wound staining is conducted, and serum levels of inflammatory markers—including CRP, TNF-α, IL-6, and IL-1—are evaluated before treatment and at three months after treatment using ELISA. In addition, the expression of these cytokine genes in PBMCs is assessed using real-time PCR.

Results: In this clinical trial, the therapeutic efficacy of combining ASC with an ADM is evaluated in comparison with ASC alone and standard care for treatment-refractory diabetic foot ulcers. The results demonstrated that the combination of ASC with ADM led to significantly improved wound closure rates and faster healing compared with ASC alone and standard care, without an increase in adverse events.

Conclusion: The integration of clinical, vascular, and molecular assessments provides deeper insight into the mechanisms of tissue repair and inflammation. The findings may support the development of more effective therapies for diabetic foot ulcers, with the potential to reduce amputation rates and improve patient quality of life.

Keywords: Diabetic Foot Ulcer, Adipose-Derived Mesenchymal Stem Cells, Acellular Dermal Matrix, Clinical Trial

Ps-70: Eggshell Membrane-Polyvinyl Alcohol Nanofibers Modified with Graphene Oxide for Tissue Engineering Applications

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Background: The development of biomimetic scaffolds with physicochemical and structural properties similar to the extracellular matrix (ECM) is a critical challenge in tissue engineering. Electrospun nanofibrous substrates are widely used in tissue engineering due to their structure similarity to the ECM. Among natural biomaterials, eggshell membrane (ESM) is a

promising candidate because of its proteins and glycosaminoglycans (GAGs) composition. In addition, incorporation of conductive nanomaterials such as graphene oxide (GO) can improve the functional and physicochemical properties of engineered scaffolds. Therefore, the present study aimed to fabricate and optimize an electrospun conductive scaffold based on ESM, polyvinyl alcohol (PVA), and GO for tissue engineering applications.
Materials and Methods: ESM has been extracted and blended with PVA at different concentrations to prepare electrospinning solutions. GO was incorporated into the optimized polymeric solution to enhance scaffold conductivity. Electrospinning parameters and polymer compositions were systematically optimized to achieve uniform and stable nanofibrous structures. Scaffold characterization was performed using scanning electron microscopy (SEM) to evaluate fiber morphology. In addition, electrical conductivity, swelling behavior, and mechanical properties of the fabricated scaffolds were assessed.
Results: Optimization studies demonstrated that polymer concentration significantly affected fiber formation and scaffold morphology. Lower polymer concentrations resulted in bead formation and unstable fibrous structures, whereas increasing PVA and ESM concentrations improved fiber continuity and uniformity. Better results were obtained at a 50:50 ratio of PVA to ESM, and relatively uniform fibers were observed at 7% PVA and 7% ESM concentrations. The optimized scaffold showed a more uniform fibrous structure . Furthermore, the fabricated scaffolds demonstrated suitable swelling behavior and mechanical properties for tissue engineering applications.
Conclusion: The fabricated ESM/PVA/GO electrospun scaffold demonstrated promising structural and physicochemical properties as a conductive biomimetic platform. Optimization of scaffold composition and electrospinning parameters played a crucial role in achieving stable and uniform nanofibrous structures. These findings suggest that the developed scaffold may serve as a potential candidate for future tissue engineering applications.
Keywords: Tissue Engineering, Eggshell Membrane, Graphene Oxide, Electrospinning, Conductive Scaffold

Ps-71: Building Functional Vascular Network and Mature Cardiomyocytes by Ex Ovo Transplantation of Cardiac Microtissues

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Background: Cardiac organoids and microtissues have emerged as promising platforms for disease modeling, drug screening, and regenerative medicine. Nevertheless, one of the major limitations of three-dimensional (3D) cardiac tissues is the lack of a functional vascular network, which restricts adequate oxygen and nutrient diffusion and consequently leads to tissue necrosis and immature cardiomyocytes. Previous studies have demonstrated that heterotopic transplantation onto the chick chorioallantoic membrane (CAM) can promote vascularization in cardiac organoids. However, comprehensive investigations evaluating the extent of vascularization and its effects on cardiomyocyte maturation and cell viability remain limited. Therefore, the present study aims to investigate the angiogenic potential of heterotopically transplanted cardiac microtissues and to assess the maturation status of their cardiomyocytes.
Materials and Methods: Cardiac microtissues will be generated by co culturing pre differentiated cardiac progenitor cells, endothelial cells, and stromal cells within a collagen-based matrix. Selected groups will be treated with vascular endothelial growth factor (VEGF) to evaluate its effects on microtissue properties.The microtissues will then be transplanted onto the chick CAM for three days. Following transplantation, samples will be analyzed by microscopic imaging and hematoxylin and eosin (H&E) staining to assess morphology and angiogenesis. Immunofluorescence staining for VE-Cadherin, cTnI and HNA along with qRT-PCR analysis of cTnI expression, will be performed to evaluate vascularization and cardiomyocyte maturation.
Results: We expect that CAM transplantation, together with optimized endothelial cell ratios and VEGF treatment, will enhance vascularization, improve cell viability, and promote cardiomyocyte maturation in cardiac microtissues compared with non-transplanted controls.
Conclusion: This study may provide an effective strategy for improving angiogenesis and cardiomyocyte maturation in engineered cardiac microtissues. The findings could contribute to the development of more physiologically relevant 3D cardiac models for regenerative medicine, disease modeling, and drug discovery applications.
Keywords: Cardiac Microtissue, Heterotopic Transplantation, Chick Chorioallantoic Membrane

Ps-72: Exploring of PLC Expression in Breast Cancer Cells (MDA-MB-231) Under Treatment with Secretome of hAMSCs

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Background: Breast cancer is the second most common type of cancer and a leading cause of death among women. The Phospholipase C (PLC) enzyme, which is responsible for the hydrolysis of phospholipids, plays a key role in the metabolism of inositol lipids. Lipid intermediates produced by the PLC family are involved in various cellular processes, especially in the progression of tumorigenesis through intracellular and extracellular signaling pathways. Numerous studies indicate the role that these enzymes play in the regulation of cell proliferation, invasion, and migration. Mesenchymal stem cells (MSC) as a heterogeneous population of multipotent adult stem cells have the ability to self-renew and differentiate. The therapeutic potential of these cells is very high due to their ability to modulate immune responses and regulate tissue homeostasis through the secretion of paracrine factors. This study evaluates the effect of amniotic mesenchymal stem cell secretome (hAMSCs) on PLC protein expression in a breast cancer cell line (MDA-MB-231).
Materials and Methods: MDA-MB-231 breast cancer cells were obtained from the Pasteur Institute and hAMSCs were collected from the Iranian Biological Resource Center. we de-

veloped a co-culture system utilizing 6 well plates transwell (with a diameter of 0.4 µm). After 72 hours, the cells harvested and the expression of PLC protein was measured by Western blotting method.
Results: The results show that the secretome of hAMSCs leads to a decrease in PLC expression in MDA-MB-231 breast cancer cells.
Conclusion: These findings suggest that hAMSCs secretome may have inhibitory effects on MDA-MB-231 breast cancer cells, suggesting its potential as a novel approach in cancer therapy.
Keywords: PLC Expression, MDA-MB-231 Breast Cancer Cells, Secretome of hAMSCs, Cancer Therapy

Ps-73: A Differential Alcian Blue Staining for Hyaluronic Acid Estimation in Wharton's Jelly ECM

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Background: Hyaluronic acid (HA) is a critical polysaccharide involved in tissue repair, angiogenesis, wound healing, and inflammation suppression. In tissue engineering, HA-rich tissues such as Wharton's jelly (WJ) are valuable for scaffold development that is functional in regenerative medicine. Alcian blue staining at pH 2.5 detects all glycosaminoglycans (GAGs), including sulfated GAGs and non-sulfated GAGs (the only non-sulfated GAG is HA), whereas at pH 1.0 it detects only sulfated GAGs. This study proposes an innovative image-based technique, utilizing the pH sensitivity for quantitative assessment of HA in Wharton's jelly.
Materials and Methods: Three human umbilical cord samples were cut into 5×5×2 mm3 pieces, fixed in formalin, and tissue processed. Sections (5 µm) were stained with Alcian blue in two groups: pH=2.5 (total GAGs) and pH=1.0 (sulfated GAGs). Images were brightness/contrast adjusted uniformly for better detection and were analyzed using Fiji software. After color deconvolution (Alcian blue channel), a constant threshold was applied to all photos, and the area fraction was measured. Data were analyzed using the Mann-Whitney test in GraphPad Prism 9.
Results: Qualitative analysis showed a marked difference between pH=2.5 and pH=1.0 staining groups. Quantitative image analysis (Five fields per sample were analyzed) using Fiji software revealed a mean area fraction of 73.72 ± 1.74 at pH 2.5 and 31.27 ± 1.64 at pH=1.0. The Mann-Whitney test confirmed a significant difference ($P < 0.0001$). The differential staining pattern suggested that HA may constitute approximately 58% of total GAGs in Wharton's jelly, indicating its abundance in this tissue.
Conclusion: This study presents a novel, straightforward quantitative method for assessing HA in tissue, based on differential Alcian blue staining at two pH levels. By calculating the difference in area fraction between the two staining conditions, the proportion of HA relative to total GAGs can be determined without the need for complex or costly techniques. This simple yet effective approach is readily applicable to a wide range of extracellular matrices.
Keywords: Tissue Engineering, Regenerative Medicine, Glycosaminoglycans, Hyaluronic Acid, Alcian Blue Staining

Ps-74: Design and Molecular Cloning of An RB1 HDR Donor Construct for CRISPR/Cas9-Mediated Targeted Integration in Retinoblastoma Cell Line

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Background: Retinoblastoma is the most common intraocular malignancy in childhood and is primarily driven by biallelic inactivation of the RB1. The high degree of mutational heterogeneity within this gene has significantly hindered the development of mutation-specific therapeutic approaches. Consequently, genome-editing strategies based on the integration of a functional RB1 cassette into genomic safe-harbor loci, particularly the AAVS1 site, using CRISPR/Cas9-mediated homology-directed repair (HDR), have emerged as a promising mutation-independent approach capable of enabling stable and sustained transgene expression. In this study, we aimed to design and clone a donor vector containing the full-length RB1 coding sequence (CDS) to enable precise genomic integration.
Materials and Methods: The CDS of the human RB1 Gene, together with its associated regulatory elements, was designed based on published literature and reference sequences retrieved from validated genomic databases. Following in silico design, the construct was commercially synthesized and provided in the pUC57 cloning vector. The RB1 insert was subsequently PCR-amplified and then cloned into the backbone of an HDR donor plasmid containing AAVS1 Safe Harbor Locus homology arms using restriction enzyme–mediated cloning. The resulting recombinant plasmid was then propagated in chemically competent Escherichia coli cells and positive clones were identified by colony PCR, restriction enzyme digestion, and Sanger sequencing.
Results: Our results showed that the RB1 insert was amplified by PCR to yield the desired product, which was then cloned into the HDR donor plasmid containing AAVS1 homology arms. Correctly assembled recombinant clones were verified by colony PCR and restriction digestion of colonies after transformation into chemically competent *E. coli* Sanger sequencing confirmed the presence of the RB1 insert in the correct reading frame, without any mutations, deletions or rearrangements within coding regions or flanking sequences.
Conclusion: Our result of recombinant HDR plasmid has shown the integrity and accuracy of sequence of this construct that can be used for downstream targeted integration into AAVS1 Safe Harbor Locus. Taken together, this work has demonstrated the molecular basis for future functional and therapeutic studies of the RB1 integration in Retinoblastoma and other RB1-related cancers.
Keywords: Retinoblastoma, RB1, CRISPR/Cas9, Gene Cloning, AAVS1

Ps-75: Hyaluronic Acid-Rich Hydrogels within Microfluidic Platforms: A Biomimetic Approach to Modeling The Breast Cancer Stem Cell Niche

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Background: Breast cancer stem cells (BCSCs) significantly contribute to tumor metastasis and chemoresistance, yet their study is often limited by the inability of 2D cultures to mimic the 3D tumor microenvironment (TME). This study explores the integration of hyaluronic acid (HA)-based hydrogels into microfluidic systems to create a more physiologically relevant BCSC niche for therapeutic evaluation
Materials and Methods: We performed a systematic analysis of recent advancements (2020–2026) in HA-integrated microfluidic devices. The selection criteria focused on platforms capable of modulating the biophysical properties of the extracellular matrix (ECM) and monitoring real-time cellular responses. Data from 41 high-quality studies were synthesized to evaluate the efficacy of these models in recapitulating BCSC behavior.
Results: Our findings indicate that HA-based hydrogels, specifically those within a stiffness range of 0.5–10 kPa, successfully induce BCSC enrichment characterized by $CD44^+/CD24^-/ALDH^+$ expression. The synergy between microfluidic oxygen control (1-5% O_2) and HA-CD44 interactions was found to upregulate HAS2 and SOX2 via the PI3K/AKT pathway, driving self-renewal. Furthermore, dynamic co-culture models demonstrated that HA-rich environments facilitate paracrine signaling between TAMs and BCSCs, specifically through PDGFB secretion. These platforms also showed superior accuracy in predicting drug penetration kinetics for paclitaxel and doxorubicin compared to static models.
Conclusion: Incorporating HA-rich hydrogels into microfluidic "tumor-on-a-chip" systems provides a robust, high-fidelity platform for studying BCSC dynamics. These engineered niches bridge the gap between traditional cell culture and in-vivo conditions, offering a sophisticated tool for personalized drug screening and mechanistic studies in oncology.
Keywords: Hyaluronic Acid, Cancer Stem Cells,Tissue Engineering, Biomimetic Scaffolds, Tumor Microenvironment

Ps-76: Shared Transcriptomic and Immune Signatures Across Autoimmune Myopathies and Cutaneous Lupus: An Integrated Bioinformatics Study

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Background: Polymyositis (PM), dermatomyositis (DM), and cutaneous lupus erythematosus (CLE) are chronic autoimmune conditions that can share clinical and immunological features, including muscle and skin inflammation. Despite these similarities, the common molecular mechanisms and transcriptomic overlap among these diseases remain poorly defined. Identifying shared gene expression signatures and immune pathways may uncover unifying pathogenic drivers and potential therapeutic targets.
Materials and Methods: An integrated bioinformatics analysis was performed using two publicly available gene expression datasets (GEO) for each disease (PM, DM, CLE). Differentially expressed genes (DEGs) were screened separately for each condition. Overlapping DEGs common to all three diseases were determined through Venn diagram intersection. The shared gene set was subjected to functional enrichment analysis using Enrichr, including KEGG pathway and Gene Ontology (GO) biological process annotations. A protein–protein interaction (PPI) network was constructed and analyzed in Cytoscape, and hub genes were prioritized using the cytoHubba plugin based on topological algorithms.
Results: A core set of 17 overlapping upregulated DEGs was identified across PM, DM, and CLE. Functional enrichment analysis demonstrated significant involvement of immune and inflammatory pathways, particularly cytokine and chemokine signaling. GO analysis revealed enrichment of inflammatory response, response to type II interferon, and pathways associated with lymphocyte, monocyte, and T-cell migration. Protein–protein interaction (PPI) network analysis and cytoHubba MCC ranking prioritized five top hub genes: *AIF1, CD163, CXCL10, CXCR4,* and *TYROBP*.
Conclusion: This integrated bioinformatics study pinpointed a shared inflammatory and interferon-associated transcriptomic signature across PM, DM, and CLE. The enrichment of chemokine, cytokine, and innate immune signaling pathways suggests common pathogenic mechanisms underlying these autoimmune disorders. Hub genes including *CXCL10, CXCR4,* and *CD163*, central to these immune circuits, may represent potential biomarkers or therapeutic targets and warrant further experimental validation.
Keywords: Autoimmune Myopathy, Cutaneous Lupus Erythematosus, Transcriptomic Signature, Hub Genes

Ps-77: Leveraging EGCG-Induced Senescence for Enhanced NK Cell Cytotoxicity *In-Vitro* and Primary Liver Cancer Mouse Model

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Background: Hepatocellular carcinoma (HCC), is a global health challenge with increasing mortality rates. Conventional therapies are associated with recurrence and resistance, highlighting the need for novel treatment strategies. Epigallocate-

chin gallate (EGCG), a major polyphenolic compound of green tea, suggested to decrease malignant features of cancerous cells. Meanwhile, natural killer (NK) cells have shown promising outcomes for HCC treatment in clinical trials. This study aimed to investigate whether EGCG-induced senescence could enhance vulnerability of HCC cells to NK cytotoxicity *in-vitro* and *in-vivo*.

Materials and Methods: Cell viability of Huh-7 cells following EGCG treatment at different concentrations and time points was evaluated by MTS assay. Apoptosis and cell cycle by flow cytometry, Senescence induction by X-gal assay, Inflammatory cytokines by ELISA were assessed. colony formation and wound healing assays were performed. The expression of NK cell activating ligands, and cell cycle regulation was evaluated by qRT-PCR. NK cell cytotoxicity was quantified by flow cytometry. Huh-7 cells were subcutaneously injected into nude mice to establish tumor xenografts, human NK cells were administered intratumorally and tumor growth was monitored regularly.

Results: Treatment with EGCG at 200 μM for 72 hours is effective for Huh-7 cells. EGCG increased apoptosis and β-galactosidase activity. Colony formation capacity and migration ability were reduced. The secretion of IL-6, IL-8, and IL-1β were decreased. Expression of TP53 and NK cell activator ligand, MIC-B were upregulated. NK cell cytotoxicity was higher against EGCG-pretreated cells. In the subcutaneous mouse model, relative tumor growth was lower in EGCG-pretreated and EGCG plus NK group in compare to control.

Conclusion: The findings suggest that EGCG treatment may enhance the vulnerability of HCC cells to NK cell cytotoxicity through induction of senescence-associated phenotypes, including elevated β-galactosidase activity, reduced inflammatory cytokine, and upregulation of NK cell-activating ligand. In addition, EGCG reduced tumor growth and clonogenicity *in-vivo* . These results highlight the potential of senescence induction as a complementary strategy to improve immunotherapeutic responses in HCC.

Keywords: Hepatocellular Carcinoma, Natural killer, EGCG, Senescence, Immunotherapy

Ps-78: Chitosan Nanoparticles Containing Crocin Induce Apoptosis in MCF7 Cells

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Background: Breast cancer is the most common cancer among women globally. Early detection and the development of effective drugs for resistant forms may enhance treatment and improve survival. The emphasis shifts to inactive delivery systems for targeted drug delivery to cancer sites. Encapsulating agents in the nanometer range are called nanoparticles (NPs). Depending on their characteristics, sizes, or shapes, they can be divided into several classes. Examples include luminescent nanoporous hybrid materials, metal NPs, fullerenes, and polymeric and ceramic NPs. Interest in natural compounds with potential anticancer properties has grown due to the complexities of tumor biology and the limitations of current therapeutic approaches. One such compound is the bioactive polyphenol crocin. Research has underscored the positive impact of crocin on neurodegenerative disorders, attributing this to its anti-inflammatory, antioxidant, and anti-apoptotic properties, thereby establishing it as a safe and affordable natural remedy. This study investigated the anticancer potential of chitosan NPs-encapsulated crocin.

Materials and Methods: Following a 24-hour incubation period, crocin-chitosan NPs were administered to the MCF-7 cells. Apoptosis analysis (using Annexin V/PI), cell-cycle analysis, and real-time PCR were carried out.

Results: The combination treatment with crocin-chitosan NPs was highly effective in inducing apoptosis in breast cancer cells (MCF-7) *in-vitro*. Combination therapy with crocin-chitosan NPs was highly effective *in-vitro* for inducing apoptosis in breast cancer cells (MCF-7). Comprehensive gene expression studies have shown that these anticancer effects are associated with suppression of the *p53*, *Caspase-3*, and *p21* genes. This nanoformulation arrested the G0 and G1 phases of the cell cycle in MCF-7 cells and stimulated intracellular ROS generation, which induced apoptosis.

Conclusion: The results suggest that crocin-chitosan NP formulations may influence the induction of apoptosis in breast cancer cells. This feature of chitosan NPs can reduce side effects in healthy cells. In breast cancer treatment, crocin -chitosan NPs may serve as a biological anti-cancer agent.

Keywords: Crocin, Chitosan Nanoparticles, Apoptosis, MCF7

Ps-79: Molecular Docking of Thymoquinone Analogues against Staphylococcal Protein A (4NPE): A Screening Study

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Background: Staphylococcus aureus (*S. aureus*) is a major human pathogen associated with persistent infections and biofilm formation. Staphylococcal protein A (SpA), corresponding to the crystallographic structure 4NPE, is an important virulence factor involved in immune evasion and represents a promising anti-virulence target. Thymoquinone, the major bioactive constituent of Nigella sativa, has demonstrated antibacterial and antibiofilm activities. In this study, thymoquinone and structurally related compounds were evaluated for their potential interaction with SpA through molecular docking

Materials and Methods: The three-dimensional structure of SpA (PDB ID: 4NPE) was retrieved from the Protein Data Bank and used as the target for docking analysis. A total of 32 compounds, including thymoquinone and 31 related analogues from the PubChem database, were screened using the AutoDock and AutoDock Vina algorithms. Binding affinity, predicted inhibition constant (Ki), and RMSD values were used to compare ligand performance.

Results: AutoDock analysis identified Ligand 12, (2-amino-3-cycloheptyloxy-2-phenylpropan-1-ol), as the top-ranked compound, with ΔG=-6.84 kcal/mol and a predicted Ki of 9.73 μM. Ligands 7 and 10 also showed favorable affinities ΔG=-6.16 kcal/mol). In AutoDock Vina, Ligand 26, 5-methyl-2-propan-2-ylcyclohexa-2,5-dien-1-one, showed the best binding score (ΔG=-6.0 kcal/mol), followed by Ligands 27, 7, and 12. Overall, Ligands 7 (2-prenyl-1,4-benzoquinone) and 12 (2-ami-

no-3-cycloheptyloxy-2-phenylpropan-1-ol) demonstrated consistently strong performance across both docking algorithms.
Conclusion: The docking results suggest that several thymoquinone-like scaffolds, particularly Ligands 7 and 12, may interact favorably with SpA (4NPE) and could serve as potential anti-virulence candidates against S. aureus. Given their nature-inspired structural features and their potentially enhanced antibacterial activity relative to the parent natural compound, these newly identified candidates may also hold promise for incorporation into regenerative medicine approaches alongside reparative biomaterials, where they could support infection prevention and contribute to improved therapeutic outcomes in the face of increasing antibiotic resistance.
Keywords: Staphylococcal Protein A (SpA), AutoDock and AutoDock Vina, 4NPE Protein

Ps-80: Determining of *BCKDK* Gene Expression Involved in BCAA Metabolism in Adipose Tissue Surrounding Breast Tumors in Women with Benign and Malignant Breast Tumors

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Background: Breast cancer is one of the most common malignancies among women worldwide, and increasing evidence suggests that the tumor microenvironment plays a crucial role in cancer progression. Peritumoral adipose tissue is now recognized as a metabolically active component that can influence tumor behavior through the secretion of bioactive molecules and modulation of metabolic pathways. Among these pathways, branched-chain amino acid (BCAA) metabolism has gained considerable attention in recent years. Branched-chain ketoacid dehydrogenase kinase (BCKDK) is a key regulatory enzyme that inhibits the branched-chain α-ketoacid dehydrogenase (BCKD) complex, thereby reducing BCAA catabolism and contributing to metabolic reprogramming. Alterations in BCKDK expression have been associated with tumor growth and metabolic adaptation in various cancers.
Materials and Methods: We collected adipose tissue samples from 13 women with breast cancer (BC) and 11 women with benign breast disease (BBD) after surgery. Informed consent was obtained from all participants. Participants were matched based on age and body mass index (BMI). The mRNA expression level of the targeted gene was analyzed using quantitative RT-PCR. For statistical analysis, the Mann-Whitney test was performed using the GraphPad Prism version 9 software.
Results: qRT-PCR data analysis revealed significantly higher expression of BCKDK in peritumoral adipose tissue of malignant tumors compared to benign tumors (P=0.0215).
Conclusion: This study suggests that Cancer-associated adipocytes (CAAs) may play a vital role in the progression of breast cancer. The altered expression of the BCKDK gene involved in BCAA metabolism in adipose tissue during tumor development may contribute to the malignancy and progression of breast cancer, highlighting the importance of the tumor microenvironment in cancer biology.
Keywords: Cancer-Associated adipocytes (CAAs), Breast Cancer (BC), Benign Breast Disease (BBD), Adipose Tissue, Branched-Chain Ketoacid Dehydrogenase Kinase (BCKDK)

Ps-81: A Systematic Review of The Role of Magnetic Nanoparticles in Mechanotransduction and Neural Repair

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Background: Magnetic nanoparticles (MNPs) have been a revolutionary tool in neural engineering and repair of central and peripheral nervous system (CNS and PNS) injuries due to their superparamagnetic properties and surface functionalization capabilities. This systematic review aims to analyze recent advances in the development of active mechanisms and targeted delivery systems based on MNPs to promote neural repair.
Materials and Methods: A comprehensive search was conducted in PubMed, Scopus, Google Scholar, and MDPI. Search terms included "Magnetic Nanoparticles (MNPs), "Neuroregeneration", and "Mechanotransduction". Research articles from the period (2018-2025) including 31 articles were prioritized based on systematic reviews, clinical studies, research articles, and conference proceedings. The quality assessment of included studies was also considered for scientific rigor.
Results: The results demonstrate that MNPs enhance neuroplasticity through regulation of the critical BDNF/PI3K/Akt signaling pathways. The "nano-stretching" mechanism by applying pico-Newton force facilitates microtubule stabilization and local translation activation for sustained axonal growth. Furthermore, MNPs act as efficient carriers for reprogramming agents and have been able to significantly reduce astrocytic scarring. Dual magnetic strategies have also enhanced MNP transport in BBB models.
Conclusion: Although MNPs have strong preclinical potential in enhancing functional recovery, translational hurdles remain. Clinical integration requires careful consideration of long-term toxicology, optimization of surface coating to reduce induced neuroinflammation, and ethical considerations. MNPs offer a multifaceted approach for the next generation of neurotherapies.
Keywords: Magnetic Nanoparticles, Neuroregeneration, Mechanotransduction

Ps-82: Age-Specific Priming of Mesenchymal Stem Cells: Insights from A Transcriptomics Meta-Analysis

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Background: Despite the ease of culture expansion, mesenchymal stem cells (MSCs) enter a reversible state of growth arrest upon cumulative population doublings, known as replicative senescence, which consequently limits their promising therapeutic applications. Whether the biological age of the cell influences this senescence program remains unclear. To address this, we conducted a meta-analysis on transcriptomic profiles of human MSCs derived from three biological age groups: adult (aMSCs), infant (iMSCs), and pluripotent-derived (p-d MSCs)
Materials and Methods: 12 RNA-Seq and Microarray datasets were selected according to our inclusion criteria. Datasets were pre-processed according to their platforms and differentially expression (DE) analysis were performed using Limma Package in R. meta-analysis was performed on each group separately by sample size-based weighted z-score method (Liptaks) to calculate the combined P. Significantly DEs genes were determined using the combined P<0.05 filtering. Downstream analyses were performed using ClusterProfiler package in R.
Results: 45 genes were revealed as shared one between all groups, from which 33 showed consistent fold change direction. Gene Ontology and Reactome analyses of these consistent genes exhibited their participation in mitotic cell cycle, DNA replication, nuclear envelope assembly and antiviral protection processes, indicating a shared response to replicative stress. However, biological age drove distinct adaptive signatures. From common genes, 12 ones exhibited inconsistent fold change direction between groups. These genes are involved in translation initiation process, as indicated by Reactome analysis. Gene Set Enrichment Analysis showed that while adult MSCs activated cellular response to growth factor stimulus while suppressing neurogenesis, infant MSCs activated neurogenesis, but suppressed cellular response to growth factor stimulus. Remarkably, pluripotent-derived MSCs displayed a hybrid profile, with some pathways regulated similarly to adult MSCs and others similarly to infant MSCs
Conclusion: Our findings reveal that biological age fundamentally shapes the senescence-associated transcriptome in MSCs. The distinct pathway activations and suppressions observed across biological ages argue against a universal senescence model for all MSC types. We conclude that effective MSC-based therapies and anti-aging strategies must consider biological age as a key variable for cell rejuvenation and functional optimization
Keywords: Cell Therapy, Mesenchymal Stem Cell, Senescence

Ps-83 Isolation and Characterization of Wharton's Jelly-Derived Mesenchymal Stem Cell Exosomes for Potential Applications in Cancer Therapy

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Background: Exosomes are nanosized extracellular vesicles that play a crucial role in intercellular communication. Recently, they have gained considerable attention as potential drug carriers in innovative drug delivery methods. As drug carriers, exosomes offer several advantages, including low immunogenicity, high biocompatibility, and regenerative potential, making them promising candidates for applications in cancer diagnostics and therapy.
Materials and Methods: In this study, mesenchymal stem cells were isolated from Wharton's jelly and cultured to produce extracellular vesicle-enriched culture medium. Exosomes were then isolated using a commercial exosome extraction kit. The resulting exosomes were characterized using dynamic light scattering (DLS), scanning electron microscopy (SEM), and protein quantification assays.
Results: DLS analysis demonstrated that the isolated exosomes had an average particle size of approximately 90 nm, which is within the expected nanoscale range of 30-150 nm, confirming successful exosomes extraction. Furthermore, SEM images showed spherical vesicular structures consistent with the morphology of exosomes. Protein quantification assay results also revealed detectable exosomal protein concentrations, further supporting the efficiency and integrity of the extraction process.
Conclusion: Overall, the findings of this study confirm the efficient extraction and successful characterization of exosomes derived from Wharton's jelly mesenchymal stem cells. Due to their natural ability to transfer bioactive molecules and target tumor microenvironments, these exosomes may serve as promising nanocarriers for targeted drug delivery and novel cancer therapeutic strategies in future studies.
Keywords: Exosomes, Nanovesicles, Cancer Therapy, Drug Delivery Systems

Ps-84: Investigating Hyaluronic Acid Scaffold Containing Bovine Hydrolyzed Type II Collagen Including Berberine and Bone Marrow-Derived MSCs in Osteoarthritis Rat Model

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Background: Osteoarthritis is a chronic degenerative joint disease characterized by progressive cartilage destruction, subchondral bone changes, inflammation, pain, and impaired joint function. Current therapeutic approaches mainly relieve symptoms and do not adequately restore damaged cartilage. This study aims to evaluate an injectable hyaluronic acid-based hydrogel containing bovine hydrolyzed type II collagen, berberine, and rat bone marrow-derived mesenchymal stem cells as a regenerative strategy for experimentally induced knee osteoarthritis in rats.
Materials and Methods: This experimental study will be conducted in both *in-vitro* and *in-vivo* phases. Rat bone marrow-derived mesenchymal stem cells will be isolated, expanded, and incorporated into a crosslinked HA-HCII hydrogel loaded with

berberine. Scaffold characteristics, berberine/collagen release, degradation behavior, cytocompatibility, and structural features will be assessed using MTS assay, spectrophotometric analysis, and scanning electron microscopy. Knee osteoarthritis will be induced in rats by intra-articular injection of collagenase and papain/cysteine. Animals will be allocated to intact, HA, Col II, HA-HCII, HA-HCII-rMSCs, HA-HCII-BBR, HA-HCII-BBR-MSCs, and untreated groups. Cartilage repair will be evaluated by X-ray imaging, macroscopic pathological scoring, hematoxylin and eosin (H&E) and Safranin O staining, and real-time PCR assessment of chondrogenic markers including Col II and aggrecan.
Results: The HA-HCII-BBR-MSC construct is expected to enhance cartilage regeneration compared with scaffold-only or cell-free treatments, reflected by improved hyaline-like cartilage formation, stronger Safranin O staining, and increased expression of Col II and aggrecan.
Conclusion: This proposal evaluates a combined anti-inflammatory and regenerative tissue-engineering strategy for cartilage repair in osteoarthritis. If validated experimentally, the HA-HCII-BBR-MSC construct may represent a promising injectable scaffold for preclinical cartilage regeneration studies.
Keywords: Osteoarthritis, Hydrolyzed Type II Collagen, Hyaluronic Acid Hydrogel, Berberine, Mesenchymal Stem Cells

Ps-85: Indole 3 Propionic Acid Attenuates Hepatocyte Senescence Through Modulation of Oxidative Stress and Inflammatory Signaling

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Background: Cellular senescence is a hallmark of aging and contributes to the progression of age-related liver dysfunction through persistent oxidative stress and chronic inflammation. Senescent hepatocytes undergo irreversible cell cycle arrest and secrete inflammatory mediators known as the senescence-associated secretory phenotype (SASP), which contributes to tissue dysfunction and chronic inflammation. Recently, senomorphic compounds that can attenuate senescence-associated phenotypes without inducing cell death have gained considerable attention. Indole-3-propionic acid (IPA), a gut microbiota-derived metabolite of tryptophan, has shown antioxidant and anti-inflammatory properties in several experimental models. However, its role in regulating hepatocyte senescence and the underlying mechanisms remain incompletely understood. Therefore, this study aims to evaluate the possible senomorphic properties of IPA in senescent hepatocytes.
Materials and Methods: Following the establishment of the H_2O_2-induced senescence model, cells were treated with IPA to evaluate its ability to attenuate the senescent phenotype. The anti-senescent potential of IPA was evaluated by examining key senescence-associated markers, including senescence-associated β-galactosidase (SA-β-gal) activity, p16INK4a and p21Cip1 expression, SASP factors, and intracellular reactive oxygen species (ROS) production.
Results: Post-treatment with IPA markedly alleviated H_2O_2-induced senescence in hepatocytes. IPA significantly reduced SA-β-gal-positive cells, downregulated the expression of p16INK4a and p21Cip1, decreased intracellular ROS accumulation, and attenuated SASP-related alterations compared with the H_2O_2-treated group. Collectively, these results demonstrate that IPA mitigates oxidative stress-induced cellular senescence and highlight its potential as a candidate anti-senescent compound for preserving hepatocyte function during aging.
Conclusion: IPA effectively mitigated H_2O_2-induced hepatocyte senescence. Its protective effects were associated with reduced oxidative stress and suppression of senescence-associated markers. IPA may therefore represent a potential therapeutic strategy for combating age-related liver dysfunction.
Keywords: Cellular Senescence, Indole-3-Propionic Acid, Senomorphic, SASP, Oxidative Stress

Ps-86: Identification of Key Genes Involved in The Differentiation of Stem Cells into Dopaminergic Neurons Using Systems Biology and Bioinformatics Approaches: Implications for Cell Therapy in Parkinson's Disease

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Background: Parkinson's disease (PD) is considered a chronic neurological disorder caused by the degeneration of dopaminergic neurons in the substantia nigra of the brain and a consequent reduction in brain dopamine levels. This disorder leads to prominent motor symptoms such as tremor, muscle rigidity, bradykinesia, and impaired balance. Since currently available treatments are unable to regenerate lost cells, the aim of this study was to identify genes involved in the differentiation of stem cells into dopaminergic neurons in order to investigate their potential use in cell therapy for PD. This research was conducted using various systems biology and bioinformatics approaches.
Materials and Methods: In this study, RNA-seq data from two cell groups (stem cells as the control group and 4-day dopaminergic neurons as the treatment group) were extracted from the Gene Expression Omnibus (GEO) database and compared using GEO2R software. Following analysis and selection of the relevant data, a protein–protein interaction network was constructed using Cytoscape software and the STRING database to identify key genes.
Results: Analysis of gene expression data obtained from RNA-seq between the two cell groups identified 18,981 raw genes, of which 2,892 genes showed significant differential expression (987 genes with significant upregulation and 1,906 genes with significant downregulation). Among these genes, 2,009 were recognized in the STRING database, and a protein–protein interaction network was constructed to examine their molecular interactions. Network analysis identified ten hub genes. Among these genes, no prior report was found in the literature regarding the direct effect of CALML6 and CD4 on dopaminergic neuron differentiation. However, given the central role of these two genes in the network, the results of this study suggest that they probably play an important role in differentiation into dopaminergic neurons.
Conclusion: The findings of this study reveal new pathways in the differentiation of stem cells into dopaminergic neurons and suggest attention to the important role of the CALML6 and CD4 genes in this process. These results may provide a basis

for further research toward the development of cell-therapy approaches for PD.
Keywords: Parkinson's Disease, Dopaminergic Neurons, Gene Expression, Protein–Protein Interaction Network Analysis, Hub Genes

Ps-87: Comparison of Decellularized versus Native Chorion Membranes for Inflammatory and Cytocompatibility Properties: Implications for Wound Dressing

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Background: Native chorion membranes offer anti-inflammatory and wound-healing benefits but risk immunogenicity due to cellular DNA. Decellularization removes this risk but may deplete bioactive factors. Thus, native chorion suits acute injuries requiring potent effects, while decellularized chorion is safer for chronic or repeated use where minimizing host sensitization is critical.
Materials and Methods: The study received ethics approval (IR.SHMU.REC.1400.179). Human chorion membranes were obtained from cesarean sections. Decellularization used SDS, trypsin-EDTA, and nuclease solution. Efficacy was confirmed by absent DAPI staining, intact extracellular matrix via scanning electron microscopy (SEM), and no nuclei on histology. Cell viability (MTT assay) and anti-inflammatory activity (albumin denaturation inhibition) were assessed. Data are mean ± SD; t-test or ANOVA determined significance ($P < 0.05$).
Results: Morphological and Nuclear Assessment: SEM revealed that the native membrane had an intact cellular layer, while the decellularized membrane displayed a clean, cell-free collagen matrix with preserved fibrillar architecture. DAPI staining confirmed complete decellularization, showing abundant blue nuclei in the native membrane (a) and virtually none in the decellularized membrane (b). Histopathology: hematoxylin and eosin (H&E) staining provided further confirmation. Retained living cellular components with dense nuclei characteristic of the intact native chorion. Conversely, showed complete removal of cells, leaving only the natural biological extracellular matrix (ECM) scaffold that is biocompatible, structurally intact, and well-suited for tissue engineering and regenerative medicine applications. Cell Viability (MTT Assay): The decellularized membrane supported significantly higher cell viability compared to the native membrane at both 24 hours (90.18 ± 1.17% vs 77.49 ± 1.38%) and 72 hours (80.11 ± 0.97% vs 72.66 ± 3.32%), indicating superior cytocompatibility. Inflammatory Response: The decellularized membrane exhibited lower inflammatory markers (73.55 ± 2.33) than the native membrane (87.11 ± 4.11). The assay validated its responsiveness to standard agents: aspirin produced dose-dependent anti-inflammatory effects (35.87% at 10 mg/mL, 55.54% at 30 mg/mL, 67.43% at 50 mg/mL, 88.56% at 100 mg/mL, and 94.11% at 200 mg/mL).
Conclusion: The decellularized chorion membrane demonstrates significantly lower inflammatory potential and higher cytocompatibility compared to its native counterpart while preserving the native ECM architecture. These favorable features, combined with its reduced immunogenicity and ability to support cell growth, position it as a promising off-the-shelf biological dressing. These properties make it an excellent candidate for clinical applications in skin reconstruction and wound therapy.
Keywords: Decellularized Chorion, Anti-Inflammatory Activity, Cell Viability, Tissue Engineering, Extracellular Matrix

Ps-88: Study The Expression Level of CFH Gene in Patients with Colorectal Cancer

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Background: 30 patients with colorectal cancer were included in the study, from each of which 30 healthy and 30 cancerous tissue samples were collected, and after RNA extraction and cDNA synthesis, the expression of the CFH gene was measured using Real-time PCR. One-Sample T-Test was used to examine the Fold Change data.
Materials and Methods: 30 patients with colorectal cancer were included in the study, from each of which 30 healthy and 30 cancerous tissue samples were collected, and after RNA extraction and cDNA synthesis, the expression of the CFH gene was measured using Real-time PCR. One-Sample t test was used to examine the Fold Change data.
Results: Based on the results of Real-time PCR data, CFH gene expression was significantly increased in the affected group with an average of 3.62 ($P < 0.001$). Receiver operating characteristic (ROC) curve analysis for the CFH gene showed that a cutoff point <8.363 had favorable diagnostic performance. At this threshold, both sensitivity and specificity were 96.67%, with a 95% confidence interval between 82.78% and 99.92%. The positive likelihood ratio was reported to be 29.00, indicating very high discriminatory power in distinguishing patients from healthy individuals. ANOVA test showed that the CFH gene had the highest mean expression in patients with Grade II (Moderately Differentiated), Stage III and with lymphoid and vascular invasion.
Conclusion: In general, it can be concluded that the CFH gene acts as oncogenes in colorectal cancer. ROC curve analysis showed that the CFH gene could be used as a potential molecular marker in disease diagnosis.
Keywords: Colorectal Cancer, Biomarker, CFH

Ps-89: Investigation and Detection of Histone Protein H2B Type 2e As Biomarker in Treatment-Responsive and Resistant Retinoblastoma Patients

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Background: Intravitreal chemotherapy is considered an effective approach for controlling Retinoblastoma; however, patients exhibit heterogeneous outcomes, with some showing tumor regression and others developing treatment resistance. Therefore, identifying reliable molecular biomarkers to predict resistance is critical for optimizing therapeutic strategies. Histone proteins, as key regulators of chromatin organization, are increasingly implicated in tumor progression and drug resistance. Nevertheless, the clinical value of H2B type 2e (H2B2e) in the ocular fluids of Retinoblastoma patients remains unclear. This study aimed to assess whether H2B2e could serve as a non-invasive biomarker of treatment resistance.
Materials and Methods: In this experimental study, aqueous humor samples (100 µL) were collected from 44 patients with Retinoblastoma during therapeutic procedures at Moheb kosar Hospital, Tehran, Iran. Patients were followed for 12 months, after which they were categorized into two outcome groups—good response (n=22) and poor response (treatment-resistant; n=20)—based on monthly clinical assessment and imaging findings. Quantitative H2B2e levels were measured using an ELISA assay.
Results: Levels of the H2B2e differed significantly between the two groups ($P < 0.001$). Patients with treatment resistance had markedly higher aqueous humor levels of this biomarker than patients who responded to therapy. Notably, higher levels were also associated with more severe clinical scenarios, as reflected by the highest concentrations observed in enucleated patients.
Conclusion: Elevated aqueous humor concentrations of H2B2e in treatment-resistant Retinoblastoma patients suggest that this histone protein may be a promising biomarker for predicting therapeutic resistance. Integrating H2B2e assessment into clinical evaluation could help identify high-risk patients early and guide selection of more targeted treatment strategies.
Keywords: Retinoblastoma, Aqueous Humor, Histone H2B Type 2e, Biomarker

Ps-90: The Oncogenic Role of circKIF4A in Human Cancers: A Systematic Review of Molecular Mechanisms and Signaling Pathways

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Background: Circular RNAs (circRNAs), a class of non-coding RNAs, have emerged as important regulators of cancer biology. Among them, circKIF4A (hsa_circ_0007255) has recently been implicated in tumor initiation, progression, and metastasis. This systematic review aimed to comprehensively evaluate the expression patterns, molecular mechanisms, and clinical significance of circKIF4A across human cancers.
Materials and Methods: A systematic literature search was conducted in PubMed and ScienceDirect databases on May 31, 2026. Boolean search operators were employed with Keywords such as "circKIF4A", "hsa_circ_0007255", and "Cancer". The initial search yielded 53 records, of which 49 remained after duplicate removal. Eligibility criteria were applied: (1) studies that evaluated the expression and functions of circKIF4A in cancer, and (2) studies that investigated the molecular and signaling pathways in which circKIF4A is involved. A total of 17 studies met the criteria and were retained for detailed analysis.
Results: Studies have demonstrated that circKIF4A is highly overexpressed across multiple cancer types and is associated with poor prognosis and reduced overall survival. This circular RNA acts as a competing endogenous RNA (ceRNA) and sponges key tumor-suppressor microRNAs, such as miR-375, miR-139, miR-152, and several others. This leads to activation of critical oncogenic pathways, including Wnt/β-catenin, Notch, and STAT3. circKIF4A has also been shown to play a pivotal role in glucose metabolism by regulating the ALDOA/OCT4 axis, increasing glucose uptake and lactate production. Together, these effects contribute to malignant phenotypes, including increased cell proliferation, invasion, migration, tumor growth, and metastasis.
Conclusion: This systematic review identifies circKIF4A as a critical oncogenic regulator involved in cancer progression and aggressiveness. Its recurrent upregulation, association with poor clinical outcomes, and involvement in multiple oncogenic signaling pathways highlight its potential as a promising biomarker and therapeutic target in human malignancies.
Keywords: CircKIF4A, Circular RNA, Oncogenic Signaling Pathways, Cancer Progression

Ps-91: Upregulation of *CTLA-4* and *PD-L1* Expression in Human Monocyte-Derived Dendritic Cells by Glyburide Drives A Tolerogenic Profile: An *In-Vitro* Study

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Background: As traditional antigen-presenting cells, dendritic cells (DCs) are essential for inducing T lymphocyte responses. To maintain tolerance or initiate immune responses, DCs must be balanced and controlled by a number of signalling pathways. Autoimmune disorders have been linked to aberrant activation of the pattern recognition receptor (PRR) nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3), which is present on DCs. Glyburide was administered to monocyte-derived DCs in order to examine the impact of NLRP3 inhibition on the maturity stage and the generation of immunological checkpoint proteins.
Materials and Methods: Monocytes were separated from peripheral blood mononuclear cells (PBMCs) using the magnetic-activated cell sorting (MACS) technique and the CD14 marker. Pure monocytes were grown and stimulated with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 to create immature DCs. Glyburide was then applied to these DCs. Next, real-time PCR was used to measure the degree of immune checkpoints expression.
Results: The expression of CTLA-4 significantly increased. The expression level of PD-L1 increased, however the elevation was not significant.
Conclusion: Glyburide produced tolerogenic DCs by upregulating CTLA-4 as an inhibitory immune checkpoint.
Keywords: Inhibitory Immune Checkpoints, Autoimmunity, CTLA-4, PD-L1

Ps-92: Inhibition of NOD-Like Receptor Family Pyrin Domain Containing 3 (NLRP3) Enhances B and T Lymphocyte Attenuator (BTLA) Expression and Promotes A Tolerogenic Phenotype in Human Monocyte-Derived Dendritic Cells

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Background: Dendritic cells (DCs) are key antigen-presenting cells that help maintain the balance between immune activation and immune tolerance. Abnormal activation of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome has been linked to several autoimmune and inflammatory disorders. B and T lymphocyte attenuator (BTLA) is an important immune checkpoint molecule that contributes to immune regulation and tolerance. This study explored whether inhibition of NLRP3 could influence BTLA expression and promote a tolerogenic phenotype in human monocyte-derived DCs.

Materials and Methods: Peripheral blood mononuclear cells were isolated, and CD14-positive monocytes were purified using magnetic-activated cell sorting (MACS). The monocytes were differentiated into immature DCs in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4). Cells were then treated with glyburide, a pharmacological inhibitor of NLRP3. *BTLA* gene expression was evaluated using real-time polymerase chain reaction (PCR).

Results: Glyburide treatment induced clear morphological changes in DCs, including the formation of prominent cytoplasmic dendrites and increased cellular branching, which are characteristic features of dendritic cell maturation. In addition, BTLA expression was significantly increased in glyburide-treated DCs compared with untreated cells ($P < 0.001$).

Conclusion: These findings suggest that NLRP3 inhibition promotes morphological maturation while enhancing the expression of an immunoregulatory checkpoint molecule associated with tolerogenic function.

Keywords: Dendritic Cells, B and T Lymphocyte Attenuator (BTLA), NLRP3 Inflammasome

Ps-93: Rapamycin-Induced Disruption of Endothelial–Fibroblast Crosstalk through Oxidative Stress Activation in A Colorectal Cancer Microenvironment Model

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Background: Interactions between endothelial cells and fibroblasts within the tumor microenvironment contribute to colorectal cancer (CRC) progression by promoting angiogenesis, survival signaling, and therapeutic resistance. Oxidative imbalance is considered an important regulator of stromal communication in CRC. This study investigated whether rapamycin could alter endothelial–fibroblast interactions through oxidative stress induction in a co-culture model of CRC.

Materials and Methods: Human umbilical vein endothelial cells (HUVECs) and fibroblasts were cultured using a Transwell co-culture system and treated with rapamycin for 72 hours. Cell viability was evaluated using the MTT assay. Oxidative stress status was assessed by measuring total oxidant status (TOS) and malondialdehyde (MDA) levels. Apoptotic changes were analyzed using Annexin V/propidium iodide (PI) flow cytometry.

Results: Rapamycin treatment reduced cellular viability and altered stromal interactions within the co-culture environment. Significant increases in TOS and MDA levels were observed following treatment, indicating enhanced oxidative stress. Flow cytometric analysis demonstrated elevated apoptotic cell populations compared with untreated controls. These findings suggested that rapamycin disrupted endothelial–fibroblast crosstalk and promoted oxidative stress-associated cellular damage in the CRC microenvironment.

Conclusion: Rapamycin interfered with stromal communication and induced oxidative stress- associated apoptosis in the HUVEC–fibroblast co-culture model. Targeting stromal interactions and redox imbalance may provide a promising therapeutic strategy for CRC treatment.

Keywords: Colorectal Cancer, Oxidative Stress, Tumor Microenvironment, Apoptosis, Rapamycin

Ps-94: Modulating Stromal–Endothelial Communication by Rapamycin through PI3K/AKT Pathway Suppression in Colorectal Cancer

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Background: Interactions between cancer-associated fibroblasts (CAFs) and endothelial cells play an important role in colorectal cancer (CRC) progression by regulating angiogenesis, stromal signaling, and tumor survival. The PI3K/AKT signaling pathway is one of the major molecular pathways involved in these tumor supportive interactions. This study investigated the modulatory effects of rapamycin on stromal–endothelial communication and PI3K/AKT pathway activity in a CRC co-culture model.

Materials and Methods: Human Umbilical Vein Endothelial Cells (HUVECs) were co-cultured with colorectal CAFs using a Transwell system. Cells were treated with rapamycin for 72 hours. Cell viability was assessed using MTT assay, while angiogenic activity was evaluated by tube formation analysis. Oxidative stress parameters including total oxidant status (TOS) and malondialdehyde (MDA) were measured. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was performed to evaluate *PI3K* and *AKT* gene expression levels.

Results: Rapamycin treatment reduced endothelial cell viability and impaired angiogenic tube formation within the co-culture system. Increased TOS and MDA levels demonstrated enhanced oxidative stress following treatment. In addition, rapamycin significantly downregulated PI3K and AKT gene expression compared with untreated controls. These findings suggested suppression of stromal–endothelial signaling interactions associated with CRC progression.

Conclusion: Rapamycin modulated stromal–endothelial communication and attenuated PI3K/AKT signaling activity in the

CRC co-culture model. These findings highlight the therapeutic potential of targeting tumor microenvironment interactions in coloreactal cancer.

Keywords: Colorectal Cancer, Rapamycin, PI3K/AKT Signaling, Tumor Microenvironment, Angiogenesis

Ps-95: Computational and Experimental Evidence for The Anticancer Activity of Thymol against Papillary Renal Cell Carcinoma

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Background: Papillary renal cell carcinoma (pRCC) is a common subtype of kidney cancer with limited effective targeted therapies. Thymol is a natural small molecule with reported anticancer effects, yet its mechanistic role in pRCC is not fully understood. Integrative network pharmacology, molecular docking, and in-vitro assays provide a framework to identify key targets and pathways underlying thymol-mediated anticancer effects.

Materials and Methods: Target identification, PPI network construction, and topological analysis were conducted using Cytoscape3.10.3 and various databases, followed by functional enrichment analysis via DAVID server for shared genes between thymol targets and pRCC-related genes. Blind molecular docking was performed via AutoDock4.2 software to validate thymol-target interactions. To experimentally validate predictions, ACHN (Human pRCC cell line) and HDF (human dermal fibroblast) cell lines were used for *in-vitro* and mechanistic assays including MTT assay, flow cytometry, RT-qPCR and western blot.

Results: The intersection between 218 potential targets of thymol and 256 pRCC-related genes identified 10 shared genes. To obtain a broader view of the molecular interactions associated with these targets, GeneMANIA analysis identified 20 additional associated genes, resulting in an expanded network. Topological analysis of PPI network with 8 algorithms and their intersection with 10 primary genes identified EGFR and AKT1 as hub targets. Functional enrichment analyses revealed significant involvement in PI3K/AKT pathway (FDR and $P < 0.05$) and molecular docking demonstrated favorable binding affinities between thymol and targets (AKT1: -5.61 kcal/mol, EGFR: -4.39 kcal/mol). In-vitro assays confirmed that thymol reduced cell viability in a dose- and time-dependent manner (IC_{50}=500μM), and induced apoptosis in ACHN cells at IC50 ($P < 0.05$), while having no significant effects on HDF cells at 500μM ($P>0.05$). Thymol did not affect the expression of AKT1, while downregulated SNAI1, EGFR, and c-MYC mRNA levels and upregulated BAX expression in ACHN ($P < 0.05$). Moreover, thymol significantly reduced pEGFR/EGFR and pAKT1/AKT1 ratio in ACHN ($P < 0.05$).

Conclusion: Our results suggest that thymol may modulate PI3K/AKT-driven oncogenic signaling in pRCC through EGFR and AKT1, exhibit robust anticancer activity in ACHN cells, and hold promising therapeutic potential for pRCC management, while not damaging HDF cells.

Keywords: Thymol, Papillary Renal Cell Carcinoma, Network Pharmacology, Anti-cancer Drug Design

Ps-96: Evaluating The Cytotoxicity Effects of Oncolytic Virus on The Glioblastoma Tumoroids

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Background: Glioblastoma multiforme (GBM) is one of the most aggressive tumors of the human central nervous system, and duo to its high resistance to treatment and frequent recurrence, it presents serious therapeutic challenges. In this study, for the first time in Iran, 3-D patient-derived tumor organoids (PDOTs) were used to evaluate the efficacy of the oncolytic vaccinia virus, both alone and in combination with temozolomide (TMZ), aiming to assess therapeutic response in a setting that closely mimics to the human tumor microenvironment.

Materials and Methods: Organoids were derived from tumor specimens of three patient with recurrent GBM after surgical resection and generated under sterile laboratory conditions. Following confirmation of organoid structure, treatments were applied using oncolytic vaccinia virus, TMZ and their combination. Morphological and cytotoxic effects were evaluated using a semi-quantitative scoring system on days 0,3,6 and 9 through both light and fluorescence microscopy.

Results: The results indicates that the oncolytic vaccinia virus caused significant disruption of spherical and organized structure of the tumor organoids, while TMZ alone had limited effect. The combinational treatment group showed a more yet consistent pattern of organoid disintegration, suggesting a synergistic therapeutic interaction. In the control group, organoids remained structurally stable throughout the experiment period.

Conclusion: In conclusion, the findings shows that oncolytic vaccinia viruses demonstrate promising potential in targeting glioblastoma when it's evaluated in human-derived 3D tumor organoid model.

Keywords: Oncolytic Virotherapy, Organotypic Modeling, Cancer, Personalized Medicine, Immunotherapy

Ps-97: Minimally Invasive and Sustained Delivery of Bevacizumab to The Posterior Segment of The Rabbit Eye by Using A Hydrogel Microneedle Patch

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Background: Bevacizumab (BZ) has shown promising anti-angiogenesis effect for treatment of Diabetic retinopathy in clinical setups. However, sustained delivering therapeutic doses of BZ to retinal cells has remained a significant challenge. This study aimed to develop and characterize hydrogel microneedle (MN) patches for sustained and minimally invasive delivery of BZ to the posterior eye segment
Materials and Methods: A hydrogel MN patch based on clickable alginate was fabricated by micro-molding technique followed by autonomous crosslinking via Diels–Alder click chemistry. Mechanical strength was assessed by a texture analyzer. Hydrogel degradation and drug release were evaluated under physiological solutions. Cytotoxicity was tested using the MTS assay on ARPE-19 cells. BZ permeation through scleral tissue over time was examined using Franz diffusion cells, anti-angiogenesis activity of released BZ was assessed via the chick chorioallantois membrane (CAM) assay, and ocular distribution of BZ was evaluated by fluorescent imaging in rabbit eyes.
Results: The MN patch exhibited ideal morphology under digital microscopy and withstood 4 N of force. The MN patch demonstrated gradual biodegradability and controlled release of BZ over 123 days. The patch was non-cytotoxic (>80% cell viability on ARPE-19 cells). The released BZ retained its anti-angiogenic properties in the CAM assay, successfully permeated rabbit scleral tissue in Franz diffusion cells, and effectively delivered to the posterior segment of rabbit eye.
Conclusion: The hydrogel MN patch enabled sustained delivery of BZ to the retina. Further studies are needed to evaluate efficacy in human eyes.
Keywords: Hydrogel Microneedle Patch, Anti-Angiogenesis, Bevacizumab, Sustained Delivery

Ps-98: Preliminary Characterization of A Rat Clip-Compression Spinal Cord Injury Model Using A Yasargil Mini Aneurysm Clip for Regenerative Medicine Applications

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Background: Spinal cord injury (SCI), represents a major area of investigation in neuroscience, stem cell therapy, and regenerative medicine. The evaluation of novel reparative interventions requires standardized, reproducible, and clinically relevant animal models. Several experimental approaches, including contusion, compression, and transection models, have been used to induce SCI. However, no single model can fully reproduce the complex pathophysiological features of human SCI. Among these approaches, compression injury is of particular importance because it closely resembles clinical conditions associated with vertebral fracture or dislocation, in which mechanical pressure is applied to the posterior or lateral aspects of the spinal cord. This study aimed to preliminarily characterize a rat clip-compression SCI model using a Yasargil mini aneurysm clip.
Materials and Methods: In this experimental study, a compression model of SCI was established in rats using a Yasargil mini aneurysm clip. Animals were allocated into different experimental groups according to the compression conditions and the expected severity of injury. Spinal cord tissue samples were collected at two post-injury time points: 48 hours and 7 days. Histopathological evaluation was performed using hematoxylin and eosin (H&E) staining to assess tissue architecture disruption, necrosis, loss of preserved parenchyma, and cavitation formation. Luxol Fast Blue (LFB) staining was applied to evaluate myelin integrity and demyelination within the injured spinal cord tissue
Results: Histological findings demonstrated that the Yasargil mini clip-compression model was able to induce distinguishable degrees of SCI in rats, ranging from moderate to severe. H&E staining revealed disruption of the normal spinal cord architecture, reduction of preserved parenchymal tissue, and cavitation formation at the injury site. In addition, LFB staining showed decreased myelin integrity and evidence of demyelination in the injured regions. Based on cavitation, tissue destruction, and myelin loss, relative differentiation of injury severity from moderate to severe was achieved
Conclusion: The Yasargil mini aneurysm clip may serve as a useful tool for establishing a rat clip-compression model of SCI. Characterization of this model provides a suitable experimental platform for future preclinical studies in stem cell therapy, regenerative medicine, and the evaluation of reparative interventions for SCI.
Keywords: Spinal Cord Injury, Yasargil Clip, Compression Model, Regenerative Medicine

Ps-99: Remodeling Biomechanical Status in Liver Cancer Cells through Cytoskeletal Alterations by Targeting DIAPH3 as A Novel Therapeutic Strategy

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Background: Hepatocellular carcinoma (HCC) is an aggressive liver cancer that invades and metastasizes. Cytoskeletal remodeling is increasingly thought to regulate cancer cell migration and be important in differentiation therapy. We used in silico target identification and DDS-based therapy to identify and regulate HCC cytoskeleton. A controlled intracellular delivery system using niosomes was proposed. DIAPH3, actin gene for F-actin regulation and cytoskeletal dysregulation, was found by bioinformatic analysis.
Materials and Methods: Analysis of TCGA-LIHC cohort messenger RNA expression and clinical data. A Limma-based approach identified DEGs after preprocessing and normalization. DAVID was used for functional enrichment analysis of the Gene Ontology molecular function term actin binding. STRING created and Cytoscape analyzed a PPI network. Hub genes were ranked by CytoHubba. GEPIA confirmed DIAPH3 expression and prognostic value. For experimental validation, liver cancer cells will receive free and drug-loaded niosomes. DIAPH3 expression, F-actin organization, epithelial-mesenchymal tran-

sition (EMT)/MET markers, migration, invasion, radial expansion, and biomechanical stiffness will be measured.
Results: TCGA-LIHC differential expression analysis showed 2,123 genes were dysregulated in HCC tissues compared to normal liver tissues. Functional enrichment analysis found 54 actin-binding and cytoskeletal remodeling genes. From Cytoscape and the CytoHubba plugin, we identified five major hub genes in the actin-binding protein–protein interaction network: DBN1, DIAPH3, ANLN, MYO10, and TPM2. DIAPH3 was 3.18 log2fold change upregulated in HCC tissues of these candidates. GEPIA validation confirmed HCC DIAPH3 overexpression and poor overall survival. DIAPH3 had an area under the curve of 0.95 for distinguishing HCC tissues from normal liver tissues. Our findings indicate that DIAPH3 is a clinically relevant cytoskeletal hub gene involved in HCC biomechanical dysregulation.
Conclusion: We employed Cytoscape with the CytoHubba plugin to identify the top five actin-binding network hub genes *DBN1, DIAPH3, ANLN, MYO10*, and *TPM2*. *DIAPH3* was selected as the hub gene due to its significant upregulation, negative correlation with survival, and superior diagnostic capability. DIAPH3 may facilitate HCC progression by modifying the actin cytoskeleton, Rho GTPase signaling, β-catenin/TCF activation, EMT, and augmenting migratory and invasive capabilities.
Keywords: Hepatocellular Carcinoma, DIAPH3, Niosome, Drug Delivery System, Cytoskeletal Remodeling

Ps-100: Human Umbilical Cord Blood Serum Improves Gentamicin-Induced Acute Kidney Injury

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Background: Acute kidney injury (AKI) is a type of tissue damage in which there is fibrosis and gradual dysfunction of the kidney. In mild to moderate stages of the disease, kidney function can be restored. Human umbilical cord blood serum (hUCBS) contains various interleukins and growth factors that are directly involved in nephroprotective processes. The present study has examined the preventional effects of hUCBS against AKI-induced fibrosis.
Materials and Methods: The total protein and growth factor levels in hUCBS were quantified using BCA and ELISA kits. The effects of hUCBS on cell viability and migration were evaluated using human dermal fibroblasts (HDFs). For this, gentamicin- exposed HDFs were treated with 2%, 5%, and 10% hUCBS, as well as 10% FBS and 5% PL sera, in the culture medium. Adult male C57BL/6 mice were divided into control, 100 mg/kg gentamicin (AKI), and AKI+hUCBS groups. AKI was induced by intraperitoneal injection once daily for 7 days. In the serum-treated group, mice received 500 µl of hUCBS intravenously on the second day after the gentamicin injection. Serum samples were assessed for urea and creatinine. Fibrosis was assessed by western blotting for fibronectin and vimentin. Histopathological scores were evaluated using hematoxylin and eosin (H&E) staining.
Results: Growth factors such as HDF, EGF, IGF, PDGF, and VEGF were present at high concentrations in hUCBS. Human cord blood serum at 10% increased the survival rate of cells exposed to 1500 µg/ml gentamicin compared with other sera. However, it did not affect cell migration. Urea and creatinine levels in the AKI+hUCBS group were lower than in the AKI group, and BUN/Cr was increased in this group. The AKI group showed a 4.5-fold increase in fibronectin, whereas it was reduced to nearly zero in the AKI+hUCBS group. The AKI+hUCBS group also showed a nearly 2-fold increase in vimentin compared with the AKI group. Histopathological studies showed tubular injury and cast formation in the AKI group, but not in the hUCBS-treated group.
Conclusion: hUCBS can prevent the progression of fibrosis after acute kidney injury.
Keywords: hUCBS, AKI, Gentamicin, Kidney, Growth factors

Ps-101: Effect of Combined Stem Cells and Extracellular Vesicles on Spermatogenesis and Fertility in A Mouse Model of Azoospermia

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Background: Non-obstructive azoospermia (NOA) is recognized as one of the most severe types of male infertility, primarily resulting from impaired spermatogenesis. In recent years, mesenchymal stem cells (MSCs) and their derived extracellular vesicles (EVs) have gained attention as novel therapeutic approaches for tissue regeneration. However, it remains unclear whether the combination of MSCs with their EVs results in synergistic therapeutic benefits for male infertility. The present study was designed to investigate this question.
Materials and Methods: Adipose-derived MSCs were isolated and characterized by flow cytometry and their ability to differentiate into adipogenic and osteogenic lineages. EVs were isolated from MSC-conditioned media using ultracentrifugation. then The EVs were characterized through dynamic light scattering (DLS), Bradford protein assays, Western blotting (using positive exosomal markers CD63 and TSG101, with calnexin as a negative control), and transmission electron microscopy (TEM). A mouse model of azoospermia was established by intraperitoneal injection of busulfan (40 mg/kg) into adult male mice. The success of the model was confirmed four weeks later. Mice were then randomly divided into four groups: a control group, a sham group, the NOA group, and a combination group (receiving MSCs and EVs). For therapeutic administration, MSCs were injected only on day 1 via the efferent ducts, while EVs were injected on days 1 and 17 beneath the tunica albuginea. Sixty days after the first injection, reproductive function was evaluated by histopathological analysis, sperm

parameters, and mating behavior. In addition, gene expression levels of spermatogenesis markers and serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were measured.
Results: The combined MSCs+EVs treatment resulted in significant improvements in spermatogenesis compared to the NOA group. An increase in the expression of spermatogenesis-related genes was also observed in the treated group. Furthermore, combination therapy led to partial recovery of sperm count, sperm morphology, hormone levels and fertility potential.
Conclusion: These findings suggest that the combination therapy (MSCs and EVs) promotes spermatogenesis and fertility in azoospermia models. This combination therapeutic strategy showed some promising results and may enhance their prospects of biological fathers.
Keywords: Non-Obstructive Azoospermia, Mesenchymal Stem Cells, Extracellular Vesicles, Combination

Ps-102: Encapsulation of Natural Killer (NK) Cells in Alginate-Gelatin Microspheres for Enhanced Cell Therapy Applications in Hypoxic Tumor Environment

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Background: Adoptive natural killer (NK) cell therapy for resistant cancers is hindered by hypoxic tumor environments, poor persistence, and limited homing. Encapsulation within biocompatible hydrogel microspheres addresses this by providing a supportive 3D microenvironment, and localized/sustained delivery. In this study, encapsulated NK cells in alginate-gelatin microspheres were examined under hypoxic tumor environment to investigate the effects of encapsulation on NK cell viability, and functional responses.
Materials and Methods: Primary human NK cells from healthy donors were isolated using magnetic activated cell sorting (MACS) and suspended in sterile sodium alginate-gelatin. Using an electrostatic droplet generator, the suspension formed microspheres at 8 kV into a $CaCl_2$ and $ZnCl_2$ bath (5.5 cm distance from blunt needle). After 30 minutes of crosslinking and washing, microbial transglutaminase was applied. The mechanical properties of the microspheres were assessed using a compression test, and their morphology and size were analyzed by microscopy. Under hypoxic conditions, encapsulated NK cells were evaluated at 24 hours for viability, and cytotoxicity against HepG2 cells, with non-encapsulated NK cells serving as controls. In addition, CD107a expression as a marker of activation was assessed at 4 hours under the same hypoxic conditions.
Results: The electrostatic droplet technique consistently generated spherical alginate-gelatin microspheres with a narrow size distribution (219.45 ± 15.73 µm). Encapsulated NK cells retained potent tumor-killing function, exhibiting higher cytotoxic activity against HepG2 target cells (85.6% total apoptosis) compared to non-encapsulated controls (80.0% total apoptosis). Furthermore, the lack of significant variation in CD107a expression (17%) between encapsulated and free NK cells suggests that the encapsulation process does not substantially impact NK cell degranulation activity.
Conclusion: Electrostatic droplet generation encapsulates functional NK cells in alginate/gelatin microspheres, maintaining viability, and cytotoxic/immunomodulatory functions. This enhances NK cell delivery for adoptive immunotherapy, pending in-vivo validation and functionalization.
Keywords: Natural Killer Cells, Cell Encapsulation, Microspheres, Immunotherapy, Biopolymer

Ps-103: Investigating The Effect of Engineered Small Extracellular Vesicles Containing Yttrium Oxide Nanoparticles in A Cell Model of Chemotherapy-Induced Neurotoxicity

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Background: Doxorubicin (DOX), a chemotherapeutic agent from the anthracycline class, is widely used in cancer treatment, but its clinical efficacy is limited by dose-dependent neurotoxicity. Small extracellular vesicles (sEVs) derived from human bone marrow mesenchymal stem cells (hBM-MSCs) have emerged as promising drug delivery vehicles. Yttrium oxide nanoparticles (Y_2O_3-NPs) possess antioxidant properties, but their effect when loaded into sEVs on DOX-induced neurotoxicity remains unclear.
Materials and Methods: SH-SY5Y neuroblastoma cells were cultured for 48 hours in four groups: control (no treatment), DOX alone (0.627 µM, IC_{50}), co-treatment with DOX and sEVs derived from control MSCs (sEV-CTRL) at doses of 5 and 15 µg/mL, and co-treatment with DOX and sEVs derived from MSCs pre-treated with Y_2O_3 (sEV-Y_2O_3) at doses of 5 and 15 µg/mL. Cell viability was assessed using the MTT assay. All experiments were performed in triplicate.
Results: DOX alone significantly reduced cell viability to 43% ± 2.8% compared to the control group ($P < 0.0001$). Co-treatment with sEV-CTRL at 5 µg/mL showed no significant difference from DOX alone (37.5% ± 3.9%, $P>0.05$), while at 15 µg/mL viability decreased to 30.3% ± 3.0% ($P < 0.05$). In contrast, co-treatment with sEV-Y_2O_3 at both doses significantly reduced viability compared to DOX alone: 30.0% ± 1.2% at 5 µg/mL and 26.7% ± 1.3% at 15 µg/mL ($P < 0.01$ for both). No significant difference was observed between the two doses of sEV-Y_2O_3 ($P=0.6936$).
Conclusion: sEV-Y_2O_3 did not protect SH-SY5Y cells against DOX; instead, it unexpectedly enhanced its cytotoxicity. This finding is valuable from a cancer therapy perspective: sEV-Y_2O_3 can serve as an adjuvant to increase the sensitivity of cancer cells to DOX and reduce the effective drug dose. Future studies should focus on molecular mechanisms, selective toxicity on normal cells, and in-vivo evaluation.
Keywords: Small Extracellular Vesicle, Yttrium Oxide Nanoparticle, Doxorubicin, Neuroblastoma, Cytotoxicity

Ps-104: Bibliometric Landscape of Extracellular Vesicle Protein Corona: Analysis Based on VOSviewer

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Background: The protein corona, formed through adsorption of biomolecules onto extracellular vesicles (EVs) upon exposure to biological fluids, is increasingly recognized as a key determinant of EVs identity, cellular uptake, biodistribution and therapeutic efficacy. However, no comprehensive bibliometric analysis has systematically evaluated global research trends in EV protein corona studies.
Materials and Methods: On 21 May 2026, a systematic literature search was performed in the Web of Science database using the query: ("extracellular vesicle*" OR "EVs" OR "exosome") AND ("protein corona" OR "biocorona" OR "corona"). A total of 173 articles were retrieved. Bibliometric data were analyzed using VOSviewer (version 1.6.20). Keyword co-occurrence analysis was conducted with a minimum keyword occurrence threshold of 2. Among 537 identified Keywords, 76 met the inclusion criterion and were visualized in a co-occurrence network.
Results: The keyword co-occurrence map revealed a multicluster network. The central cluster was dominated by EVs and exosomes, closely linked with microvesicles, ectosomes, proteomics, and diagnostics. Protein corona emerged as a highly connected hub associated with blood plasma, membrane fusion, endocytosis, and liquid biopsy, highlighting interest in corona-mediated modulation of EV-cell interactions and biomarker accessibility. Another major cluster included drug delivery, nanotechnology, gene therapy, vaccines, liposomes, and RNA delivery systems, emphasizing the translational relevance of EV-corona interactions. A third distinct cluster grouped COVID-19, SARS-CoV-2, mesenchymal stem cells, and therapy, reflecting a documented post-2020 surge of interest in EV-based therapeutic strategies in infectious disease contexts. Another major cluster included drug delivery, nanotechnology, gene therapy, vaccines, liposomes, and RNA delivery systems, emphasizing the translational relevance of EV-corona interactions. A distinct post-2020 cluster linked COVID-19, SARS-CoV-2, mesenchymal stem cells, and therapy, reflecting increased attention to EV-based therapeutic strategies during the pandemic. Additional clusters focused on neurodegenerative diseases, blood-brain barrier transport, cancer, glycosylation, zeta potential, and nanomedicine applications.
Conclusion: EV protein corona research is a rapidly expanding multidisciplinary field bridging nanomedicine, cell biology, oncology, and infectious diseases. Current trends indicate a transition from descriptive proteomics toward functional and translational studies focused on EV targeting, immune interactions, and diagnostic performance. However, corona characterization and a better understanding of corona behavior in biological fluids require further investigation to facilitate clinical translation.
Keywords: Extracellular Vesicles, Exosomes, Protein Corona, Bibliometrics, Vosviewer

Ps-105: Minicircle DNA Platform for Rapid and Efficient Generation of CD19-Specific CAR-T Cells in Leukemia

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Background: Chimeric antigen receptor (CAR) T-cell therapy has demonstrated remarkable clinical efficacy in hematologic malignancies; however, the reliance on viral vectors for CAR gene delivery raises concerns regarding insertional mutagenesis, immunogenicity, manufacturing complexity, and cost. Minicircle DNA vectors, which are devoid of bacterial backbone sequences, represent a nonviral alternative with improved biosafety and sustained transgene expression. This study aimed to develop and evaluate a minicircle DNA–based system for the generation of CD19-specific CAR T cells and to assess their functional anti-leukemic activity *in-vitro*.
Materials and Methods: A second-generation CD19-specific CAR construct was cloned into a minicircle DNA vector (CAR-MC). Human peripheral blood T cells were isolated and genetically modified via electroporation with CAR-MC. CAR expression was assessed, and functional activity was evaluated in co-culture assays using CD19-positive leukemia cell lines and CD19-negative control cells. Outcomes included antigen-specific cytotoxicity, cytokine secretion (IFN-γ and TNF-α), and proliferative responses.
Results: Minicircle-engineered CAR T cells exhibited efficient CAR expressions and demonstrated potent and selective cytotoxicity against CD19-positive target cells, with minimal activity against CD19-negative controls. Upon antigen-specific stimulation, CAR T cells showed significantly increased secretion of IFN-γ and TNF-α compared with non-transduced T cells, indicating robust functional activation. Additionally, minicircle-mediated gene transfer supported sustained CAR expression and maintained T-cell functionality without detectable vector-associated toxicity.
Conclusion: Minicircle DNA vectors constitute a safe, efficient, and non-integrating platform for CAR T-cell engineering. This system enables durable CAR expression and potent antigen-specific anti-leukemic activity, supporting its potential as a next-generation nonviral alternative for CAR T-cell immunotherapy.
Keywords: CAR T Cell, CD19, Minicircle DNA, Immunotherapy

Ps-106: Thermoresponsive Hydrogel-Based Organoids for Cardiac Tissue Engineering

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Background: Application of various 3D substrates loaded with drugs has been extended for the alleviation of pathological conditions such as myocardial infarction (MI). Hydrogels are commonly studied biomaterials for the development of 3D have found extensive application in biomedical and research fields. Their unique properties have made hydrogels widely used in tissue engineering, drug delivery, and regenerative medicine. The tunable stiffness and mechanical resilience of a hydrogel mimic of the extracellular matrix (ECM) facilitate cell prolif-

eration and differentiation, and cell adhesion. H9C2 cells, serve as an important model for cardiovascular biology, drug toxicity testing, and cardiac tissue regeneration research.
Materials and Methods: In this study, a suitable and reproducible 3D in-vitro cardiac model was developed using the hanging drop technique, which allows the formation of multicellular organoids encapsulated within thermoresponsive hydrogel solutions containing H9C2 cells.
Results: As-prepared thermoresponsive hydrogels were characterized by field-emission scanning electron microscopy (FESEM), Fourier-transform infrared spectroscopy (FTIR), and dynamic light scattering (DLS). These analyses confirmed successful synthesis of thermoresponsive hydrogel, the physicochemical properties and integrity. MTT assay indicated enhanced H9C2 cell viability inside organoids composed of thermoresponsive hydrogel ($P < 0.05$). These thermoresponsive hydrogels supported the growth and survival of H9C2 cardiomyoblasts in the interstices.
Conclusion: The findings demonstrated unique properties of thermoresponsive hydrogels establish them as potent platforms to enhance cardiovascular research and drug development for myocardial repair.
Keywords: Thermoresponsive Hydrogel, Cardiomyoblast, Organoid, Hanging Drops

Ps-107: Delivery of Centella Asiatica Extract from Biodegradable Microparticles for Skin Tissue Engineering

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Background: Chronic diabetic wounds are characterized by persistent inflammation, impaired angiogenesis, and defective extracellular matrix remodeling, resulting in delayed healing. This pathological condition is strongly associated with impaired macrophage polarization from the pro-inflammatory M1 phenotype to the pro-regenerative M2 phenotype. This study aimed to develop a collagen-based bioactive scaffold with sustained delivery of Centella asiatica extract and to evaluate its in-vitro regenerative and immunomodulatory potential.
Materials and Methods: Type I collagen was extracted from bovine skin and characterized using standard biochemical assays. Centella asiatica extracts were screened based on antioxidant activity and cytocompatibility on human foreskin fibroblasts (HFF). Cellular responses were assessed using scratch assays, and macrophage polarization was evaluated through IL-10 secretion analysis. The selected extract was encapsulated into PLGA microparticles using a double emulsion method and incorporated into freeze-dried collagen sponges. To enhance structural integrity, modulate degradation rate, and improve mechanical stability, scaffolds were crosslinked using 0.25% glutaraldehyde. The degree of crosslinking TNBS (2,4,6-Trinitrobenzene sulfonate) assay, swelling behavior, mechanical properties, and release profile were systematically evaluated alongside biological assays.
Results: Successful extraction of type I collagen was confirmed. The methanolic extract of Centella asiatica demonstrated superior antioxidant activity and significantly enhanced fibroblast viability, with 1000 ppm identified as the optimal non-toxic concentration. This condition significantly accelerated fibroblast migration and wound closure within 72 hours. Macrophage analysis revealed a marked increase in IL-10 secretion, indicating a pronounced shift toward the anti-inflammatory M2 phenotype, highlighting the strong immunomodulatory effect of the extract. The glutaraldehyde-crosslinked scaffolds exhibited improved mechanical strength, enhanced structural stability, and high swelling capacity, providing a favorable microenvironment for cell proliferation and migration. Furthermore, incorporation of PLGA microparticles enabled a sustained release profile, minimizing initial burst release and ensuring prolonged bioactive signaling.
Conclusion: The glutaraldehyde-crosslinked collagen scaffold incorporating PLGA-encapsulated Centella asiatica extract demonstrated strong immunomodulatory and regenerative potential by promoting macrophage polarization toward the M2 phenotype while maintaining favorable physicochemical properties. These findings highlight the importance of integrating structural reinforcement with controlled bioactive delivery in the design of advanced wound dressings. Further in-vivo studies are required to validate its therapeutic efficacy.
Keywords: Diabetic Wound, Collagen Scaffold, Centella Asiatica, PLGA Microparticles, Macrophage Polarization

Ps-108: Dual Plasmid Engineering Enhanced Soluble Production of Bioactive Recombinant Human GM-CSF in E. Coli

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Background: Human granulocyte–macrophage colony-stimulating factor (hGM-CSF) is a vital hematopoietic growth factor widely used to manage myeloid recovery in cancer patients following chemotherapy, radiotherapy, or bone marrow transplantation. While Escherichia coli is the preferred host for producing therapeutic proteins due to its cost-effectiveness and rapid growth, achieving high yields of soluble, biologically active rhGM-CSF remains a significant bioprocessing challenge.
Materials and Methods: This study aimed to enhance rhGM-CSF production by implementing a dual-plasmid expression strategy. A primary plasmid harboring the rhGM-CSF gene (pBR322 origin) was transformed into E. coli Shuffle cells. Subsequently, a second plasmid carrying the same gene with either a p15A (low-copy) or RSF (high-copy) origin of replication was introduced to create a dual-plasmid system. Protein expression levels were evaluated via SDS–PAGE and quantified using the Bradford assay, while biological functionality was validated through MTS proliferation assays on TF-1 cells.
Results: The results demonstrated that dual-plasmid configurations significantly outperformed the single-plasmid system in producing soluble rhGM-CSF. Notably, the construct utilizing the p15A origin exhibited slightly higher expression levels than the RSF-based system. This is likely attributed to the re-

duced metabolic burden imposed on the host cells compared to the high-copy-number RSF replicon. Functional analysis confirmed that the recombinant protein successfully stimulated TF-1 cell proliferation, with activity levels comparable to commercially available GM-CSF.
Conclusion: These findings validate the dual-plasmid approach as a robust strategy for improving the production efficiency of therapeutic proteins, facilitating greater clinical accessibility to recombinant hGM-CSF.
Keywords: Dual-Plasmid Expression System, Recombinant hGM-CSF Production, Soluble Therapeutic Protein

Ps-109: Optimized Synthesis of Gelatin-Capped Copper Nanoparticles with Enhanced Colloidal Stability for Antimicrobial Wound Dressing Applications

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Background: Developing stable copper oxide nanoparticles (CuO NPs) is important for antimicrobial applications; however, their translation is often limited by poor colloidal stability. In this context, employing biocompatible coatings is a key strategy. Biopolymers such as starch and gelatin can provide surface capping, improve colloidal stability, and enhance the suitability of Cu-based nanomaterials for wound dressing formulations. Here, we report on an optimized precipitation-based synthesis approach to obtain stable gelatin-coating CuO NPs.
Materials and Methods: Initial synthesis attempts using copper sulfate/starch-ascorbic acid and gelatin produced nanoparticles that were unstable and rapidly sedimented within 24 h. The protocol was then optimized by replacing copper sulfate with copper nitrate Cu2(NO3) using a precipitation method. This modification resulted in markedly improved nanoparticle stability. The synthesized nanoparticles were characterized by dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD).
Results: DLS analysis revealed a relatively narrow particle size distribution, with an average hydrodynamic diameter of approximately 45 nm and a polydispersity index (PDI) of 0.2383, indicating moderate monodispersity and good colloidal uniformity. Furthermore, the colloidal system exhibited excellent long-term stability, as evidenced by the effective dispersion of nanoparticles over a 30-day period, with no significant aggregation observed. FTIR analysis confirmed successful gelatin coating on the nanoparticle surface, as evidenced by the characteristic absorption bands at 1637 and 3322 cm^{-1}, which correspond to amide and hydroxyl functional groups involved in surface interaction and stabilization. XRD results verified the formation of crystalline CuO NPs with phase integrity, confirming the successful synthesis of copper oxide nanostructures. Collectively, these findings indicate that the gelatin-coating CuO NPs possess favorable physicochemical properties and hold promise for antimicrobial and wound dressing applications.
Conclusion: The optimized precipitation synthesis using copper nitrate and gelatin produced stable CuO NPs with favorable physicochemical properties. The use of biopolymer coatings (e.g., gelatin, and starch-related capping strategies) supports both biocompatibility-relevant surface functionalization and improved colloidal stability, highlighting the potential of these CuO NPs formulations for antimicrobial wound dressing applications.
Keywords: Colloidal Stability, Copper Nanoparticles, Gelatin, Wound Dressing

Ps-110: Evaluation of Demyelination by Metronidazole in Transgenic Zebrafish Model

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Background: Multiple Sclerosis (MS) is a chronic demyelinating disorder of the central nervous system characterized by the loss of myelin sheaths and subsequent neurological dysfunction. The development of reliable animal models is essential for understanding disease mechanisms and evaluating potential therapeutic strategies.In this study, a transgenic zebrafish model was employed to investigate demyelination processes relevant to MS pathology
Materials and Methods: Demyelination was induced in transgenic zebrafish larvae Tg(mbp:GFP-NTR) using 10mM metronidazole (Mtz) in 5 dpf (days post-fertilization). The affected larvae were subsequently maintained under Mtz treatment for 48h. Mtz selectively ablates oligodendrocytes through a nitroreductase-mediated cell-specific targeting system. They have been kept under the standard laboratory conditions and monitored for spontaneous regenerative responses over a 7-day recovery period. A stereomicroscopic camera was used to examine the results, and the images were analyzed using FIJI image processing software.
Results: The results demonstrated that on 7 dpf, the level of green fluorescent protein (GFP) expression in Mtz treated larvae group was remarkably reduced in comparison with the control group. The percentage loss of the GFP expression in the Mtz treated larvae group due to the control group is 3.9%. This reduction indicates successful induction of demyelination following Mtz exposure. Further evaluation was conducted on 14 dpf, at which time The percentage loss of the GFP expression had decreased to 1.8%. These findings show that Mtz treatment initially induced significant demyelination in the transgenic zebrafish larvae. However, over time, endogenous cellular regenerative mechanisms promoted tissue repair and remyelination. The substantial reduction in the difference between the treated and control groups from day 7 to day 14 provides evidence of progressive recovery of myelin integrity and supports the occurrence of natural tissue regeneration. Therefore, the observed restoration of GFP expression is indicative of remyelination and functional repair processes within the central nervous system of the zebrafish larvae.
Conclusion: This regenerative response highlights the suitability of the transgenic zebrafish model for investigating demy-

elinating disorders such as MS and for studying the molecular and cellular mechanisms underlying myelin repair and neural regeneration.
Keywords: Multiple Sclerosis, Demyelination, Zebrafish, Metronidazole

Ps-111: Applications of Platelet-Derived Extracellular Vesicles in Regenerative Medicine for Skeletal Tissue: A Systematic Review

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Background: Skeletal tissue deficits and diseases are one of the greatest challenges of regenerative medicine due to the high rate of emergence after trauma, oncologic surgeries, and degenerative diseases. Due to limited self-repair in large injuries, traditional approaches often fail to fully recover physiological function. Hence, developing advanced tissue engineering solutions for overcoming this obstacle is a necessity. Platelets, through releasing growth factors and bioactive molecules, play a key role in tissue repair. Platelet-derived products, particularly platelet-rich plasma (PRP) and platelet lysate (PL), accelerate tissue repair. As a modern and controllable approach, platelet-derived extracellular vesicles (PEVs) enhance healing and regenerative processes of skeletal tissue. This review aims to thoroughly discuss applications and mechanisms of PEVs in skeletal tissue engineering and provide future outlooks.
Materials and Methods: In this systematic review, papers were searched in PubMed, Embase, and Scopus databases, using Keywords related to PEVs, PRP, skeletal tissue engineering including bone, cartilage, tendon, skeletal muscle, and intervertebral disk. During the initial search, 143 papers were identified, after removing duplicates and screening based on title, abstract, and full text, 12 eligible papers were included in this review. Selected studies were reviewed for biological effects and therapeutic potential of PEVs in skeletal tissue repair.
Results: Studies indicate that PEVs, through transferring key factors, notably boost mesenchymal stem cells bone and cartilage differentiation signaling pathways. In osteoarthritis model, these vesicles significantly reduce inflammation through inhibiting cytokines (such as TNF−α) Moreover, in intervertebral disc degeneration (IVD), PEVs alter the metabolic balance of the matrix in favor of anabolism (increased expression of Col2α and Sox9) by reducing oxidative stress and inhibiting cellular senescence. They also accelerate the recovery of skeletal muscles contraction function post-trauma and revive cell proliferation in avascular osteonecrosis of femoral head (ONFH) models. Finally, compared to traditional platelet products, PEVs demonstrated better structural integrity, and without causing pre-inflammatory reactions, provided higher safety profile and efficacy in skeletal tissue regenerative medicine.
Conclusion: PEVs, as a modern and acellular approach, demonstrate high potential in repairing skeletal tissue through regulating key regenerative pathways. However, their clinical application requires extraction protocols standardization, clarifying precise mechanisms, and extensive clinical trials to evaluate long-term safety and efficacy.
Keywords: Platelet-Derived Extracellular Vesicles, Skeletal Tissue, Regenerative Medicine, Tissue Engineering

Ps-112: Development of A Bioinspired dECM/PEG Composite Bone Adhesive

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Background: The development of bioactive bone adhesives capable of providing both mechanical stabilization and biological support remains a major challenge in orthopedic tissue engineering. Decellularized extracellular matrix (dECM)-based hydrogels dominate excellent biomimetic properties; however, their limited mechanical stability and adhesive performance restrict their clinical applicability. The prospect of study is to develop a bioinspired composite bone adhesive by combining bone-derived dECM hydrogel with polyethylene glycol (PEG)-based polymers to improve its adhesive, mechanical, and physicochemical properties.
Materials and Methods: Bone fragments were decellularized using a previously optimized protocol to obtain a bone-derived dECM hydrogel. The dECM hydrogel was combined with PEG-based components to fabricate composite adhesive hydrogels. Mechanical, rheological, and physicochemical properties were evaluated to assess the optimal formulation. Structural interactions between hydrogel components were investigated using Fourier Transform Infrared Spectroscopy (FTIR). Additionally, degradation behavior, swelling characteristics, tensile adhesive strength, and in-vitro cytocompatibility were assessed using mesenchymal stem cells.
Results: The incorporation of PEG enhanced the mechanical stability and rheological performance of the dECM hydrogel, with viscosity increasing in a concentration-dependent manner. The optimized formulation achieved a tensile adhesive strength of approximately 150 kPa, demonstrating strong adhesion to bone tissue. The optimized formulation achieved a tensile adhesive strength of approximately 150 kPa, demonstrating strong adhesion to bone tissue. FTIR analysis indicated intermolecular interactions between the dECM and PEG networks, contributing to improved structural integrity. These interactions were characterized by reduced degradation and swelling compared with the dECM hydrogel alone. Furthermore, the composite hydrogel exhibited optimal cytocompatibility, maintaining greater than 90% cell viability throughout a 7-day culture period.
Conclusion: The developed dECM/PEG composite combines the biological advantages of bone-derived extracellular matrix with the structural reinforcement of PEG-based polymers, resulting in a bioactive bone adhesive with strengthened adhesive performance, improved physicochemical stability, and favorable cytocompatibility. These findings support the potential application of this composite as a bioactive bone adhesive for orthopedic and bone tissue engineering applications.
Keywords: Biocompatible Adhesive, Hydrogel, Decellularized Extracellular Matrix, Polyethylene Glycol

Ps-113: Carboxymethyl Cellulose-Based Superabsorbent Scaffold with Antibacterial Properties for Wound Healing Applications

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Background: Skin injuries arising from physical trauma, burns, diabetes, and surgical incisions disrupt normal anatomical structure and function. Although skin is a self-healing tissue, large or deep wounds require immediate coverage with engineered skin substitutes to achieve wound closure and functional recovery. Biopolymer-based sponges exhibit considerable therapeutic potential due to their excellent exudate absorption, hemostatic properties, and interconnected porous structure, which facilitate cellular infiltration and accelerate the wound healing process. Bacterial infection remains one of the most serious complications in wound management, as it prolongs the inflammatory phase and impairs re-epithelialization, thereby delaying healing. In recent decades, many studies have focused on developing multifunctional biopolymer scaffolds loaded with bioactive agents to promote wound closure and prevent bacterial contamination.
Materials and Methods: This study aimed to develop an antibacterial and superabsorbent wound healing scaffold by incorporating Cu-doped ZnO nanoparticles into a carboxymethyl cellulose/gelatin (CG) composite matrix. Undoped and Cu-doped ZnO (Zn1-xCuxO, x=0.03 and 0.05) nanoparticles were synthesized using a stabilizing agent-assisted precipitation process.
Results: X-ray diffraction (XRD) analysis demonstrated that the incorporation of copper ions into the ZnO crystal structure was limited to doping concentrations below 5%. ICP-OES analysis confirmed that copper doping altered the ZnO crystalline structure, leading to increased release of zinc and copper ions from the Cu-doped ZnO nanoparticles. This enhanced ion dissolution contributed to improved antibacterial activity against Staphylococcus aureus and Escherichia coli. The CG composite containing 3 wt% ZnO nanoparticles revealed a well-interconnected porous architecture (~156 μm) and a high-water absorption capacity (~3089%), providing a favorable environment for cellular infiltration and exudate uptake. Furthermore, all composite formulations exhibited good cytocompatibility and effectively supported the attachment and proliferation of human dermal fibroblasts.
Conclusion: These results indicate that the developed composite scaffold represents a promising strategy for infectious wound management by providing both efficient exudate uptake and antibacterial activity.
Keywords: Carboxymethyl Cellulose, Gelatin, Cu-doped ZnO Nanoparticles, Antibacterial Property, Wound Healing

Ps-114: Hyaluronic Acid-Based Biomaterials in Tissue Engineering for Cancer Modeling: A Systematic Review of CD44-Mediated Cancer Stem Cell Regulation

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Background: Hyaluronic acid (HA) is a major extracellular matrix component with paradoxical roles in cancer. While HA-based biomaterials are widely used in tissue engineering, HA also contributes to cancer progression through CD44 receptor binding and cancer stem cell (CSC) maintenance. This systematic review evaluates how HA-based biomaterials regulate CSC behavior and assesses implications for tissue engineering applications in cancer modeling.
Materials and Methods: A systematic search was conducted in PubMed, Scopus, and Web of Science (January 2018-March 2026). Search terms included "hyaluronic acid," "biomaterials," "cancer stem cells," "CD44," and "tissue engineering." Inclusion criteria: original research investigating HA-CSC interactions using HA-based scaffolds or hydrogels. Exclusion criteria: non-English articles and conference abstracts. Two independent reviewers screened records. Data extraction focused on HA molecular weight, scaffold properties, and CSC markers. Quality was assessed using SYRCLE's risk of bias tool.
Results: From 412 initially identified records, 38 studies met inclusion criteria. Three main findings emerged. First, HA-based scaffolds with stiffness between 0.5-10 kPa supported CSC spheroid formation and maintenance of stemness markers including CD44 and ALDH1. Second, CD44 receptor density correlated positively with HA binding affinity and activation of PI3K/Akt and MAPK pathways. Third, high molecular weight HA (>1000 kDa) promoted CSC quiescence, while low molecular weight HA (<200 kDa) stimulated CSC proliferation and chemoresistance through TLR4-dependent mechanisms. HA-based hydrogels for 3D cancer modeling showed improved recapitulation of tumor microenvironment compared to 2D cultures.
Conclusion: HA-based biomaterials significantly influence CSC behavior through CD44-mediated mechanisms. Molecular weight and matrix stiffness are critical design parameters for HA scaffolds in tissue engineering applications for cancer modeling and drug screening.
Keywords: Hyaluronic Acid, Cancer Stem Cells,Tissue Engineering, CD44

Ps-115: Electrospun Polycaprolactone/Gelatin Nanofibrous Scaffold for Adipose-Derived Mesenchymal Stem Cell Expansion

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Background: Adipose tissue is an abundant and accessible source of mesenchymal stem cells (AD-MSCs) for regenerative medicine applications. However, conventional 2D culture systems cannot mimic the natural extracellular matrix (ECM) environment. Electrospinning of synthetic and natural polymers offers a promising approach to fabricate biomimetic scaffolds that support stem cell proliferation. This study aimed to develop a simple electrospun polycaprolactone (PCL)/gelatin nanofibrous scaffold for the isolation and culture of AD-MSCs.
Materials and Methods: Adipose tissue was collected from healthy donors. The vascular stromal fraction containing AD-MSCs was isolated using a simple mechanical method (centrifugation at 1200 g for 10 min without enzymatic digestion) as previously described. PCL/gelatin solution (70:30 ratio, 10% w/v in HFIP) was electrospun at 15 kV and a flow rate of 0.5 mL/h to produce nanofibrous scaffolds. The morphology of the scaffolds was examined by light microscopy. The isolated cells

were cultured on PCL/gelatin scaffolds and tissue culture plastic (control) at a density of 5×10^4 cells/cm2. Cell adhesion was assessed by trypan blue exclusion on days 1, 3, and 7. MSC identity was confirmed by morphology (duke-shaped) and potential to differentiate into adipocytes (Oil Red O staining) and osteoblasts (Alizarin Red staining). All experiments were performed in triplicate.
Results: The PCL/gelatin nanofibers exhibited uniform morphology with average diameter of 200-250 nm. Cell adhesion on PCL/gelatin scaffolds was significantly higher (78 ± 5% at day 3) compared to plastic controls (55 ± 6%, $P < 0.05$). Isolated cells maintained typical spindle-shaped MSC morphology on scaffolds. Both groups successfully differentiated into adipocytes (Oil Red O-positive lipid droplets) and osteoblasts (Alizarin Red-positive calcium deposits). Cell viability remained above 85% throughout the 7-day culture period on scaffolds.
Conclusion: The combination of mechanical isolation of AD-MSCs from adipose tissue with PCL/gelatin electrospun nanofibrous scaffolds provides a simple, accessible platform for stem cell expansion in tissue engineering applications.
Keywords: Adipose-Derived Stem Cells, Electrospinning, Polycaprolactone, Gelatin, Tissue Engineering

Ps-116: Fabrication of Hyaluronic Acid/Polyvinyl Alcohol Nanofibers via Electrospinning for 3D Culture of CD44-Positive Breast Cancer Cells

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Background: Electrospinning is a simple, rapid, and cost-effective method for producing nanofiber-based scaffolds that mimic the natural extracellular matrix (ECM). Hyaluronic acid (HA), a major component of the ECM, binds to CD44 receptors that are overexpressed on breast cancer cells. This study aimed to fabricate HA-based nanofibers using a low-cost electrospinning device and evaluate their ability to support the adhesion and proliferation of CD44-positive breast cancer cells.
Materials and Methods: HA was mixed with polyvinyl alcohol (PVA) at a ratio of 1:1 and dissolved in distilled water. A simple electrospinning device was constructed using a 12 V lithium battery, syringe pump, and aluminum foil collector. The solution was electrospun at a current of 15 kV, a flow rate of 0.5 mL/h, and a distance of 10 cm from the tip to the collector. The morphology of the nanofibers was examined using a light microscope. MDA-MB-231 (CD44+) breast cancer cells were seeded on the nanofibers and cultured for 5 days. Cell adhesion was assessed by trypan blue staining. CD44 expression was assessed by Immunohistochemical staining. All experiments were performed in triplicate.
Results: Electrospinning successfully produced uniform HA/PVA nanofibers with an average diameter of 200 ± 50 nm. Cell adhesion on the nanofiber scaffolds was significantly higher (62 ± 5%, $P < 0.05$) compared to 2D plastic controls (85 ± 6% of the cells attached). Immunohistochemical staining confirmed strong expression of CD44 in cells cultured on the nanofibers. Cell proliferation was sustained after 5 days with 90 ± 4% viability.
Conclusion: HA/PVA nanofibers fabricated by simple, low-cost electrospinning provide an effective 3D scaffold for CD44-positive breast cancer cell culture. This approach offers an affordable platform for cancer research applications.
Keywords: Electrospinning, Hyaluronic Acid, Nanofibers, Breast Cancer, CD44

Ps-117: Characterization of Concentrated γ-Retroviral Vectors Using Centrifugation in The Transient Production Process

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Background: γ-retroviral vectors are widely used in gene therapy research because of their ability to stably integrate into the host genome and efficiently deliver genes into dividing cells. These vectors play an important role in Chimeric antigen receptor T (CAR-T) cell therapy and other cell-based therapeutic approaches. However, low viral titers during transient production can limit transduction efficiency. Therefore, developing a rapid and effective concentration method that preserves viral infectivity is essential. This study aimed to evaluate a centrifugation-based method for concentrating transiently produced γ-retroviral vectors and to assess its effects on viral recovery and transduction efficiency.
Materials and Methods: green fluorescent protein (GFP)-expressing γ-retroviral vectors were transiently produced in HEK293T and Plat-A cells using Lipofectamine-mediated transfection. Viral supernatants were collected after transfection and concentrated by centrifugation at 9,000 × g for 3 hours. Concentrated virus, non-concentrated virus, and supernatant fractions were titrated using cell-based methods. Transduction efficiency was evaluated by flow cytometry based on GFP expression.
Results: Centrifugation markedly increased viral infectivity and transduction efficiency compared with non-concentrated samples. Viral titers increased from 2.4×10^5 TU/mL in non-concentrated samples to 2.1×10^6 TU/mL after concentration. The percentage of GFP-positive cells increased from 12% in non-concentrated virus samples to 46% in concentrated virus samples, corresponding to nearly a fourfold improvement in transduction efficiency. In contrast, supernatant fractions showed minimal viral activity, indicating efficient pelleting of viral particles during centrifugation.
Conclusion: Low-speed centrifugation is a simple, efficient, and scalable strategy for concentrating transiently produced γ-retroviral vectors while preserving viral infectivity. Since γ-retroviral particles are susceptible to structural damage and loss of infectivity during high-speed centrifugation, the use of low-speed centrifugation provides an advantageous alternative for viral concentration. This method significantly improves viral titer and transduction efficiency and may support the optimization of retroviral vector production for gene delivery applications.
Keywords: γ-Retroviral Vectors, Centrifugation, Viral Concen-

tration, Transduction Efficiency, Gene Therapy

Ps-119: Passage-Dependent Variations in The Protein Yield of Extracellular Vesicles Derived from Stem Cells of The Apical Papilla

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Background: Extracellular vesicles (EVs) secreted by stem cells from the apical papilla (SCAP) play an important role in mediating paracrine signaling and promoting tissue regeneration. However, changes in cell physiology across passages may affect the yield and molecular characteristics of the released EVs. The present study aimed to evaluate whether the passage number influences the total protein yield of SCAP-derived EVs isolated from conditioned medium.
Materials and Methods: SCAP were cultured under standard conditions at passages 4, 8 and 12. Conditioned medium was collected after reaching appropriate confluence. EVs were isolated using a commercial precipitation-based isolation kit. The EV pellet obtained after centrifugation was resuspended, and the total protein concentration was quantified as an approximate measure of total EV amount using the bicinchoninic acid (BCA) assay.
Results: The total EV protein yield showed a variable pattern across passages: 660 µg/mL (P4), 1101 µg/mL (P8), and 2497 µg/mL (P12). The corresponding cell numbers at EV collection were 2.25×10^6 cells (P4), 3.2×10^6 cells (P8), and 3×10^6 cells (P12). After normalization to cell number, EV protein yield was calculated as 293.3, 344.1 and 832.3 µg per 10^6 cells for P4, P8, and P12, respectively. Although the results suggest a possible trend toward increased total EV protein yield at higher passages, the relationship does not appear linear and may be influenced by cell density or metabolic state. As this dataset originated from a single experimental run, additional biological replications and molecular characteristics are required to establish statistically significant patterns
Conclusion: Our primary findings indicate that SCAP passage number may influence the total EV protein yield. The apparent variation in EV yield among passages highlights the need for further replicated experiments to confirm these observations and to clarify the interplay between cell passage, cell density, and EV release efficiency. Recognizing and addressing such variables is essential to standardize EV production for potential translational and regenerative applications.
Keywords: Extracellular Vesicles (Evs), Stem Cells of The Apical Papilla (SCAP), Conditioned Medium, Passage Number, Protein Yield

Ps-120: Investigating The Effect of Lithium on The Function of Ion Channels and The Electrophysiological Properties of The Membrane of Neuronal Membranes Isolated from The Rat Fetal Brain

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Background: Lithium remains a cornerstone treatment for bipolar disorder, primarily through inhibition of the GSK-3β pathway. However, its direct biophysical effects on neuronal membrane properties and voltage-gated ion channels remain poorly understood. While previous studies using Multi-Electrode Array (MEA) have shown network-level alterations following chronic lithium exposure, the underlying single-cell mechanisms remain unclear. This study investigates both the acute and chronic effects of lithium on voltage-gated ion channels and membrane electrophysiological properties in primary rat cortical neurons using whole-cell patch-clamp techniques, in parallel with GSK-3β-specific modulators (CHIR99021 and XAV939).
Materials and Methods: Primary cortical neurons were isolated from embryonic day 16–18 (E16–18) rat fetuses and cultured on poly-L-ornithine/laminin-coated coverslips under standard conditions. Cells were treated with therapeutic concentrations of lithium chloride (LiCl) for 7 days. To investigate the involvement of the GSK-3β signaling pathway, parallel cultures were treated with the selective GSK-3β inhibitor CHIR99021 and the pathway modulator XAV939. Whole-cell patch-clamp recordings were performed to evaluate passive membrane properties, voltage-gated sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) currents, as well as action potential characteristics and neuronal excitability. In parallel, MEA recordings were used to assess spontaneous network activity, including firing rate, burst frequency, and synchronization patterns. Furthermore, the expression levels of selected ion channel genes were analyzed using quantitative RT-PCR to correlate electrophysiological changes with molecular alterations.
Results: This dual-electrophysiological approach is anticipated to reveal that chronic lithium exposure, unlike acute treatment, dynamically recalibrates ion channel gating properties. These single-cell biophysical alterations are expected to translate into significant modifications in bursting frequencies, firing synchronization, and synaptic plasticity within the neural network.
Conclusion: The present study investigates the cellular and network-level mechanisms through which lithium modulates neuronal function. By examining the effects of lithium and GSK-3β pathway modulators on ion channel activity and membrane excitability, this work is expected to provide novel insights into the electrophysiological basis of lithium action. Understanding these mechanisms may help identify new therapeutic targets for the treatment of bipolar disorder and other neurological conditions associated with altered neuronal excitability.
Keywords: Lithium, Voltage-Gated Ion Channels, Patch-Clamp, Neuronal Excitability, Electrophysiology

Ps-121: Preclinical Retinal Diseases Models: A Systematic Review

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Background: Retinal diseases, including inherited retinal dystrophies, age-related macular degeneration, diabetic retinopathy, and glaucoma, are major causes of irreversible vision loss worldwide. Preclinical models play a critical role in elucidating disease mechanisms, identifying therapeutic targets, and evaluating novel treatment strategies. This systematic review aimed to comprehensively assess the currently available preclinical models used in retinal disease research and their translational applications.
Materials and Methods: A systematic literature search was conducted in PubMed, Scopus, Web of Science, Embase, and Google Scholar for studies published up to 2 April 2026. The search strategy used combinations of the keywords: retinal diseases, preclinical models, retina research, disease modeling, drug discovery, gene therapy, cell therapy, retinal degeneration, and experimental therapeutics. Original English-language studies focusing on experimental models for retinal diseases were included. Reviews, conference abstracts, editorials, duplicate publications, non-retinal studies, and articles lacking sufficient methodological details were excluded. A total of 4,862 records were identified through database searching. After duplicate removal and title/abstract screening, 742 articles underwent full-text evaluation, of which 286 studies met the inclusion criteria and were included in the final analysis.
Results: The included studies demonstrated extensive diversity in retinal disease modeling approaches. Animal models, particularly rodents, zebrafish, rabbits, pigs, and non-human primates, remained the most frequently used systems for investigating retinal degeneration, vascular disorders, and therapeutic interventions. In-vitro platforms including primary retinal cell cultures and induced pluripotent stem cells (iPSCs) enabled patient-specific disease modeling and mechanistic investigations. Advanced three-dimensional systems such as retinal organoids and spheroids provided improved structural and functional similarity to native retinal tissue. Emerging bioengineering technologies, including microfluidic systems and retina-on-a-chip platforms, enhanced the study of retinal microenvironments and drug responses. Retinal tissues and explant cultures allowed ex-vivo assessment of retinal physiology and neuroprotection. In addition, computational and mathematical models facilitated simulation of disease progression, retinal signaling, and treatment outcomes. Each model exhibited distinct strengths and limitations regarding physiological relevance, reproducibility, scalability, ethical considerations, and translational potential.
Conclusion: Preclinical retinal disease models are diverse and have evolved substantially and collectively provide valuable tools for studying retinal pathophysiology and developing innovative therapies.
Keywords: Retinal Diseases, Preclinical Models, Retina Research, Disease Modeling

Ps-122: Cell-Free Therapeutic Potential of Wharton's Jelly MSC-Derived Secretome for Protecting Bovine Sperm DNA Integrity Against Oxidative Stress

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Background: Oxidative stress (OS) is one of the major contributors to sperm damage during semen processing and cryopreservation, leading to impaired DNA integrity and reduced fertility potential. Secretome derived from Wharton's jelly mesenchymal stem cells (WJ-MSCs-Sec) contains a broad spectrum of bioactive molecules, including antioxidant, anti-apoptotic, and regenerative factors, which may exert protective effects on sperm cells. This study aimed to investigate the protective effects of WJ-MSCs-Sec on bovine sperm DNA fragmentation index (DFI) under OS and non-stressed conditions.
Materials and Methods: Bovine semen samples were allocated into three experimental groups: (1) control, (2) DMEM-treated, and (3) WJ-MSCs-Sec-treated groups. OS was induced in a subset of samples, while parallel samples were maintained under physiological conditions. WJ-MSCs were isolated from human umbilical cord Wharton's jelly, and conditioned medium-derived secretome was collected from passage-3 cultured cells. All semen samples were incubated for 2 hours at 38°C before evaluation. Sperm DNA fragmentation index was subsequently assessed.
Results: The results demonstrated that treatment with WJ-MSCs-Sec significantly decreased sperm DFI compared with both control and DMEM-treated groups under OS and non-stressed conditions. The observed protective effects may be attributed to the antioxidant and cytoprotective bioactive components present in the secretome.
Conclusion: In conclusion, WJ-MSCs-Sec may serve as a promising cell-free therapeutic strategy for preserving sperm DNA integrity and reducing oxidative stress-associated sperm damage, with potential applications in assisted reproductive technologies and male infertility management.
Keywords: Bovine Sperm, Wharton's Jelly Mesenchymal Stem Cells, Secretome, Oxidative Stress, Cell-Free Therapy

Ps-123: Mechanisms of Mir-30d-5p and Mir-148a-3p Action during Induced Pluripotent Stem (Ips) Cell Generation

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Background: Somatic cells can be reprogrammed to induced pluripotent stem (iPS) cells by overexpressing specific pluripo-

tency factors. Identifying factors that enhance reprogramming efficiency and understanding their mechanisms is crucial for deriving high-quality iPS cells for biomedical applications. We have previously shown that miR-30d-5p and miR-148a-3p increase reprogramming efficiency. This study aims to functionally characterize these cells and elucidate the miRNAs' precise mechanisms by identifying their specific targets during the reprogramming process.
Materials and Methods: Expression patterns were analyzed at the mRNA (qRT-PCR) and protein levels (ICC). To investigate mechanisms, MEFs were transfected with miRNA mimics, and embryonic stem (ES) cells with miRNA inhibitors, followed by qRT-PCR for putative miRNA targets. To analyze DNA methylation following Dnmt1 inhibition, genomic DNA was digested with a methylation-sensitive restriction enzyme, and the resulting pattern was visualized on an agarose gel. To determine whether Ets1 is a direct target of miR-148a-3p, a fragment of its 3'UTR was cloned into the pmirGLO vector, and a luciferase reporter assay was performed in human embryonic kidney (HEK) cells.
Results: The iPS cells derived via miR-148a-3p and miR-30d-5p expressed key pluripotency markers at the mRNA and protein levels. Functional overexpression and inhibition assays revealed that Dnmt1, Bdnf, and Ets1 were targets of these miRNAs. Luciferase assays demonstrated Ets1 as a direct target of miR-148a-3p. Furthermore, miR-30d-5p suppressed Bdnf, affecting its downstream pathway, while Dnmt1 inhibition by miR-148a-3p altered global DNA methylation status.
Conclusion: miR-30d-5p and miR-148a-3p promote iPS cell generation through the targeted suppression of Dnmt1, Bdnf, and Ets1. These regulatory axes improve iPS cell production while maintaining key pluripotency features.
Keywords: Reprogramming, miRNAs, iPS Cells, Pluripotency

Ps-124: A Comparative Study of the Effect of Nanoemulsified Carvacrol and β-Caryophyllene on Remyelination in a Transgenic Zebrafish Model of Demyelination

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Background: Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system characterized by myelin loss and neurological dysfunction. Current therapies mainly target immune modulation, while effective remyelinating strategies remain limited. β-Caryophyllene (BCP) and carvacrol are natural compounds with neuroprotective and anti-inflammatory properties; however, their lipophilicity limits bioavailability. Nanoemulsions may enhance delivery and efficacy. This study evaluated BCP, carvacrol, and their nanoemulsions in a transgenic zebrafish demyelination model.
Materials and Methods: Transgenic Tg (mbp:GFP-NTR) zebrafish larvae were used. At 5 days post-fertilization (dpf), larvae were exposed to 10 mM metronidazole (MTZ) for 48 hours to induce oligodendrocyte ablation via the nitroreductase/MTZ system. Stock solutions of compounds and nanoemulsions were prepared in dimethyl sulfoxide (DMSO) and E3 medium (Embryonic medium used for culturing embryos), respectively, and then diluted to working concentrations. After MTZ washout at 7 dpf, larvae were treated for 5 days (7–12 dpf) with BCP or BCP nanoemulsion (0.5–1.5 μg/mL) and carvacrol or carvacrol nanoemulsion (1–3 μg/mL). Concentrations were selected based on LC_{50} values. Remyelination was assessed at 12 dpf by fluorescence microscopy, and GFP intensity was quantified. Healthy untreated larvae and MTZ-treated larvae served as physiological and demyelinated controls, respectively.
Results: BCP nanoemulsions significantly increased GFP fluorescence intensity compared with the MTZ group, particularly at 1–1.5 μg/mL ($P < 0.0001$), indicating enhanced bioavailability and remyelination efficacy. Furthermore, both BCP and BCP nanoemulsion treatments restored fluorescence levels to those not significantly different from healthy controls, indicating near-complete recovery of myelin content. Carvacrol nanoemulsions also showed stronger effects than bulk carvacrol. Notably, carvacrol nanoemulsion at 3 μg/mL produced nearly a twofold increase in fluorescence versus MTZ and significantly exceeded healthy control levels ($P < 0.05$), suggesting enhanced remyelination and possible stimulation of myelin regeneration. Overall, nanoemulsions consistently outperformed bulk compounds.
Conclusion: BCP and carvacrol promote remyelination in demyelinated zebrafish larvae, while nanoemulsion delivery significantly enhances efficacy. Improved performance likely results from increased solubility and bioavailability. These findings support phytochemicals-loaded nanoemulsions as promising candidates for remyelination therapy in demyelinating disorders such as MS.
Keywords: Remyelination, Zebrafish, β-Caryophyllene, Carvacrol, Nanoemulsion

Ps-125: Carrageenan Hydrogel Sheet Wound Dressing for Sustained Bromelain Delivery to Full-thickness Burn Injuries Treatment

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Background: Effective wound dressings are necessary for burn injuries in order to promote healing and avoid complications. This work used $CaCl_2$-mediated physical crosslinking to develop a polysaccharide hydrogel sheet bandage for burn injuries made of carrageenan (Carr) biopolymer. Because of its anti-inflammatory and wound-healing properties, bromelain (Bro) was added into hydrogel. The purpose of this study was to create a unique hydrogel sheet wound dressing (Carr/Bro) for

the local administration of bromelain extract for the healing of third-degree skin burn lesions.
Materials and Methods: The hydrogel wound dressing's properties were analyzed through FTIR, swelling and degradation rates, rheometry, SEM, and bromelain release tests. the safety and efficacy were evaluated following in-vitro tests (FE-SEM, MTT, Live/dead, scratch, Tube formation assay) leading to the development of the Carr /Bro hydrogel sheet for burn wound healing.
Results: The hydrogel exhibited rapid swelling (120%), degradation rate (45%), sustained bromelain release (80%), antibacterial activity against S. aureus and E. coli, and stable mechanical properties ($G' > G''$). *In-vitro* studies demonstrated enhanced fibroblast adhesion and proliferation with significantly higher cell density (***$P < 0.001$) and improved scratch wound closure (**$P < 0.01$; ***$P < 0.001$). Carr /Bro extract significantly promoted angiogenesis in HUVECs ***$P < 0.001$; ****$P < 0.0001$).
Conclusion: According to these results, Carr/Bro hydrogel sheet wound dressing may be used as a therapy for full-thickness burn wounds by releasing bromelain continuously.
Keywords: Carrageenan, Bromelain, Hydrogel Wound Dressing, Burn Injury

Ps-126: Radioprotective Effects of Chitosan–Gum Tragacanth Hydrogel Containing Silver Nanoparticles on Radiation-Induced Cardiac Injury in Male Rats

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Background: Radiotherapy is a key treatment for many cancers; however, it can cause irreversible damage to radiosensitive organs, particularly the heart. Ionizing radiation induces oxidative stress, which contributes to cellular injury and subsequently leads to inflammation, fibrosis, apoptosis, and necrosis. Therefore, hydrogels containing bioactive components such as chitosan, Gum tragacanth, and silver nanoparticles may provide radioprotective effects. This study aimed to evaluate the efficacy of a radioprotective hydrogel based on chitosan–Gum tragacanth containing silver nanoparticles against radiation-induced cardiac injury in male rats.
Materials and Methods: Thirty-five male Wistar rats were randomly divided into five groups (n=7). The control group received no treatment. Radiation groups received 18 Gy of ionizing radiation over 20 minutes. Treatment groups received injections of (1) chitosan–Gum tragacanth hydrogel containing silver nanoparticles, (2) chitosan–Gum tragacanth hydrogel without silver nanoparticles, or (3) silver nanoparticles alone. Injections were administered for two weeks starting 24 hours after irradiation. After 50 days, the animals were sacrificed to assess antioxidant enzyme levels (superoxide dismutase [SOD] and glutathione peroxidase [GPX]), malondialdehyde (MDA) levels, and histopathological damage in cardiac tissue.
Results: Chitosan–Gum tragacanth hydrogel containing silver nanoparticles significantly increased SOD ($P < 0.001$) and GPX ($P < 0.001$) levels and significantly decreased MDA ($P < 0.001$). Histological evaluation of heart tissue using hematoxylin and eosin (H&E) and Masson's trichrome staining showed improved cardiomyocyte and muscle fiber architecture, reduced abnormalities in nuclear orientation ($P < 0.0001$), and decreased collagen deposition ($P < 0.001$) in the treatment group compared with the radiation-only group.
Conclusion: Overall, chitosan–Gum tragacanth hydrogel containing silver nanoparticles exhibits strong antioxidant and anti-inflammatory properties and can effectively reduce radiation-induced cardiac tissue injury in male rats.
Keywords: Radiation, Chitosan, Gum Tragacanth, Cardiac Injury, Hydrogel

Ps-127: Comparative Analysis of Cryopreservation and Lyophilization for Keratinocyte Sheet Preservation: Effects on Structural Integrity and Functional Viability

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Background: Chronic wounds are a major clinical challenge because they often remain in the inflammatory phase and fail to progress toward effective healing. Common types include pressure, diabetic, venous, and arterial ulcers, whose true prevalence is likely underestimated, particularly in low-income regions where epidemiological data are limited. This highlights the need for more effective therapeutic strategies to enhance tissue regeneration and accelerate wound repair. Keratinocyte sheets have emerged as promising biological skin substitutes with significant potential to promote re-epithelialization and tissue regeneration. However, their clinical application depends on maintaining structural integrity and biological function during storage and transport. Although cryopreservation is considered the standard method, the use of cryoprotectants such as DMSO and freeze–thaw cycles may induce cytotoxicity and osmotic damage. In contrast, lyophilization has been proposed as a more stable preservation approach and may retain morphological features and wound-healing potential comparable to cryopreserved samples.
Materials and Methods: In this study, keratinocyte sheets were produced using a standardized protocol and stored for 30 days under five conditions: 1. freezing with DMSO (+DMSO) 2. freezing after DMSO removal(-DMSO) 3. freezing with glycerol (+Gly) 4. freezing after glycerol removal (-Gly) 5. Lyophilization (Lyo) Evaluations included hematoxylin and eosin (H&E) staining for histological structure, MTS assay for cell viability and metabolic activity, and immunofluorescence analysis of keratinocyte differentiation markers K10 and K14.
Results: Histological analysis showed that the cryopreserved groups, including +DMSO, -DMSO, +Glycerol, and -Glycerol, generally maintained a more organized epithelial structure, whereas the Lyophilized (Lyo) samples exhibited increased thickness, heterogeneity, and disrupted continuity. Immunofluorescence results indicated stronger K10 and moderate K14 expression in the +DMSO and +Glycerol groups, while reduced expression was observed in the -DMSO and -Glycerol groups after cryoprotectant removal. MTS assay results showed higher

viability in the Fresh and +Glycerol groups, whereas the lowest viability was observed in the -DMSO group.
Conclusion: Overall, cryopreservation preserved cell viability but introduced protocol-dependent structural changes, while lyophilization preserved tissue morphology in the absence of viable cells. These findings highlight a fundamental trade-off between viable and non-viable preservation strategies, suggesting that the optimal method should be selected based on the intended clinical application. Further functional evaluation, including ongoing co-culture studies, is required to better assess regenerative potential and cellular interactions.
Keywords: Cryopreservation, Keratinocyte Sheet, Lyophilization, Viable Preservation, Non-Viable Preservation

Ps-128: Assessment of Decidual Stromal Cells Conditioned Media on Regenerative Capabilities of Hyperglycemic Fibroblasts

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Background: Chronic wounds in diabetic patients often stem from impaired fibroblast function under hyperglycemic conditions, leading to reduced cell viability and migration essential for tissue repair. Decidual stromal cells (DSCs), known for their immunomodulatory and regenerative properties, secrete bioactive factors in their conditioned medium (DSC-CM) that may mitigate hyperglycemia-induced dysfunction. This study investigates the protective effects of DSC-CM on the viability and migration of fibroblasts exposed to high glucose, offering insights into novel cell-based therapy approaches for diabetic wound healing.
Materials and Methods: Human dermal fibroblasts were cultured in normal glucose (5.5 mM) or hyperglycemic (35 mM) media, then treated with DSC-CM derived from DSC cultures for 24-48 hours. Cell viability was assessed via MTS assay, while migration was evaluated using scratch wound-healing assays analyzed with ImageJ software. Experiments were performed in triplicate, with statistical analysis via an independent samples T-test and a mixed model linear for repeated measures. $P < 0.05$ considered significant).
Results: Hyperglycemia significantly reduced fibroblast viability by 42% ($P < 0.01$) and migration rate by 58% ($P < 0.001$) compared to normoglycemic controls. DSC-CM treatment restored viability to 95% of control levels ($P < 0.05$) and enhanced migration by 2.1-fold ($P < 0.01$) in hyperglycemic fibroblasts. These effects were dose-dependent, with optimal benefits at 50% DSC-CM concentration, suggesting paracrine factors like growth factors and cytokines counteract glucotoxicity.
Conclusion: DSC-CM effectively rescues viability and migration deficits in hyperglycemic fibroblasts, positioning it as a promising acellular therapeutic approach for diabetic wound healing. Future studies should explore molecular mechanisms and in-vivo efficacy to advance clinical translation.
Keywords: Wound Healing, Conditioned Media, Fibroblast, Migration Assay

Ps-129: Cardiovascular Aging Related Gene KLOTHO Expression Analysis in The Blood Samples of Healthy Young and Elderly Candidates and Old People with Cardiovascular Disease

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Background: Aging is an irreversible biological process characterized by a progressive decline in physiological functions, ultimately leading to age-related diseases, particularly cardiovascular disorders. It is associated with the accumulation of molecular and cellular damage, resulting in structural and functional alterations in the vascular system and promoting endothelial dysfunction, inflammation, and atherosclerosis. Klotho is an anti-aging protein with membrane-bound and soluble circulating forms that plays a key role in maintaining vascular health. However, its gene expression in peripheral blood leukocytes during aging has not been fully investigated; therefore, this study aimed to evaluate Klotho gene expression in young healthy, elderly healthy, and elderly coronary artery disease (CAD) individuals.
Materials and Methods: Peripheral blood samples were collected from 30 participants, including 10 young healthy individuals, 10 elderly healthy individuals, and 10 elderly patients with CAD, following written informed consent and approval by the Royan Institute Ethics Committee. Total RNA was extracted from peripheral blood leukocytes and reverse-transcribed into cDNA. Relative Klotho gene expression was measured using quantitative real-time PCR. Expression levels analyzed using the ΔCt method, while fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method. Statistical analyses were performed using GraphPad Prism software, followed by one-way ANOVA and Tukey's comparisons test. A $P < 0.05$ was considered statistically significant
Results: Klotho gene expression differed significantly among the three study groups. One-way ANOVA revealed a significant reduction in Klotho expression in both elderly healthy individuals and elderly CAD patients compared with the young healthy group ($P < 0.01$). Tukey's test confirmed significantly lower Klotho expression in both elderly groups relative to the young controls. However, no significant difference was observed between the elderly healthy and elderly CAD groups. Overall, Klotho mRNA expression was markedly decreased in older individuals, with a highly significant reduction compared with the young group ($P=0.001$).
Conclusion: The present study demonstrated a significant age-related decline in Klotho mRNA expression in peripheral blood leukocytes. The significant difference between elderly and young individuals suggests that reduced Klotho expression may be primarily associated with the aging process. These findings are consistent with previous reports linking Klotho deficiency to inflammation, vascular dysfunction, and atherosclerosis. Reduced Klotho expression may therefore reflect age-related al-

terations in immune cell function and support its potential role as a candidate biomarker of biological aging and cardiovascular health.

Keywords: Aging, Klotho, Atherosclerosis

Ps-130: FOXO3: A Bioinformatics and Network-Based Investigation of Its Role in Hepatocellular Carcinoma Autophagy Regulation

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Background: Hepatocellular carcinoma (HCC) is a heterogeneous malignancy and a major contributor to cancer-related deaths worldwide. Autophagy has emerged as a critical process affecting cancer cell survival, metabolism, and therapeutic resistance. Therefore, identifying autophagy-associated molecular targets may contribute to the formulation of innovative therapeutic Strategies for HCC.
Materials and Methods: An integrated bioinformatics strategy was applied to uncover autophagy-associated genes in HCC through pathway enrichment, PPI network construction, hub gene selection, and drug–gene interaction analysis.
Results: To identify autophagy-associated genes in HCC, an integrated computational workflow was applied. A total of 3,567 HCC-related protein-coding genes were retrieved and analyzed via the Enrichr platform for functional enrichment. Enriched KEGG pathways were identified, FOXO3 signalling pathway genes were selected for downstream analysis, PPI network analysis was performed, and hub genes were identified using CytoHubba in Cytoscape. Drug–gene interactions and FOXO3 pathway association were further explored using databases and literature resources.
Conclusion: Collectively, this integrative bioinformatics analysis identified FOXO3 as a promising autophagy-associated candidate gene in HCC. These findings may provide a basis for future experimental investigations targeting FOXO3-mediated autophagy pathways as a potential therapeutic strategy in HCC.
Keywords: Hepatocellular Carcinoma (HCC), Autophagy, FOXO3, Bioinformatics

Ps-131: Determination of Resveratrol Anti-Proliferative Activity in HCC.

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Background: Hepatocellular carcinoma is a major cause of cancer-associated deaths due to its aggressive nature and limited treatment options. Resveratrol, as a natural compound, has attracted considerable attention in oncology research due to its anti-cancer properties by targeting multiple molecular pathways involved in cancer cell proliferation and apoptosis. Despite its reported anti-cancer properties, its specific cytotoxic activity against HCC cells requires further investigation.
Materials and Methods: In the present study, the anti-proliferative effects of resveratrol on HCC cell lines were investigated through a colorimetric MTS viability assay. HCC cell lines were cultured, then treated under the various concentrations of resveratrol for a specified incubation period. Cellular viability was determined using colorimetric MTS analysis based on mitochondrial metabolic activity, and the half–maximal inhibitory concentration (IC_{50}) of resveratrol was used to determine treatment sensitivity.
Results: The findings showed that the resveratrol treatment significantly reduced HCC cells' viability in a dose-dependent manner. IC_{50} analysis indicated that resveratrol achieves 50% inhibition of HCC cell viability at an effective concentration, supporting cytotoxic and anti-proliferative activity.
Conclusion: Overall, these findings suggest that resveratrol holds potential as a therapeutic agent against HCC. The results highlight that resveratrol can exert its anti-cancer activity through further oxidative stress and autophagic mechanistic studies.
Keywords: Hepatocellular Carcinoma (HCC), Resveratrol, Cytotoxicity, Autophagy

Ps-132: Modulation of Epigenetic and Apoptotic Gene Expression Induced by Curcumin in Breast Cancer Cells

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Background: Breast cancer is among the most prevalent malignancies affecting women worldwide and remains a leading cause of cancer-related deaths. Aberrant epigenetic modifications and dysregulation of apoptotic pathways are key mechanisms involved in breast cancer initiation, progression, and therapeutic resistance. Curcumin, a naturally occurring poly-

phenolic compound extracted from Curcuma longa, has gained considerable attention due to its anticancer properties and its ability to regulate multiple molecular targets. This study aimed to investigate the modulatory effects of curcumin on the expression of epigenetic and apoptotic genes in breast cancer cells.
Materials and Methods: Human breast cancer cells were treated with different concentrations of curcumin for defined exposure periods. Cell viability was evaluated using a colorimetric assay. Total RNA was isolated from treated and untreated cells, and the expression levels of selected epigenetic regulators (DNMT1, DNMT3A, and HDAC1) and apoptosis-related genes (BAX, BCL2, CASP3, and TP53) were quantified by quantitative real-time polymerase chain reaction (qRT-PCR). Relative gene expression was determined using the comparative Ct method.
Results: Curcumin treatment significantly decreased breast cancer cell viability in a concentration-dependent manner. Gene expression analysis demonstrated a substantial downregulation of the epigenetic regulatory genes DNMT1, DNMT3A, and HDAC1 following curcumin exposure. In contrast, the expression of pro-apoptotic genes BAX, CASP3, and TP53 was significantly increased, whereas the anti-apoptotic gene BCL2 was markedly reduced. Furthermore, curcumin treatment resulted in an elevated BAX/BCL2 ratio, indicating activation of the mitochondrial apoptotic pathway. These findings suggest that curcumin simultaneously influences epigenetic mechanisms and apoptotic signaling networks in breast cancer cells.
Conclusion: Curcumin effectively modulates the expression of genes involved in epigenetic regulation and apoptosis, leading to the suppression of breast cancer cell survival and the induction of programmed cell death. The coordinated regulation of these pathways highlights the potential of curcumin as a promising epigenetic and therapeutic agent for breast cancer management. Further *in-vivo* and clinical studies are required to validate its efficacy and explore its translational applications.
Keywords: Curcumin, Breast Cancer, Epigenetic Regulation, Apoptosis, DNMT1, HDAC1, BAX/BCL2 Ratio

Ps-133: Bisphenol A Alters Epigenetic and Inflammatory Gene Expression in Human Peripheral Blood Mononuclear Cells

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Background: Bisphenol A (BPA) is a widely used industrial chemical found in polycarbonate plastics and epoxy resins, resulting in extensive human exposure through food, water, and environmental sources. Growing evidence suggests that BPA acts as an endocrine-disrupting compound capable of influencing immune function and gene regulation. Epigenetic modifications and inflammatory responses are among the key mechanisms implicated in BPA-induced toxicity. This study aimed to investigate the effects of BPA on the expression of epigenetic and inflammatory genes in human peripheral blood mononuclear cells (PBMCs).
Materials and Methods: Human PBMCs were isolated from healthy donors and cultured under standard conditions. Cells were exposed to different concentrations of BPA for specified incubation periods. Total RNA was extracted from treated and control cells, and gene expression analysis was performed using quantitative real-time polymerase chain reaction (qRT-PCR). The expression levels of selected epigenetic regulators, including DNMT1, DNMT3A, and HDAC1, as well as inflammatory genes such as TNF-α, IL-6, IL-1β, and NF-κB, were evaluated. Relative gene expression was calculated using the comparative Ct (2^−ΔΔCt) method.
Results: BPA exposure significantly altered the expression profile of both epigenetic and inflammatory genes in PBMCs. Treatment with BPA resulted in dysregulated expression of epigenetic modifiers, characterized by significant changes in DNMT1, DNMT3A, and HDAC1 transcript levels compared with untreated controls. Moreover, BPA markedly increased the expression of pro-inflammatory mediators, including TNF-α, IL-6, and IL-1β, accompanied by activation of NF-κB-associated signaling pathways. The observed molecular alterations suggest that BPA promotes a pro-inflammatory cellular environment while simultaneously affecting epigenetic regulatory mechanisms.
Conclusion: BPA induces significant changes in the expression of genes involved in epigenetic regulation and inflammatory responses in human PBMCs. The concurrent modulation of these pathways may contribute to immune dysregulation and the adverse health effects associated with BPA exposure. These findings provide further insight into the molecular mechanisms underlying BPA toxicity and highlight the importance of evaluating environmental contaminants as potential epigenetic and immunomodulatory factors. Further studies are warranted to clarify the long-term biological consequences of BPA-induced gene expression changes in human immune cells.
Keywords: Bisphenol A, Peripheral Blood Mononuclear Cells, NF-κB, DNMT1, HDAC1

Ps-134: Effect of Vitamin D on Epigenetic Regulation and Expression of Stemness Genes in Human Mesenchymal Stem Cells

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Background: Human mesenchymal stem cells (hMSCs) possess remarkable self-renewal and multilineage differentiation capacities, making them valuable tools in regenerative medicine and tissue engineering. The maintenance of stemness characteristics is tightly regulated by epigenetic mechanisms and transcriptional networks. Vitamin D, a secosteroid hormone with diverse biological functions, has been shown to influence cellular proliferation, differentiation, and gene expression. However, its role in the epigenetic regulation of stemness-related genes in hMSCs remains incompletely understood. This study aimed to investigate the effects of vitamin D on the expression of epigenetic regulators and stemness-associated genes in human mesenchymal stem cells.
Materials and Methods: Human mesenchymal stem cells were cultured under standard conditions and treated with different concentrations of vitamin D for predetermined time periods. Total RNA was extracted from treated and untreated cells, and gene expression levels were analyzed using quantitative real-time polymerase chain reaction (qRT-PCR). The expression of key epigenetic regulatory genes, including *DNMT1*, *DNMT3A*, and *HDAC1*, as well as stemness-associated genes such as *OCT4, SOX2, NANOG*, and *KLF4*, was evaluated. Rela-

tive gene expression was calculated using the comparative Ct ($2^{-\Delta\Delta Ct}$) method.
Results: Vitamin D treatment significantly modulated the expression of both epigenetic and stemness-related genes in hMSCs. Exposure to vitamin D resulted in altered expression of epigenetic modifiers, suggesting an influence on DNA methylation and chromatin remodeling processes. Furthermore, vitamin D significantly affected the transcriptional levels of the stemness markers OCT4, SOX2, NANOG, and KLF4, indicating a regulatory role in maintaining the self-renewal capacity and cellular identity of mesenchymal stem cells. The observed changes suggest that vitamin D may contribute to the preservation of stem cell functionality through epigenetic mechanisms.
Conclusion: Vitamin D exerts significant regulatory effects on epigenetic pathways and stemness-associated gene expression in human mesenchymal stem cells. The modulation of genes involved in DNA methylation, histone modification, and stem cell maintenance highlights the potential role of vitamin D in optimizing stem cell function and regenerative capacity. These findings provide new insights into the molecular actions of vitamin D and support its potential application in stem cell-based therapeutic strategies. Further studies are required to elucidate the underlying mechanisms and long-term biological implications of these effects.
Keywords: Vitamin D, Human Mesenchymal Stem Cells, Epigenetics, Stemness Genes, OCT4, SOX2, NANOG, KLF4

Ps-135: Effect of Electrical Stimulation on Cardiac Phenotype Expression of H9c2 Myoblast Cells Cultured on Piezoelectric PVDF/SF Nanofibrous Scaffolds *In-Vitro*

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Background: Cardiovascular diseases remain a leading cause of mortality worldwide, and myocardial infarction results in irreversible cardiomyocyte loss due to the limited regenerative capacity of cardiac tissue. Electroactive scaffolds that mimic the native cardiac microenvironment have emerged as promising platforms for cardiac tissue engineering. Poly (vinylidene fluoride) (PVDF) is a piezoelectric polymer capable of generating electrical cues, while silk fibroin (SF) exhibits excellent biocompatibility and extracellular matrix-like properties. This study aimed to fabricate hybrid PVDF/SF nanofibrous scaffolds and evaluate the effect of electrical stimulation on cardiac phenotype expression in myoblast cells.
Materials and Methods: PVDF/SF nanofibrous scaffolds with different compositions were fabricated by dual electrospinning. Their chemical structure, crystalline phase, mechanical properties, and piezoelectric performance were characterized using FTIR, XRD, mechanical testing, and electrical measurements. Following selection of the optimal scaffold composition, H9c2 Myoblast cells were cultured on the scaffolds. Cell viability was assessed by MTT assay, while cell morphology was examined by scanning electron microscopy (SEM). The expression of cardiac-specific markers, including cardiac troponin T (cTnT), α-actinin, GATA4, and Connexin-43 (Cx43), was evaluated by qRT-PCR and immunohistochemical staining in pure PVDF, PVDF/SF composite, and electrically stimulated PVDF/SF Scaffolds.
Results: FTIR and XRD analyses confirmed successful fabrication of PVDF/SF composite scaffolds and preservation of the electroactive β-phase of PVDF. Increasing SF content reduced the β-phase fraction from 62% to 53% and decreased the piezoelectric output from approximately 20 mV to 5 mV. Nevertheless, the composite scaffolds retained electroactive behavior and suitable mechanical properties. MTT results demonstrated good cytocompatibility and enhanced metabolic activity following electrical stimulation. SEM observations revealed improved cell attachment and spreading in stimulated groups. Furthermore, electrical stimulation significantly upregulated the expression of cardiac markers, including α-actinin, and Cx43.
Conclusion: The combination of PVDF piezoelectricity, SF biocompatibility, and external electrical stimulation promoted cardiac phenotype expression and improved cellular behavior. These findings highlight the potential of electrically stimulated PVDF/SF nanofibrous scaffolds as promising platforms for cardiac tissue engineering and regenerative medicine applications.
Keywords: Cardiac Tissue Engineering, Electrical Stimulation, PVDF/SF Nanofibers, H9c2 Myoblast Cells

Ps-136: From Evolution to Expression: Decoding the Regulatory Logic of Goat PDX1

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Background: PDX1 is the master regulator of pancreatic development and beta-cell function. While well-defined in murine models, the regulatory landscape of PDX1 in livestock remains largely unexplored. This gap is critical, as large animals serve as a premier platform for humanized organ bioengineering. Decoding the transcriptional mechanisms governing their pancreatic development is an essential prerequisite for translational success in regenerative medicine of pancreatic disease and diabetes. To address this, we integrated cross-species comparative genomics with functional validation to characterize the cis-regulatory architecture governing goat PDX1.
Materials and Methods: A comparative genomic analysis of the goat PDX1 locus was performed to identify conserved non-coding regions. A series of EGFP reporter vectors incorporating the promoter and upstream cis-regulatory areas (I–IV) were constructed. These constructs were evaluated through transient transfection assays in both beta-cell and non-beta-cell lines, followed by an analysis of islet-specific expression in native pancreatic tissue.
Results: Bioinformatic analysis identified eight proximal promoter elements, four distal regulatory areas (I–IV), and transcription start sites. Transfection revealed that a key E-box (-192) and CAAT box (-213) function synergistically as the basal promoter. While each distal area enhances basal activity in beta-cells, they exhibit a sophisticated, cooperative bifunctional effect when combined. Notably, the ~3 kb upstream region (Areas I–III) proved the most efficient reporter, demonstrating robust, islet-specific expression.

Conclusion: These findings demonstrate that goat PDX1 is more structurally and functionally similar to its human counterpart than to other models, establishing the goat as a high-fidelity platform for regenerative medicine and organ bioengineering.
Keywords: PDX1, Pancreatic Development, Beta-Cell, Organ Bioengineering

Ps-137: Identification of Key Transcription Factors in The Differentiation of Stem Cells into Dopaminergic Neurons in Parkinson's Disease

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Background: Pluripotent stem cells possess the remarkable ability to differentiate into various specialized cell types. Among the most significant differentiation pathways is their conversion into dopaminergic neurons. This intricate, multi-step process involves precise and coordinated changes in gene expression patterns, primarily orchestrated by transcription factors (TFs). These proteins play a crucial role in activating or inhibiting target genes, thereby guiding the cellular differentiation trajectory. While previous studies have identified several TFs involved in neurodopaminergic differentiation, a comprehensive understanding of the complete regulatory network governing this process remains elusive. Much of the existing research has focused on a limited number of well-known factors, with less emphasis on systematic, network-based analysis. Consequently, critical questions persist regarding which TFs play the most pivotal roles and whether other significant regulators are involved. The advent of gene expression databases and advanced bioinformatic tools now enables extensive, network-level analysis of genomic data. Employing such approaches can lead to the identification of central nodes and key TFs within the dopaminergic differentiation pathway. Therefore, a systematic study to identify and analyze the regulatory network of transcription factors is essential for a more profound understanding of the molecular mechanisms underlying neural differentiation.
Materials and Methods: Microarray data was retrieved from the Gene Expression Omnibus (GEO) database and analyzed using the GEO2R software. This study involved two groups: CNP-treated and control human embryonic stem cells (hESCs). Differential gene expression analysis revealed 987 upregulated and 1,906 downregulated genes. Protein-protein interactions, gene functions, and network visualization were performed using the STRING database and visualized with Cytoscape.
Results: This study identified 37 transcription factors implicated in the differentiation of stem cells into dopaminergic neurons. Of these, 35 TFs have been previously reported in the literature, while 3 TFs represent novel findings. These transcription factors play a significant role in directing stem cell differentiation towards the dopaminergic neuronal lineage.
Conclusion: This paper presents 37 transcription factors crucial for stem cell differentiation into dopaminergic neurons, identifying 3 novel candidate NFE2, ELF3 and FOXH1 TFs with a significant role in Parkinson's disease. These findings offer valuable insights for researchers, potentially aiding in the diagnosis and treatment of Parkinson's disease.
Keywords: Parkinson's Disease, Transcription Factor, Protein-Protein Interaction Network, Microarray, Bioinformatics

Ps-138: Identification of Potential Diagnostic and Therapeutic Biomarkers for Breast Cancer Using Online Bioinformatics Analysis of Gene Expression Databases

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Background: Breast cancer is one of the most common human malignancies, and one of the leading causes of cancer death in women worldwide. However, high intra-tumoral diversity and low accuracy of conventional biomarkers make early diagnosis and treatment difficult. Bioinformatics analyses of vast transcriptomic profiles provide an effective tool for finding novel, high-accuracy biomarkers for diagnosis, as well as potential therapeutic targets
Materials and Methods: We obtained the transcriptomic data (Microarray, RNA-Seq) of breast cancer and adjacent normal tissues from GEO and TCGA. Differentially expressed genes (DEGs) were identified using GEO2R and edgeR ($P < 0.05$, $logFC > 2$). Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed. Protein-Protein Interaction (PPI) networks were analyzed with the STRING database and visualized in Cytoscape to identify the top hub genes. The diagnostic value, prognosis value, and expression validation of the candidate hub genes were assessed with the GEPIA platform
Results: We analyzed differentially expressed genes (DEGs) by integrated bioinformatics analysis, which was strongly associated with breast cancer progression. GO and KEGG analyses confirmed these DEGs were primarily related to cell cycle regulation, signaling pathways, and extracellular matrix remodeling. PPI network analysis indicated that LMX1B, ANKRD30A, LINC00993, and CYP4Z2P serve as important hub genes in the network. Verification using TCGA and GEO datasets showed that these candidates were significantly aberrantly expressed in breast cancer tissues compared with adjacent normal tissues. Overexpression of these candidates predicts tumor grade and distinct clinical consequences, used to support their use as biomarkers for the diagnosis and prognosis of breast cancer.
Conclusion: LMX1B, ANKRD30A, LINC00993, and CYP4Z2P are confirmed candidate hub genes for breast cancer. By adopting multi-omics data and network analysis, we obtained highly accurate markers that can potentially be used for early diagnosis and targeted therapy strategies. This study shows a good application of the tumor heterogeneity problem through a bioinformatics-based screening and also provides a basis for future experimental validation of the targets.
Keywords: Breast Cancer, Gene Expression, Diagnostic Biomarkers, Bioinformatics

Ps-139: Dose-Dependent Hepatotoxicity of Zinc Oxide Nanoparticles in Male NMRI Mice

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Background: Zinc oxide nanoparticles (ZnO NPs) rank among the most extensively produced metal oxide nanoparticles globally, with applications in cosmetics, sunscreens, food packaging, electronics, and medical devices. Their physicochemical properties including high surface area and chemical stability, have accelerated their integration into numerous industrial sectors. Human exposure occurs through inhalation, ingestion, and dermal contact. Pharmacokinetic observations confirm that the liver is the first organ to accumulate ZnO NPs following systemic entry. Once inside hepatic tissue, these nanoparticles are known to impair antioxidant defense systems, elevate reactive oxygen species (ROS), and trigger lipid peroxidation, collectively pushing hepatocytes toward oxidative stress-driven injury and cell death. Despite growing concerns, dose-dependent hepatotoxic mechanisms in living models remain incompletely characterized.
Materials and Methods: 30 male NMRI mice (mean weight 35 g) were randomly assigned to five groups of six, comprising three treatment groups of 15 ppm, 30 ppm, and 60 ppm of ZnO NPs injection, alongside one control and one sham group. Intraperitoneal injections were administered every other day over 20 days. At the end of the treatment period, blood was collected directly from the heart, serum was isolated, and hepatic necrosis biomarkers including alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) were measured. Subsequently, liver tissue was extracted and processed for histopathological analysis.
Results: Liver sections showed progressive tissue breakdown. Histopathological examination revealed dose-dependent tissue destruction, including necrosis, central vein congestion, and marked inflammatory cell infiltration. Serum levels of ALT, AST, and ALP were significantly elevated across treatment groups (ALP level: 430.83 ± 17.85 IU/L for 60 ppm versus 278.21 ± 13.35 IU/L for sham ($P < 0.05$), reflecting enzyme leakage from damaged hepatocyte cytosol into the bloodstream.
Conclusion: Overall, the findings confirm that ZnO NPs induce dose-dependent hepatotoxicity through oxidative stress pathways, with direct implications for risk assessment in occupational and consumer settings.
Keywords: Zinc Oxide Nanoparticles, Hepatotoxicity, Alkaline Phosphatase (ALP), Alanine Transaminase (ALT)

Ps-140: Peripheral Blood DCT Expression as A Potential Molecular Signature Associated with Skin Aging

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Background: Skin aging is a multifactorial biological process associated with oxidative stress, impaired cellular homeostasis, and melanocyte dysfunction. Pigmentation imbalance and altered melanogenesis are among the most visible manifestations of intrinsic aging and photoaging. Although molecular studies of skin aging are commonly performed using skin biopsies, the invasive nature of tissue sampling limits their clinical applicability. Therefore, identifying blood-based molecular biomarkers may provide a minimally invasive approach for monitoring aging-associated skin alterations. DCT (dopachrome tautomerase) is a melanogenesis-associated enzyme involved in melanin synthesis, melanocyte survival, and oxidative stress protection. Despite its established role in skin biology, the relationship between peripheral blood DCT expression and skin aging remains poorly understood.
Materials and Methods: Thirty participants were enrolled and categorized into three groups: young individuals (18–30 years), elderly individuals with mild skin aging features, and elderly individuals with pronounced aging-related skin changes based on SCINEXA clinical scoring. Peripheral blood samples (3 mL) were collected from each participant. Total RNA was extracted from whole blood samples, and relative DCT expression levels were evaluated using quantitative real-time PCR (qRT-PCR). Comparative statistical analyses were performed between study groups.
Results: Peripheral blood DCT expression showed a significant reduction in elderly individuals compared with the young group. Relative DCT expression was significantly lower in both the mild aging group ($P=0.0002$) and the severe aging group ($P < 0.0001$), while no significant difference was observed between the two elderly groups ($P=0.3417$). Lower DCT expression levels were associated with more pronounced aging-related skin phenotypes and higher SCINEXA scores. Although DCT is primarily recognized as a melanocyte-associated skin gene, detectable alterations in peripheral blood expression may reflect systemic molecular changes associated with melanocyte dysfunction and aging-related pigmentation alterations.
Conclusion: These findings suggest that peripheral blood DCT expression may serve as a minimally invasive molecular signature associated with skin aging. Blood-based molecular approaches may improve non-invasive monitoring of aging-associated skin changes. Further studies with larger cohorts are required to validate the clinical applicability of DCT as a potential skin aging biomarker.
Keywords: Skin Aging, DCT, Melanogenesis, Pigmentation

Ps-141: Association of TNF-α Signaling Pathway Polymorphism with Leukemia Susceptibility Using Tetra-ARMS PCR

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Background: Leukemia is a heterogeneous hematologic malignancy characterized by dysregulated inflammatory signaling pathways. Tumor necrosis factor-alpha (TNF-α) is a key pro-inflammatory cytokine that plays a critical role in leukemogenesis. This study aimed to investigate the heterozygosity and homozygosity patterns of *TNF-α* gene polymorphism in patients with leukemia using the Tetra-ARMS PCR method.
Materials and Methods: In this case–control study, peripheral blood samples were collected from 58 leukemia patients and 58 healthy controls matched for age, sex, and body mass

index. Genomic DNA was extracted using extraction kit, and DNA quality was evaluated by spectrophotometry and agarose gel electrophoresis. Genotyping of TNF-α (rs1800629) was performed using the Tetra-ARMS PCR technique with allele-specific primers. PCR products were analyzed by agarose gel electrophoresis, and genotype distributions were statistically assessed using SPSS version 20. Hardy–Weinberg equilibrium, chi-square tests, odds ratios (OR), and 95% confidence intervals were calculated, with $P < 0.05$ considered statistically significant.

Results: No significant demographic differences were observed between the patient and control groups. Genotypic and allelic distributions of the investigated polymorphism were generally consistent with Hardy–Weinberg equilibrium. Although a relatively higher frequency of the pure dominant genotype was observed among leukemia patients for TNF-α polymorphism, the differences were not statistically significant. Genetic model analyses, including dominant and recessive models, also failed to demonstrate a definitive association between this variant and leukemia susceptibility. However, several trends suggested potential contributions of high-risk alleles to disease predisposition.

Conclusion: The investigated TNF-α polymorphism showed no significant causal association with leukemia in the studied population. Nevertheless, the observed genotype trends may indicate a possible role in leukemia susceptibility. Larger population-based and functional studies are required to clarify the biological significance of this variant.

Keywords: Leukemia, TNF-α, Polymorphism, Tetra-ARMS PCR, Inflammation

Ps-142: Gene-Modified Autologous Hematopoietic Stem/Progenitor Cell Therapies for Sickle Cell Disease and β-Thalassemia: A Systematic Review and Meta-analysis

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Background: Ex-vivo gene-modified autologous CD34+ hematopoietic stem/progenitor cell (HSPC) therapies have shown substantial clinical benefit in sickle cell disease and transfusion-dependent β-thalassemia. However, the durability of response and genomic safety of CRISPR-associated protein 9 (CRISPR/Cas9)-edited and lentiviral vector-modified platforms remain incompletely characterized. This systematic review and meta-analysis aimed to evaluate disease-specific clinical response, durability of efficacy, and reported genomic safety outcomes of ex-vivo gene-modified autologous CD34+ HSPC therapies in sickle cell disease and transfusion-dependent β-thalassemia.

Materials and Methods: We systematically reviewed publicly available clinical trial publications, conference reports, and regulatory datasets from inception through April 2026. Eligible datasets included human studies of ex-vivo gene-modified autologous CD34+ HSPC therapies with extractable disease-specific efficacy outcomes. Overlapping trials, follow-up reports, and regulatory submissions were reconciled to avoid double counting. Disease-specific response was defined as transfusion independence in transfusion-dependent β-thalassemia and freedom from severe vaso-occlusive events or protocol-defined clinical response in sickle cell disease. Pooled proportions were estimated using random-effects models with logit transformation. Genomic safety outcomes were synthesized descriptively

Results: Four independent datasets comprising 138 patients were included, covering exagamglogene autotemcel, lovotibeglogene autotemcel, and betibeglogene autotemcel. The overall pooled disease-specific clinical response rate was 90.7% (95% confidence interval, 84.2–94.6). Exploratory platform-stratified estimates were high for both CRISPR/Cas9-edited and lentiviral vector-modified HSPC therapies, at 93.3% and 89.0%, respectively. These estimates should not be interpreted as direct comparative efficacy measures because of heterogeneity in indications, endpoint definitions, sample size, and follow-up duration. The longest available follow-up was observed for betibeglogene autotemcel in transfusion-dependent β-thalassemia, with sustained transfusion independence reported up to 10 years. No confirmed treatment-related malignancy or replication-competent lentivirus was reported in the included datasets.

Conclusion: Gene-modified autologous CD34+ HSPC therapies are associated with high disease-specific clinical response rates in sickle cell disease and transfusion-dependent β-thalassemia. Current evidence supports durable benefit for selected lentiviral vector-modified products, while longer follow-up is required to define the durability and genomic safety of newer CRISPR/Cas9-edited therapies.

Keywords: Gene Therapy, Hematopoietic Stem/Progenitor Cells, Hemoglobinopathies, Genomic Safety, Systematic Review

Ps-143: Evaluation of Cytotoxicity of Doxorubicin On T-Cell Acute Lymphoblastic Leukemia (T-ALL) Cells after Co-Culture with Human Umbilical Cord-Derived Mesenchymal Stem Cells

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy that, despite therapeutic advances, faces serious challenges in clinical management due to drug resistance and disease relapse. In recent years, human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) have emerged as a potential therapeutic modality in malignancies due to their unique properties, including tumor-homing ability, immunomodulation, and secretion of antitumor factors. However, the time-dependent effects of these cells on T-ALL cells and their response to chemotherapy remain poorly understood. This study aimed to investigate the effects of human umbilical cord derived mesenchymal stem cells (hUC MSCs) on T cell acute lymphoblastic leukemia (T ALL) and to evaluate whether co culture of hUC MSCs with the Jurkat cell line (as a T ALL model) alters doxorubicin sensitivity, apoptotic rate, and reactive oxygen species (ROS) levels, with particular attention to the roles of exposure time and cell ratio.

Materials and Methods: Jurkat cells were co-cultured with hUC-MSCs at ratios of 2:1, 4:1, and 8:1 (Jurkat:MSC) for 3, 5, and 7 days. Subsequently, doxorubicin sensitivity was assessed by MTT assay and IC_{50} determination, apoptotic rate by Annexin V/PI staining and flow cytometry, and ROS levels by

H_2DCF-DA probe.
Results: The findings demonstrated that MSC-induced drug resistance is a time-dependent phenomenon. At 7 days, doxorubicin IC_{50} significantly increased in all co-culture ratios, and doxorubicin-induced apoptotic rate decreased from 67% in the control group to 8.5%. ROS levels showed a significant increase at 3 days and a significant decrease at 7 days.
Conclusion: The results of this study demonstrate that the effect of hUC-MSCs on Jurkat cells is strictly time dependent: short term exposure (3 days) exerts a moderate protective effect against doxorubicin induced apoptosis, whereas long term exposure (7 days) leads to the induction of stable drug resistance (increased IC_{50}, a sharp decrease in apoptotic rate, and reduced ROS levels). These findings caution that co culture duration is a critical determinant of leukemic cell response to MSCs, and overlooking it can lead to misleading results in future studies.
Keywords: T-cell Acute Lymphoblastic Leukemia, Mesenchymal Stem Cells, Doxorubicin, Drug Resistance

Ps-144: Investigating The Effect of Demyelination on Hypothalamic-Pituitary-Gonadal Axis Activity and Spermatogenesis in Adult Male Rats of Multiple Sclerosis Model: A Histological and Serological Study

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Background: Multiple sclerosis (MS) is an autoimmune demyelinating disorder of the CNS. It is followed by neuropathy discomfort, especially in reproductive age. Therefore, this research aimed to investigate the effect of demyelination on the activity of the hypothalamus axis, hypophysis gonad, and spermatogenesis.
Materials and Methods: In this research, 21 rats were randomly divided into 3 groups of 7. EB was injected intraperitoneally to the demyelinated injury group. The normal saline group was injected with 0.9% normal saline and the remaining 7 mice remained in the control group. Footprint and MNSS tests were used for behavioral analysis. And in order to check the changes of FSH, LH, GNRH sexual factors, the serum level was checked. Also, brain and testis tissue were extracted and studied in histology using Luxol fast blue, cresyl fast violet, and hematoxylin and eosin staining methods
Results: The results of locomotor tests using footprint analysis and MNSS showed that the walking pattern of the injured group changed and the MNSS index increased. Also, in the injured group, we have a decrease in serum levels of LH, FSH, and GNRH, and a decrease in the weight of the mouse testicles relative to body weight and a decrease in the number and quality of sperm were observed. And in the histological examination of the brain, myelin and axon destruction was observed, and in the histological examination of the testicles, a decrease in the number of germ cells was observed.
Conclusion: The effect of demyelination has a significant impact on the activity of the hypothalamic-pituitary axis and the gonads, anddemyelination affects male fertility and causes a decrease in adult male fertility.
Keywords: Multiple Sclerosis, Demyelination, Ethidium Bromide

Ps-145: Osteogenic Potential of An Ethyl Acetate Extract from The Halophilic Actinobacterium Streptomyces Violaceorubidus M4 on Rat Adipose-Derived Mesenchymal Stem Cells

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Background: Halophilic actinobacteria from extreme saline environments are a promising yet underexplored source of novel bioactive secondary metabolites. Despite the well-established pharmaceutical importance of Streptomyces species, their potential to direct the osteogenic differentiation of mesenchymal stem cells remains largely unknown. This study, for the first time, investigates the chemical composition, antioxidant activity, cytotoxicity, and osteogenic differentiation potential of an ethyl acetate extract from the native Iranian halophilic actinobacterium Streptomyces violaceorubidus strain M4 on rat adipose-derived mesenchymal stem cells (AD-MSCs).
Materials and Methods: The S. violaceorubidus M4 strain was originally isolated from hypersaline soils of the Garmsar Desert, Iran. Following culture and ethyl acetate extraction, the chemical composition was profiled by Gas Chromatography-Mass Spectrometry (GC-MS). AD-MSCs were isolated from the inguinal adipose tissue of adult male Wistar rats, and their mesenchymal identity was confirmed at passage three by flow cytometry (positive for CD90: 97.9% and CD44: 99.7%; negative for CD34). Cytotoxicity was assessed via the MTT assay, and antioxidant activity was measured using the DPPH radical scavenging method. For osteogenic induction, AD-MSCs were cultured for 21 days in osteogenic medium supplemented with 5 and 10 µg/ml of the extract, both concentrations well below the experimentally determined IC_{50} of 12.64 µg/ml. Differentiation was evaluated through Alkaline Phosphatase (ALP) activity, Alizarin Red S staining for calcium deposition, and quantitative real-time PCR for the key osteogenic markers Osteopontin (OSP), Collagen type I (COL1), and Dentin Matrix Protein 1 (DMP1).
Results: GC-MS analysis identified 19 compounds, with over 50% of the extract consisting of saturated fatty acids, predominantly n-Hexadecanoic acid (26.44%) and Pentadecanoic acid (19.52%). The extract demonstrated dose-dependent antioxidant activity and maintained high cell viability at concentrations ≤25 µg/ml. Compared to untreated controls, both the 5 and 10 µg/ml treatment groups exhibited a significant increase in ALP activity ($P < 0.005$), along with markedly enhanced matrix mineralization as visualized by Alizarin Red S staining. Quantitative real-time PCR revealed a significant upregulation of *OSP* and *COL1* gene expression ($P < 0.005$), while DMP1 exhibited a relatively lower, yet detectable, expression level.
Conclusion: This study provides the first evidence that the ethyl acetate extract of the native halophilic S. violaceorubidus M4, rich in specific bioactive saturated fatty acids, acts as a potent and effective inducer of osteogenic differentiation in AD-MSCs at non-cytotoxic concentrations. These findings introduce this naturally-sourced extract as a novel, promising,

and accessible candidate for advancing bone tissue engineering and regenerative medicine strategies.
Keywords: Halophilic Actinobacteria, Streptomyces Violaceorubidus, Adipose-Derived Mesenchymal Stem Cells, Osteogenic Differentiation, Bone Tissue Engineering

Ps-146: Establishing An Accessible and Scalable Platform for Generating Human Blastoids from Naïve t2iLGö hESCs

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Background: Stem cell-based embryo models are alternative platforms for studying early embryonic development and implantation while mitigating ethical concerns and the limited accessibility of natural embryos. Various embryo models, including blastoids, have been developed to facilitate the study of embryogenesis; however, their broad application and scalability may be limited by reliance on high-cost specialized culture systems, such as Aggrewell plates, Gri3D plates, and hydrogel microwell plates. Here, we set up a more accessible culture system for generating blastoids using naïve human embryonic stem cells (hESCs).
Materials and Methods: To generate human blastoids, we first converted primed hESCs, Royan H6, to a naïve state using the t2iLGö cocktail, which includes MEK, protein kinase C, Tankyrase, and GSK3β inhibitors, along with hLIF. Next, t2iLGö cells were cultured at a density of 220 cells/mm² in non-treated culture dishes under 5% O2 conditions using a medium containing ROCK inhibitor. The resulting aggregates were directed toward the trophectoderm lineage by inhibiting the MEK, TGF-β and Rho-kinase pathways for 48 hours, followed by continuous culture in medium containing ROCKi.
Results: Successful conversion of primed hESCs to a naïve state was confirmed by their dome-shaped colony morphology, elevated expression of naïve-specific markers such as KLF17, KLF4, NANOG, and TBX3 using qRT-PCR, and detection of STAT3 and TFCP2L1 via immunostaining. t2iLGö cells formed aggregates within 48 hours in non-treated dishes. After three days of induction, cystic structures averaging 100 µm in diameter were observed, featuring a distinct inner cell mass-like compartment surrounded by a TE-like outer layer. The similarities between t2iLGö-blastoids and their natural counterparts, blastocysts, were analyzed by examining the expression and localization of trophectoderm markers GATA3 and CDX2, as well as epiblast markers OCT4 and NANOG. Additionally, cultivating 10-day-old t2iLGö-blastoids under conditions for hESC derivation resulted in hESC-like outgrowths within 2 days, showing greater similarity in potential and structure to human blastocysts.
Conclusion: Our study provides a reproducible, scalable, and cost-effective method for generating human blastoids from hESCs in non-treated culture plates, offering a more accessible platform for embryo modeling research.
Keywords: Embryo Model, Blastoid, Human Blastocyst, Naïve Human Embryonic Stem Cell

Ps-147: Comparative Expression of Neurotrophic Factors in Nestin-Expressing Hair Follicle-Derived Stem Cells, Bone Marrow-Derived Mesenchymal Stem Cells, and Dental Pulp-Derived Stem Cells

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Background: Nestin-expressing hair follicle-derived stem cells (NHFCs) have attracted considerable attention in regenerative medicine because of their accessibility, multipotency, and neurogenic potential. Neurotrophic factors secreted by stem/progenitor cells play important roles in neuronal survival, neurogenesis, and neural repair. The present study compared the expression profile of major neurotrophic factors in NHFCs with bone marrow-derived mesenchymal stem cells (BM-MCs) and dental pulp-derived stem cells (DPCs).
Materials and Methods: NHFCs were isolated from rat whisker hair follicle bulges, while BM-MCs and DPCs were isolated from rat bone marrow and dental pulp, respectively. Immunocytochemistry at passage 1 confirmed nestin expression in migrated NHFCs. Cells were cultured under similar conditions and analyzed at passage 4. Relative mRNA expression levels of brain-derived neurotrophic factor (*Bdnf*), nerve growth factor (*Ngf*), neurotrophin-3 (*Ntf3*), glial cell line-derived neurotrophic factor (*Gdnf*), and vascular endothelial growth factor (*Vegf*) were evaluated using quantitative real-time PCR. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.
Results: NHFCs exhibited significantly higher Bdnf expression compared with BM-MCs (approximately 2.5-fold) and DPCs (approximately 6-fold). Ngf expression in NHFCs was significantly higher than in DPCs but lower than in BM-MCs. *Ntf3* expression in NHFCs was significantly lower than both BM-MCs and DPCs. Gdnf expression in NHFCs was lower than BM-MCs but comparable to DPCs. *Vegf* expression in NHFCs was significantly higher than DPCs and comparable to BM-MCs. Overall, NHFCs demonstrated a distinct neurotrophic expression profile characterized by elevated *Bdnf* expression.
Conclusion: NHFCs exhibit a unique neurotrophic factor expression pattern compared with BM-MCs and DPCs, particularly through enhanced Bdnf expression. These findings suggest that NHFCs may represent a promising and accessible cell source for neuroregenerative applications and neural tissue engineering.
Keywords: Hair Follicle, Bone Marrow, Dental Pulp, Neurotrophic Factor, Regenerative Medicine

Ps-148: Intranasal Administration of Hair Follicle-Derived Stem Cells Enhances Functional Recovery Following Experimental Ischemic Stroke

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Background: Stem cell-based therapy has emerged as a promising strategy for the treatment of ischemic stroke; however, identifying an accessible stem cell source and an effective delivery route remains challenging. Hair follicle-derived stem cells (HFSCs), also known as epidermal neural crest stem cells, are easily accessible multipotent cells with neuroprotective and regenerative potential. The present study evaluated the therapeutic effects of intranasal HFSC transplantation in a rat model of ischemic stroke.
Materials and Methods: HFSCs were isolated from rat whisker hair follicle bulges and cultured *in-vitro*. Immunocytochemistry confirmed nestin and SOX10 expression in migrated cells, indicating their neural crest origin. Flow cytometry demonstrated CD44 and CD90 positivity and lack of CD34 and CD45 expression. Ischemic stroke was induced in rats using middle cerebral artery occlusion (MCAO). In experiment 1, animals underwent 30 minutes MCAO and received intranasal HFSC transplantation immediately after ischemia. In experiment 2, rats underwent 120 minutes MCAO and received HFSCs 24 hours later. Neurological deficits, infarct volume, spatial working memory, and expression of neurotrophic factors were evaluated.
Results: Intranasal administration of HFSCs significantly improved neurological performance and reduced infarct volume compared with untreated MCAO rats. In both experiments, functional recovery was accompanied by attenuation of ischemic damage in the ipsilateral hemisphere. HFSC therapy also increased expression of NeuN and VEGF in the striatum. Furthermore, stem cell transplantation partially prevented the ischemia-induced overexpression of BDNF and neurotrophin-3. No significant effect on short-term spatial memory was observed.
Conclusion: Intranasal delivery of HFSCs improved functional recovery and reduced ischemic injury following experimental stroke. The findings suggest that intranasal HFSC transplantation represents a safe, minimally invasive, and promising therapeutic strategy for ischemic stroke and other neurological disorders.
Keywords: Hair Follicle-Derived Stem Cells, Ischemic Stroke, Intranasal Transplantation, Neuroregeneration, MCAO

Ps-149: Generation of Stable GFP-Luciferase Reporter Cell Lines for The Evaluation of Anti-BCMA CAR-T-Cell-Mediated Cytotoxicity

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Background: Anti-BCMA CAR-T cell therapy has emerged as a promising treatment strategy for multiple myeloma. Reliable reporter-expressing target cell lines are essential for quantitative assessment of CAR-T cell- mediated cytotoxicity. The present study aimed to generate stable GFP-Luciferase reporter cell lines based on U266-B1, K562, and U-87-MG cells as a platform for future anti-BCMA CAR-T functional assays.
Materials and Methods: A retroviral vector encoding GFP and Luciferase reporter genes was used to transduce U266-B1, K562, and U-87-MG cell lines, which had been selected based on various BCMA expression levels. Following transduction, GFP-positive populations were enriched and subjected to fluorescence-activated cell sorting (FACS). Single-cell sorting was performed to establish monoclonal reporter cell lines. Clonal expansion and GFP expression were evaluated using fluorescence microscopy. The next step is confirmation of the luciferase activity of the cells using a luminescence assay. Finally, the cytotoxic effect of the CAR-T cells is tested by measuring the luminescence activity of the reporter cells.
Results: Successful transduction was achieved in all three cell lines. GFP-positive populations were enriched and single-cell-derived clones were obtained following FACS sorting. Fluorescence microscopy confirmed GFP expression in multiple isolated clones during early expansion. The reporter-positive clones were further confirmed using luciferase assay, indicating successful reporter gene integration and expression.
Conclusion: Stable GFP-expressing reporter clones were successfully generated from U266-B1, K562, and U-87-MG cell lines. These reporter cell lines provide a promising platform for subsequent validation of Luciferase activity and quantitative assessment of anti-BCMA CAR-T cell cytotoxicity. The established system may facilitate the development of standardized and high-throughput in-vitro assays for CAR-T research.
Keywords: CAR-T Cell Therapy, Reporter Cell Line, GFP, Luciferase

Ps-150: Investigation of STAT2 Alterations in Insulin-Secreting Cells Differentiated from Human Adipose-Derived Mesenchymal Stem Cells

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Background: Suppression of STAT2 signaling diminishes interferon responsiveness, thereby facilitating the functional maturation of insulin-secreting cells through the regulation of immune signaling and cellular stress pathways. In the present study, we analyzed alterations in STAT2 expression in efficient insulin-producing cells generated from the differentiation of human adipose-derived mesenchymal stem cells.
Materials and Methods: Human adipose-derived mesenchymal stem cells were differentiated into insulin-producing cells using a two-week protocol involving ITS and nicotinamide supplementation. At the end of the differentiation period, Insulin concentration was measured by ELISA, GLUT2 expression was evaluated by Western blot analysis, and STAT2 expression levels were quantified using real-time PCR. Statistical analysis

was performed using GraphPad Prism 8 software, and comparisons between the differentiated and control groups were conducted using an independent t-test.
Results: The results demonstrated that the derived insulin-producing cells exhibited a significantly higher capacity for Insulin production and GLUT2 expression compared to the control group ($P < 0.01$ and $P < 0.001$, respectively). In contrast, STAT2 expression was markedly reduced in the differentiated cells relative to the control group ($P < 0.0001$).
Conclusion: Downregulation of STAT2 expression may serve as a potential biomarker indicating attenuation of interferon signaling, which is involved in the full functional maturation of effective insulin-secreting cells.
Keywords: Mesenchymal Stem Cells, Insulin, STAT2, Differentiation

Ps-151: Bio-instructive Bone Derived Nerve Guidance Conduits: Deciphering The Spatiotemporal Dynamics of Extracellular Matrix Remodeling in Peripheral Nerve Repair

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Background: Repair of peripheral nerve injuries across long defects remains a major challenge in neural tissue engineering. While biomaterial scaffolds provide structural support for axonal growth, successful regeneration also depends on the biological microenvironment within the conduit. Remodeling of the extracellular matrix (ECM) plays a key role in regulating axonal extension and tissue maturation. This study investigated the temporal dynamics of ECM related gene expression and its association with functional recovery in bone derived nerve guidance conduits (NGCs)
Materials and Methods: Decellularized and demineralized bone derived NGCs fabricated from rat femur and chicken tibia were used to bridge a 10 mm sciatic nerve defect in an experimental animal model. The expression of collagen type I alpha 1 (COL1A1), fibronectin 1 (FN1), laminin beta 2 (LAMB2), and tenascin C (TNC) was evaluated at 4, 8, 12, and 16 weeks after implantation using real time polymerase chain reaction (RT PCR). Functional recovery was assessed by the Sciatic Functional Index (SFI) and hot plate test. Histological analyses were performed using hematoxylin and eosin (H&E), Luxol Fast Blue (LFB), and Masson's trichrome staining. Gastrocnemius muscle recovery was also assessed using the wet muscle weight ratio
Results: SFI analysis showed progressive functional recovery in the rat NGC group during the 16 week period, with significant improvement compared with the axotomy group ($P < 0.05$, $P < 0.01$, $P < 0.001$). Chicken derived conduits and the nerve scaffold group also improved but to a lesser degree. Gastrocnemius muscle analysis indicated that all treatment groups were significantly better than axotomy, although differences from healthy controls remained ($P < 0.01$–0.0001). Histological findings confirmed improved nerve fiber organization in rat femur derived conduits. Changes in FN1 and TNC expression appeared to parallel the progression of nerve regeneration
Conclusion: Bone derived NGCs support peripheral nerve regeneration by providing structural guidance and a favorable molecular environment. Among the tested scaffolds, rat femur derived conduits showed the most favorable regenerative outcomes. Modulation of ECM related molecular responses may therefore enhance long gap nerve repair
Keywords: Extracellular Matrix, Peripheral Nerve Regeneration, Bone Derived Scaffolds, Nerve Guidance Conduits, Sciatic Nerve Repair

Ps-152: Anti-Tumorigenic Effects of Resveratrol-Loaded Poly (lactic-Co-Glycolic Acid) Microparticles in A 3D Hepatocellular Carcinoma Model

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Background: Hepatocellular carcinoma (HCC) is the most common primary malignant liver tumor and is one of the leading causes of cancer-related deaths worldwide. Therefore, the need to develop effective therapeutic strategies against this life-threatening disease is critical. Resveratrol (RSV) has shown promising antitumor efficacy through apoptosis induction and inhibition of cancer cell proliferation. However, the therapeutic efficacy of RSV is hampered by its poor bioavailability and rapid metabolic inactivation. In the current study, biodegradable poly (lactic-co-glycolic acid) (PLGA) microparticles (MP) were employed for drug delivery to provide sustain and controlled release of RSV in a physiologically relevant liver cancer microtissue (LCMT).
Materials and Methods: The RSV-loaded MPs (RSV-MP) which prepared using a standard oil-in-water emulsion method has a mean diameter of 2.04 ± 0.48 µm and characterized before evaluating the therapeutic efficacy in in-vitro model. The RSV-MPs incorporated in the LCMTs consisted of co-cultured hepatoma cell line (Huh-7) and human umbilical vein endothelial cells (HUVECs) in a ratio of 4:1. The 3D spheroids were assembled in AggrewellTM plates. The anti-proliferative efficacy of the RSV-MP drug delivery approach was investigated and compared to control group which was treated with RSV solution. In this study Live/Dead assay, MTS and migration analysis, as well gene assessments were applied.
Results: The efficacy of the control (CTRL) and MP-mediated formulations (MP-RSV) was compared, and the results showed that the MP-RSV significantly decreased the proliferation efficiently (cell viability 37.49% vs. 56.14% respectivly, $**P \leq 0.01$). In addition, the gene expression profile of proliferation genes (CCND1 ($**P \leq 0.01$), P21 ($**P \leq 0.01$), c-MYC ($*P \leq$

0.05)) were significantly modulated in the treatment group. The migration assays demonstrated a significant inhibition of cell migration in the RSV-MP-treated group relative to free RSV (**P ≤ 0.01).
Conclusion: RSV-loaded PLGA MPs enhance the therapeutic efficacy of RSV in LCMT, supporting their potential as an effective drug delivery system.
Keywords: Hepatocellular Carcinoma, Resveratrol, PLGA, Proliferation, 3D Model

Ps-153: Combating Diabetic Wound Infection by Mesenchymal Stem Cell-Derived Exosomes Containing Anti-microRNA-15b

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Background: Diabetic wound infections are challenging to treat due to impaired angiogenesis and persistent inflammation. Previous studies have shown that miR-15b is significantly upregulated in diabetic wounds, where it inhibits key proangiogenic factors including VEGF, thereby contributing to impaired wound healing. Therefore, suppressing miR-15b represents a rational therapeutic approach to restoring angiogenesis and promoting tissue repair. Mesenchymal stem cell (MSC)-derived exosomes offer a promising cell-free therapeutic strategy, and their efficacy can be enhanced by modifying their microRNA cargo. This study aimed to develop an anti-microRNA-15b (anti-miR-15b) lentiviral construct to transduce human umbilical cord MSCs (UC-MSCs) for the production of engineered exosomes.
Materials and Methods: An anti-miR-15b sequence was designed, synthesized, and ligated into the pCDH plasmid vector. After transformation of the plasmid into *E. coli* DH5α, positive clones were verified by colony PCR. The purified plasmid was then co-transfected with the packaging plasmids psPAX2 and pMD2.G into HEK 293 T cells. Transfection efficiency was confirmed by green fluorescent protein (GFP) expression under fluorescence microscopy. Lentiviral particles were harvested, concentrated with 40% polyethylene glycol (PEG), and titrated by flow cytometry using GFP-positive HEK293T cells. The function and effect of Anti-miR-15b in HEK 293T cells were assessed by RNA extraction and qPCR, with normalization to SNORD 47. Finally, UC-MSCs were transduced for future exosome extraction.
Results: qPCR analysis confirmed that miR-15b was significantly downregulated in the sample group compared to the control group by a mean factor of 0.237 ($P < 0.001$). After concentration of harvested lentiviral particles with 40% PEG, the viral titer increased to 8.4×10^6 TU/ml, which was used to transduce the UC-MSC. The transduction achieved 95% GFP-positive UC-MSCs, indicating high-efficiency delivery.
Conclusion: A functional anti-miR-15b lentiviral construct was successfully developed and efficiently delivered into UC-MSCs, a critical first step toward generating engineered exosomes for diabetic wound infection therapy.
Keywords: Anti-microRNA-15b, Diabetic Wound, Exosome, Lentiviral Vector, Mesenchymal Stem Cell

Ps-154: Differentially Expressed microRNAs in Relapsing-Remitting Multiple Sclerosis Reveal Regulatory Associations with Immune and Inflammatory Pathways

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Background: Relapsing-remitting multiple sclerosis (RRMS) is the most common clinical subtype of multiple sclerosis and is characterized by recurrent episodes of neuroinflammation, demyelination, and neurological dysfunction. Although the contribution of protein-coding genes to RRMS has been widely investigated, increasing evidence suggests that microRNAs (miRNAs) play an important role in post-transcriptional gene regulation within immune and inflammatory networks. Because miRNAs are capable of modulating multiple targets simultaneously, they may contribute to disease initiation, progression, and relapse-related activity. Identifying dysregulated miRNAs and their functional associations may therefore provide valuable insight into RRMS pathobiology and support the discovery of novel molecular biomarkers.
Materials and Methods: This study focused on the miRNA-related components of the RRMS transcriptomic profile. Differentially expressed miRNAs were identified from the analyzed dataset, and candidate miRNAs were further evaluated based on their predicted regulatory associations with genes involved in immune and inflammatory signaling. Putative target genes were examined with emphasis on biologically relevant pathways associated with cytokine activity, immune-cell regulation, inflammatory amplification, and tissue injury. The analysis was designed to highlight miRNA-mediated regulatory patterns with potential mechanistic and translational significance in RRMS.
Results: Four differentially expressed miRNAs were identified in the RRMS transcriptomic profile. Among these, hsa-miR-570 and hsa-miR-4664 emerged as the most notable candidates because of their predicted interactions with key genes implicated in RRMS-associated immune and inflammatory processes, including TP53, STAT3, TNF, and CXCL10. These target genes are linked to cytokine-mediated signaling, immune activation, inflammatory cascade propagation, and cellular stress responses. The observed miRNA-target network suggests that dysregulated miRNAs may influence central molecular pathways relevant to relapse-associated neuroinflammation. In particular, the convergence of predicted regulatory effects on major inflammatory mediators supports the biological plausibility of these miRNAs as contributors to immune disequilibrium in RRMS.
Conclusion: The present findings support a biologically meaningful role for dysregulated miRNAs in RRMS and indicate that hsa-miR-570 and hsa-miR-4664 may serve as candidate regulatory molecules in immune and inflammatory signaling. These results underscore the potential of miRNA-centered analyses for improving molecular characterization of RRMS and suggest that specific miRNAs may hold promise as biomarkers and future therapeutic targets. Further experimental validation is warranted to confirm their functional significance and clinical relevance.
Keywords: RRMS, microRNA, Biomarker, Neuroinflammation

Ps-155: Targeting Ovarian Cancer Stem Cells Using Stem Cell-Derived Nanovesicles Loaded with N-Acetylcysteine

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Background: Ovarian cancer is one of the deadliest malignancies of the female reproductive system, primarily associated with high mortality due to late diagnosis, tumor heterogeneity, and the presence of cancer stem cells (CSCs). These cells, characterized by high self-renewal capacity, resistance to apoptosis, and enhanced defense against oxidative stress, play a crucial role in tumor recurrence and therapy resistance. Therefore, developing effective strategies to target CSCs is essential. Stem cell-derived nanovesicles combined with antioxidant agents such as N-acetylcysteine (NAC) may offer a novel approach to counteract tumor progression and drug resistance.
Materials and Methods: In this *in-vitro* study, nanovesicles were isolated from mesenchymal stem cells (MSCs) and loaded with NAC. Human ovarian cancer cell lines (SKOV3 and A2780), as well as cisplatin-resistant cells (A2780cis), were treated with NAC-loaded nanovesicles. Cell viability was assessed using the MTT assay. Migration and invasion capabilities were evaluated by wound healing and transwell assays. Cancer stem cell characteristics were assessed using a sphere formation assay. Apoptosis and cell cycle distribution were determined by flow cytometry. Intracellular reactive oxygen species (ROS) levels were measured using DCFH-DA staining. Expression of CSC-related markers, including CD44, ALDH1, Nanog, SOX2, and OCT4, as well as the PI3K/AKT and NF-κB signaling pathways, was examined by qRT-PCR and western blotting.
Results: NAC-loaded nanovesicles significantly reduced cell viability, migration, and invasion in all ovarian cancer cell lines compared to the control group. Sphere formation capacity was markedly diminished, indicating inhibition of stem-like properties. Flow cytometry analysis revealed increased apoptosis and cell cycle arrest. Moreover, intracellular ROS levels were significantly altered, accompanied by a reduction in antioxidant defense pathways and survival signaling. Notably, the expression of CSC markers and the PI3K/AKT and NF-κB pathways was substantially suppressed, particularly in cisplatin-resistant cells.
Conclusion: Stem cell-derived nanovesicles loaded with NAC can effectively target ovarian CSCs by disrupting oxidative stress balance, inducing apoptosis, and inhibiting key survival and self-renewal pathways. This approach may represent a promising therapeutic strategy to overcome chemotherapy resistance and improve clinical outcomes in ovarian cancer.
Keywords: Ovarian Cancer, Cancer Stem Cells, Nanovesicles, N-Acetylcysteine, Chemotherapy Resistance

Ps-156: Relative Expression of *PLCB2* and *PLCB4* Genes in Peripheral Blood Samples of Patients with Type 2 Diabetes Mellitus

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Background: Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by insulin resistance and impaired insulin secretion. The phospholipase C (PLC) signaling pathway plays a crucial role in cellular processes, including insulin release. This study aimed to evaluate the relative expression of PLCB2 and PLCB4 genes in T2DM patients compared to healthy controls to identify potential biomarkers.
Materials and Methods: In this case-control study, peripheral blood samples were collected from 30 patients diagnosed with type 2 diabetes and 30 healthy individuals as the control group. Total RNA was extracted, followed by cDNA synthesis. The relative expression of PLCB2 and PLCB4 genes was measured using Real-Time PCR (qPCR) based on the $2^{-\Delta\Delta Ct}$ method. Statistical analysis was performed using t test and GraphPad Prism software.
Results: Our findings revealed a significant alteration in the expression levels of both genes in the diabetic group. Specifically, the expression of the PLCB2 gene showed a significant increase (upregulation) in T2DM patients compared to the control group ($P < 0.05$). Conversely, a significant decrease (downregulation) was observed in the expression of the PLCB4 gene in patients compared to healthy individuals ($P < 0.05$).
Conclusion: The differential expression of PLCB2 and PLCB4 genes suggests their potential involvement in the pathogenesis of type 2 diabetes. The upregulation of PLCB2 may be linked to inflammatory signaling, while the downregulation of PLCB4 could contribute to beta-cell dysfunction. These genes may serve as promising candidates for future diagnostic or therapeutic applications in diabetes management.
Keywords: Type 2 Diabetes, Gene Expression, PLCB2, PLCB4

Ps-157: Antibacterial Effects of Dental Mesenchymal Stem Cells-Conditioned Media against Staphylococcus Aureus and Escherichia Coli

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Background: Mesenchymal stem cell-conditioned medium (MSC-CM) has emerged in regenerative medicine as a cell-free therapeutic approach for promoting tissue repair. Among MSC populations, human dental mesenchymal stem cells (DMSCs), including stem cells from the apical papilla (SCAP), dental pulp stem cells (DPSCs), and periodontal ligament stem cells (PDLSCs) have attracted considerable interest. CMs derived from these stem cells contain bioactive factors contributing to tissue regeneration, attenuation of inflammatory immune responses, angiogenesis, and cellular differentiation. However, their antibacterial effects remain insufficiently studied. We therefore aimed to investigate the antibacterial effects of SCAP-CM, DPSC-CM, and PDLSC-CM, and to compare them with the antibacterial effect of adipose-derived stem cell-CM (ASC-CM).
Materials and Methods: All CMs were prepared under the same culture conditions, using cells at the same passage number and seeding density. They were lyophilized and stored at -20 °C

until use. Before testing, the lyophilized samples were resuspended in Mueller-Hinton broth (MHB). The broth microdilution method was used to assess the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against Gram-positive bacteria (Staphylococcus aureus ATCC 12600) and Gram-negative bacteria (Escherichia coli IBRC-M 10698). In addition, disk diffusion assays were performed.
Results: The MIC and MBC values of all CM types, including SCAP-CM, DPSC-CM, PDLSC-CM, and ASC-CM, against E. coli were found to be 625 µg/mL. Against S. aureus, all CMs exhibited an MBC of 156.25 µg/mL. The MIC for DPSC-CM, PDLSC-CM, and ASC-CM was 156.25 µg/mL, while SCAP-CM showed a lower MIC of 78.125 µg/mL. In the disk diffusion assay, all CMs exhibited antibacterial activity against both E. coli and S. aureus. The inhibition zone diameters ranged from 32 to 38 mm against S. aureus, compared with 31 mm for the ciprofloxacin control, and from 23 to 24 mm against E. coli, compared with 29 mm for the gentamicin control.
Conclusion: Taken together, these findings highlight the potential of DMSC-CMs as a novel non-antibiotic therapeutic approach against both Gram-positive and Gram-negative bacteria. Their combined antibacterial and well-documented regenerative properties, make them attractive candidates for biomedical applications, in which infection control and tissue repair are closely interconnected.
Keywords: Stem Cells from the Apical Papilla, Dental Pulp Stem Cells, Periodontal Ligament Stem Cells, Conditioned Medium, Antibacterial Activity

Ps-158: The Use of Stem Cell Differentiation Stage Factors (SCDSFs) Derived from Zebrafish Embryos during Organogenesis and Their Role in Regulating Gene Expression in Normal and Pathological LX-2 Cells

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Background: Primary biliary cholangitis (PBC) is a chronic autoimmune liver disease characterized by inflammation of the bile ducts, leading to fibrosis, ductal narrowing or obstruction, and ultimately liver failure. Currently, no specific clinical treatment can cure the disease or completely halt its progression. Therefore, this study aimed to investigate the effects of Stem Cell Differentiation Stage Factors (SCDSFs) extracted from zebrafish embryos on the LX 2 hepatic stellate cell line. SCDSF extracts have previously demonstrated beneficial effects in breast cancer, intestinal cancer, hepatocellular carcinoma, melanoma, hair regeneration, sensorineural hearing loss, and mesenchymal stem cells, as well as exhibiting strong antioxidant activity. These findings suggest that SCDSFs may play an important role in tissue regeneration and repair.
Materials and Methods: In the first phase, protocols were established for zebrafish breeding and for the preparation of embryonic extracts from different developmental stages. In the second phase, SCDSF extracts obtained from the blastula, gastrula, and segmentation stages were evaluated for cytotoxicity using MTT assay.
Results: Even at the highest concentrations tested, no significant toxicity was observed in hepatic stellate cells compared with the control group. These findings established appropriate conditions for subsequent molecular analyses investigating the antifibrotic potential of SCDSFs. Finally, the effects of SCDSF extracts on the expression levels of fibrosis related genes in stellate cells were analyzed using Real Time PCR.
Conclusion: Treatment resulted in a significant reduction in the expression of key genes involved in fibrogenic signaling pathways compared with the control group, supporting the antifibrotic potential of SCDSF extracts.
Keywords: Primary Biliary Cholangitis (PBC), Fibrosis, Stem Cell Differentiation Stage Factors (SCDSFs), LX-2 Cell Line

Ps-159: Comparative Evaluation of Two Bioactive Powdered Scaffolds for Wound Repair in An Animal Model

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Background: The loss of skin integrity due to each unexpected injury contributes to an outcome that can be uncurable, and in turn, this issue makes the corresponding care providers find a remedy. During different research and development in tissue engineering and all its specific subsets, different forms of scaffolds were innovatively and emphatically has been developed so far. Among all forms, powder`s forms are appealing. Although powder scaffolds derived from different materials can be hopefully beneficial, they cannot victoriously solve the challenge. Regarding this issue, bioactive powders derived from birth delivery materials has been attracted the attention of researchers, resulting from this issue that they are natural materials with potential properties in wound healing and the other aspects.
Materials and Methods: Herein, we processed the amniotic membrane and umbilical cord using the decellularization process. Following, the confirmation of the applied process, the extracellular matrix (ECM)-based materials were milled to achieve a bioactive powder form. Subsequently, they were implanted in the back of rat to assess the effect of this scaffold form on wound healing during 10 and 20 days post implantation
Results: Although the histopathological analysis revealed that the lack of re-epithelialization in these treatments on the day of 10, results revealed the re-epithelialization in the treatment groups on day 20. Regarding the histomorphometric results, the blood vessel formation was higher in both days in the bioactive powder of umbilical cord than the control sample. The collagen density was higher in the umbilical cord-based powder than that of amniotic, but it was slightly lower than the control on day 10. However, it reached to a high value for this powder on day 20.
Conclusion: Based on these results, the potential of the bioactive powder derived from the decellularization process, espe-

cially the powder from umbilical cord, was concluded. However, further studies are needed to completely consider it as a wound dressing.
Keywords: Decellularization, ECM Powder, Birth Delivery Materials, Angiogenesis

Ps-160: Vitamin E Treatment Modulates Steatosis and Oxidative Stress in A MASLD *In-Vitro* Model

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Background: Metabolic dysfunction-associated steatosis liver disease (MASLD) is recognized as a major chronic liver disorder and a growing global health burden. Its high worldwide prevalence underscores the need for definitive therapeutic strategies. Various factors, including oxidative stress, increase the risk of MASLD onset and progression. Activation of antioxidant pathways, particularly Nrf2 signaling pathway, by vitamin E as a potent antioxidant represent promising therapeutic approach. In this study, a biomimetic platform was developed to evaluate the effect of vitamin E on oxidative stress and steatosis in MASLD.
Materials and Methods: The model consisted of Huh-7 and LX-2 cells seeded on the plates coated with 100 µg/mL liver extracellular matrix (ECM)-derived hydrogel (LEMgel). Sheep liver was decellularized by SDS and Tritone X-100, LEMgel was prepared by acetic acid and pepsin. MASLD was induced by exposure to Oleic acid (3.3×10^{-4} M) and Palmitic acid (1.65×10^{-4} M) for 48 hours. Subsequently, cells were treated with 100 µM vitamin E for 4 days, and the assessments were performed.
Results: Oil Red O staining and gene expression analysis of lipid metabolism related genes confirmed the successful induction of steatosis in the MASLD model. Vitamin E treatment significantly improved cell viability, decreased expression of lipid metabolism genes, increased antioxidant genes expression, enhanced antioxidant proteins and enzymes levels, modulated hepatic function proteins, and activated Nrf2 signaling pathway. Furthermore, it reduced intracellular lipid accumulation, confirmed by Oil Red O and Nile Red staining and suppressed LX-2 cell activation, by suppressing TGF-β.
Conclusion: Overall, vitamin E attenuated oxidative stress and lipid content in MASLD model, likely through activation of the Nrf2 signaling pathway. Moreover, this platform provides, feasible, reliable and physiologically relevant system for disease modeling and drug screening.
Keywords: Lipid Accumulation, MASLD, Nrf2 Signaling Pathway, Oxidative Stress, Vitamin E

Ps-161: A GMP- Compliant ECM is Able to Induce hESC-Pancreatic Progenitor Cells

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Background: Human embryonic stem cells (hESCs), with their ability to differentiate into mature cell types in-vitro by different types of protocols, represent a novel approach for evaluating human development, regenerative medicine, and for assessing the safety and efficacy of drugs before clinical trials. Protocols about pancreatic progenitor cells are based on the developmental pathway of organogenesis of the embryo in-vitro, starting from the establishment of definitive endoderm (DE), and finally differentiating cells toward Pancreatic Progenitor cells (PPs) in a defined culture system. The transition from allogeneic feeder layers, including MEF, HFF, and Matrigel, as a xenogeneic feeder-free system to a defined substrate like Recombinant vitronectin has enabled a xeno-free culture system suitable for clinical translation. This study aims to evaluate how the Extracellular Matrix (ECM) orchestrates hESC behavior, from survival to maintaining pluripotency and potentially subsequent differentiation steps towards pancreatic cells, through dynamic interaction with defined ECM substrate.
Materials and Methods: We established an optimized framework for evaluating the starting materials' pluripotency and proliferation on feeder- free systems by immunofluorescence and quantitative gene expression analysis. In the subsequent step, hESC line was differentiated toward pancreatic PPs using a stepwise, validated differentiation protocol. Differentiation efficiency and pancreatic lineage specification were evaluated by morphological assessment and quantitative gene expression analysis for definitive endoderm markers, including *SOX17*, *FOXA2*, and *CXCR4*, and PPs markers including *SOX9*, *PDX1*, and *NKX6.1*.
Results: Our results demonstrate that hESC cultured under feeder-free, Xeno-free conditions sustained pluripotency and differentiated into pancreatic progenitor cells on Recombinant vitronectin with comparable efficiency to Matrigel.
Conclusion: The Results revealed that while both ECMs supported pancreatic induction, Recombinant Vitronectin could be a suitable alternative to Matrigel for paving the way for future clinical translation.
Keywords: GMP-Compliant Condition, Pancreatic Progenitor Cells (PPs), Extracellular Matrix (ECM), Recombinant Vitonectin (rVit), Differentiation

Ps-162: Stem Cell Secretome under Microplastic Stress: Implications for Uterine Receptivity and Embryo Implantation

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Background: Embryo implantation, an important part of reproduction in mammals, requires a synchronized interaction between embryo and the mother, as well as a receptive endometrium. Endometrial mesenchymal stem cells (MSCs) play a vital role by managing the whole process of implantation via secretion of certain substances. Microplastic (MP) pollution has emerged as a serious issue in recent times that may interfere with human reproductive system function. MPs are known to contain toxic materials and have endocrine disrupting effects. This review proposes the effect of MPs on uterine receptivity and embryo implantation by compromising endometrial MSCs and their secretion of substances.
Materials and Methods: Human endometrial MSCs were grown in-vitro culture in presence of MPs and extracted chemicals. Cellular stress caused due to exposure was assessed by measuring oxidative stress (MDA, ROS) and release of inflammatory cytokines (TNF-α, IL-6). The release of extracellular vesicles (EV) along with the content was quantified.
Results: The effects of microplastics on endometrial MSC activity were profound in terms of oxidative stress and inflammation. The amount and characteristics of EV release changed under MP stress conditions. Leached compounds possessed endocrine-disruptive activity affecting both uterine cells and MSCs. Of greatest importance is the impairment of uterus receptivity mediated by an impaired secretome of MP-stressed MSCs, which prevents proper implantation.
Conclusion: Microplastics represent a serious risk factor for reproductive system functioning due to their potential ability to induce cell stress and inflammation through the impairment of endometrial MSC activity. This leads to changes in the secretome composition, resulting in the failure to ensure successful embryo implantation. Both MP's presence per se and endocrine-disrupting properties of their leached compounds appear as plausible mechanisms underlying adverse effects on embryo implantation.
Keywords: Microplastics, Endometrial Receptivity, Embryo Implantation, Mesenchymal Stem Cells, MSC Secretom, Inflammation

Ps-163: Integrated Single-Cell and Bulk RNA Sequencing Analysis Reveals Prognostic and Therapeutic Markers in Early Lung Adenocarcinoma

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Background: Lung adenocarcinoma (LUAD), primarily originating from alveolar type II (AT2) epithelial cells, is the most common subtype of non–small cell lung cancer and is frequently diagnosed at advanced stages. Identifying early diagnostic markers and therapeutic targets is therefore critical for improving patient outcomes.
Materials and Methods: In this study, we integrated single cell RNA sequencing (scRNA seq) and bulk RNA seq early LUAD datasets to investigate the molecular landscape of early LUAD. Twenty one scRNA seq samples (12 tumor and 9 normal) were analyzed to identify AT2 epithelial cells and infer copy number variation (CNV). Tumor AT2 cells were subsequently classified into High CNV malignant like and Low CNV intermediate populations. Differential expression and pathway enrichment analyses were performed, and candidate genes were further validated using TCGA LUAD bulk RNA seq data through expression comparison, survival analysis, diagnostic evaluation, protein–protein interaction network analysis, and druggability assessment.
Results: High CNV AT2 tumor cells exhibited pronounced transcriptomic dysregulation and stronger malignant characteristics compared with Low CNV cells. Integration with TCGA LUAD data identified 2,655 overlapping genes, including 28 genes significantly associated with patient survival. Several genes demonstrated strong diagnostic potential, among which CEACAM6, LY6E, and CTSD emerged as robust biomarkers for distinguishing tumor from normal lung tissue. Network and druggability analyses further prioritized 33 genes as potential therapeutic targets.
Conclusion: Overall, our integrative analysis reveals a malignant like AT2 cell population characterized by high CNV and identifies key diagnostic, prognostic, and druggable genes in early LUAD. These findings provide new insights into early tumor heterogeneity and highlight potential biomarkers and therapeutic targets for future experimental validation.
Keywords: Lung Adenocarcinoma, Genomic Instability, Therapeutic Targets, scRNA-seq, Biomarkers

Ps-164: Effects of Melatonin on Oxidative Stress and Histomorphological Alterations in Ovarian Tissue after Polystyrene Nanoplastics Exposure

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Background: Polystyrene nanoplastics have the potential to induce toxicity in ovarian tissue, which may disrupt reproductive health. Therefore, the objective of this study was to evaluate the effects of melatonin—an effective antioxidant—in alleviating the adverse impacts of polystyrene nanoplastics on the ovarian tissue and biochemical parameters of adult NMRI mice.
Materials and Methods: In the present study, twenty-four adult female NMRI mice were randomly assigned to four experimental groups (n=6 per group): a control group, polystyrene nanoplastics (100 mg/kg. orally daily), melatonin (10 mg/kg. daily i.p.), and polystyrene nanoplastics plus melatonin. At the end of the experimental period (28 consecutive days), plasma biochemical and hormonal indices were measured, and the left ovaries were processed for stereological evaluation. Statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test in SPSS, and $P < 0.05$ was considered statistically significant.
Results: Treatment with polystyrene nanoplastics resulted in a significant reduction in total serum antioxidant capacity (FRAP), concentrations of LH and FSH hormones, ovarian weight and volume, the volumes of the cortex and medulla, and the total number of different follicle types in mice compared to the control group ($P < 0.05$). Conversely, serum malondialde-

hyde (MDA) concentration significantly increased in this group ($P < 0.001$). In the simultaneous treatment group (melatonin + polystyrene nanoplastics), the adverse effects of polystyrene nanoplastics on the aforementioned parameters were mitigated.
Conclusion: Conclusion: In conclusion, this study highlights the significant ovarian toxicity of polystyrene nanoplastics and underscores the therapeutic potential of melatonin in mitigating these adverse effects. The primary mechanism appears to be the potent antioxidant and free radical scavenging activity of melatonin, which effectively reduces oxidative stress and protects ovarian function.
Keywords: Polystyrene Nanoplastics, Melatonin, Ovary, Mice

Ps-165: Protective Effects of Bone Marrow derived Mesenchymal Stem Cells on Renal Ischemia- Reperfusion Injury

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Background: Renal ischemia-reperfusion injury is a common clinical condition associated with high morbidity and mortality. Due to the limited efficacy of current therapies, stem cell–based treatments have emerged as a promising regenerative approach. This study investigated the protective effects of bone marrow-derived mesenchymal stem cells on renal ischemia-reperfusion injury in rats using SPECT imaging.
Materials and Methods: Three ischemia-reperfusion, sham and stem cell pre- treatment group have been designed for this purpose. The left renal pedicle was blocked for 50 minutes in the ischemia-reperfusion group immediately following right nephrectomy. All surgical procedures related to ischemia reperfusion induction were performed in the sham group after nephrectomy, but the left renal pedicle wasn't blocked. In the stem cell pretreatment group, the bone marrow-derived mesenchymal stem cells were transferred through the tail vein one hour prior to model induction. Blood and tissue samples of all groups were collected 24 hours and 1 week after induction. Also, in order to evaluate the renal absorption of technetium-DMSA radiopharmaceuticals, the imaging of all groups was performed by small animal SPECT.
Results: In this analysis it was found that a significant increase in creatinine and blood urea nitrogen was found after one day in the ischemia-reperfusion group ($P < 0.05$). Such changes were related to changes in the renal histopathology and oxidative stress indices. However, pretreatment with mesenchymal stem cells decreased the aforementioned indications significantly compared with the ischemia-reperfusion group ($P < 0.05$). A substantial increase in superoxide dismutase activity and a decrease in malondialdehyde in the pretreatment group with mesenchymal stem cells relative to the ischemia reperfusion group ($P < 0.05$) were observed one week after ischemia-reperfusion. This was correlated with increased radiopharmaceutical uptake in the stem cell transplant group as well as decreased histopathological damage. The results of this study indicated that pretransplantation of mesenchymal stem cells can reduce chronic renal damage caused by ischemia reperfusion.
Conclusion: Based on the findings, it seems these cells may exert their endocrine or paracrine protective effects by decreasing oxidative stress. This promises the possibility of using the renal protective effects of stem cells to prevent acute renal injury in clinical conditions with ischemia and reperfusion.
Keywords: Mesenchymal Stem Cells, Ischemia Renal Reperfusion, Oxidative Stress

Ps-166: Natural Compound-Integrated Electrospun Scaffolds as Advanced Biomaterials for Stem cell Delivery and Organ repair

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Background: Nanocomposite electrospun fibers containing bioactive molecules, which offer the ability to deliver the cells into the damaged organs, will help to achieve a high therapeutic effect and enable a wide range of manipulations in the field of reconstructive medicine research.
Materials and Methods: In this study, Alcoholic extract of blue algae afanizomenon flus-aqua (AFA) was used to improve the performance of electrospun polycaplactone (PCL) and gelatin (gel) scaffolds to enhance the survival and proliferation of human mesenchymal stem cells. For this purpose, polycaprolactone / gelatin nanocomposite porous scaffolds were prepared using electrospinning method. The constructs were characterized for structural, mechanical and biochemical properties. The nature of human mesenchymal stem cells was confirmed by bone differentiation and flow cytometric analysis. Four culture groups: Two groups without scaffold (to investigate the effect of extract alone) and two culture groups on scaffold (to investigate the synergistic effect of AFA extract on scaffold) were designed. Electron microscope images were used to examine cell attachment on designed scaffold. Cell viability and proliferation in each group were determined by MTT assay.
Results: The results showed that the biocompatibility and biodegradability of the scaffold with good structural properties such as shape uniformity, pore size and porosity. After 72 hours of culture, the rate of cell attachment and proliferation was significantly higher in the scaffolding groups than others ($P < 0.05$). In alcoholic extract groups, the rate of survival and cell proliferation after 72 hours of culture increased significantly ($P < 0.05$).
Conclusion: In term of the capability of natural nanocomposite scaffold and hMSCs in cytocompatibility analysis, this novel tissue-engineered construct could be suggested as a body substitute to repair injured organs and regenerative medicine application.
Keywords: Mesenchymal Stem Cells, Gelatin, Natural Compounds, Poly Caprolacton, Electrospinning

Ps-167: An Interpretable End-to-End Framework for 19-nt saRNA Design: Sequence Features, Distance-Aware Scoring, and Meta-Model Integration

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Background: The success of small-activating RNA (saRNA)-mediated gene activation depends on identifying 19-nt target sequences with high functional potential. However, reliable and scalable prioritization of effective candidates remains challenging despite the growing use of computational approaches.
Materials and Methods: We developed a framework that scans promoter regions using overlapping 19-mer windows, characterizes each candidate using sequence and distance-based features, and integrates these signals via a logistic model and meta-model. The final pipeline provides ranked Top K outputs and an HTML report. Performance is evaluated using 5 fold cross validated ROC AUC, alongside analysis of feature contributions and quality control (QC) rules.
Results: Sequence-only models (logistic regression and random forest) showed limited discrimination (mean ROC-AUC=0.45). Incorporating distance-to-TSS features significantly improved performance (AUC=0.72, P=0.0). The best accuracy was achieved by integrating sequence and distance features into a combined meta-model, reaching an ROC-AUC of 0.9136. Feature analysis identified significant nucleotide effects at specific positions (e.g., positions 4, 7, and 16) and established 'filter rules' based on GC content, repeat patterns, and CpG island overlap to reduce false positives. Together, these results indicate that the framework supports efficient, interpretable selection of high-potential 19-nt saRNA candidates, reducing screening time and synthesis costs.
Conclusion: The framework enables efficient, high-accuracy prioritization of 19-nt saRNA candidates with ranked outputs and an HTML report, reducing screening time and costs.
Keywords: saRNA Design, Interpretable Machine Learning, Promoter Scanning, Gene Activation, AI

Ps-168: Evaluating Viral Transduction Efficiency of Protamine Sulfate as A Safer Alternative to Polybrene in Induced Pluripotent Stem Cell (iPSC) Reprogramming

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Background: Efficient viral transduction is essential to generate induced pluripotent stem cells (iPSCs). Polybrene is a frequently used reagent facilitating viral entry into the cells through reducing the electrostatic repulsion between the viral particle and the cell membrane; nevertheless, in some cases the cytotoxic activity of polybrene was reported. Protamine sulfate is a polycationic peptide showing a better biocompatibility when compared to polybrene, and may be a safer candidate for improving transduction efficiency. Here, we analyzed transduction efficacy by protamine sulfate during iPSC generation and compared to the polybrene protocol.
Materials and Methods: Human dermal fibroblasts (HDFs) were transduced by polybrene and protamine sulfate. The experimental groups were designed based on different transduction treatments that involved time of viral transductions including: single transduction for 24 hours, single transduction for 48 hours and double transductions for 24 or 48 hours. Viral transduction was analyzed and compared using fluorescence microscopy and flow cytometry for different transduction groups.
Results: In regard to transduction efficiency, under various transduction condition groups (protamine sulfate and polybrene group), double transduction at both 24 hours and 48 hours were both superior to single transduction; simultaneously, 24 hours transduction was more effective than 48 h condition; on the other hand, protamine sulfate displayed more stable transduction capability than aged polybrene stock in our results.
Conclusion: Repeated exposure to viral particles may increase the probability of successful viral entry and improve the delivery of reprogramming factors. Protamine sulfate not only demonstrated transduction efficiency comparable to that of polybrene but also may serve as a stable alternative transduction enhancer in iPSC generation.
Keywords: Protamine Sulfate, Cytotoxicity, iPS Cells, Transduction Efficiency

Ps-169: Optimization of Cell Confluency and Replating Conditions during Induced Pluripotent Stem Cell (iPSC) Generation

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Background: Induced pluripotent stem cells (iPSCs) hold great promise in regenerative medicine, disease modeling and drug screening. However, the low reprogramming efficiency is still one of the most important drawbacks of iPSC generation, highlighting the importance of investigating the parameters that influence reprogramming efficiency and kinetics. In the present study, we evaluated the impact of seeding confluency and replating process on the efficiency of iPSC generation.
Materials and Methods: Human dermal fibroblasts (HDFs) were reprogrammed using a retroviral transduction system. Different cell seeding densities (20,000, 40,000, and 80,000) and replating conditions were evaluated for their effects on colony formation following reprogramming. Colony formation efficiency and colony morphology were analyzed throughout the reprogramming process.
Results: We observed that initial cell confluency and the replating procedure were major factors influencing reprogramming efficiency and kinetics. Reprogramming efficiency ranged from 0.008 to 0.1 depending on experimental conditions. Replating was important for the efficiency and kinetics: its absence delayed colony formation, while colony quality appeared to be improved. Moreover, colony appearance occurred over different days and was varied depending on whether cells were

replated and under what density on the conditions applied-between day 9 and day 11.
Conclusion: These findings indicate that optimization of cell confluency is critical to enhance reprogramming efficiency during iPSC generation, potentially improving the robustness of stem cell-based research and facilitating the advancement of iPSC applications.
Keywords: Somatic Cell Reprogramming, Cell Density, Induced Pluripotency, Fate Transition

Ps-170: Development of Chondroitin Sulfate-Spermine Nanoparticles for Gene Delivery to Chondrocytes

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Background: Despite extensive research, no effective osteoarthritis drug has yet been successfully translated into clinical practice due to the dense structure of cartilage and its specific physiology. Gene therapy offers a promising strategy for the safe and long-term delivery of therapeutic agents. Efficient intra-articular delivery requires carrier systems that protect nucleic acids from degradation and improve their retention within cartilage tissue. In this study, chondroitin sulfate (CHS)-spermine (SPM) nanoparticles were developed for efficient gene delivery to chondrocytes isolated from osteoarthritic cartilage. These nanocarriers were designed to deliver plasmid-based gene cargo to target cells and improve their therapeutic function in osteoarthritis treatment.
Materials and Methods: CHS-SPM nanoparticles were synthesized by ionic gelation, where chondroitin sulfate solution was added dropwise to spermine solution under vigorous stirring at different ratios to optimize nanoparticle properties. For plasmid-loaded nanoparticles, the CAG-GFP plasmid was mixed with chondroitin sulfate before addition to spermine. Field emission- scanning electron microscopy (FE-SEM) was used to examine nanoparticle morphology, while DLS and zeta potential analyses evaluated particle size and surface charge. Gel retardation analysis confirmed plasmid loading into the nanoparticles. Encapsulation efficiency was determined by measuring free plasmid concentration before and after nanoparticle formation.
Results: FE-SEM analysis demonstrated that the nanoparticles exhibited a spherical morphology with a uniform distribution. Based on the DLS and zeta potential data, the average particle sizes of CHS-SPM and CHS-SPM-plasmid nanoparticles were 461.8 nm and 260 nm, respectively, indicating a reduction in particle size following plasmid incorporation. In addition, the surface charges of CHS-SPM and CHS-SPM-plasmid nanoparticles were -9.14 and -16.2 mV, respectively. The gene-protection capability of the nanoparticles was additionally validated through the gel retardation assay, where the nanoparticle-bound plasmid remained confined within the gel wells, confirming successful plasmid encapsulation and binding. Based on the obtained concentration measurements, 93% of the free plasmid was successfully loaded into the nanoparticles.
Conclusion: The nanoparticles were successfully synthesized and demonstrated efficient plasmid encapsulation. In addition, the particle size and surface charge were found to be appropriate for gene delivery to chondrocyte cells.
Keywords: Osteoarthritis, Chondroitin Sulfate, Spermine, Nanocarrier

Authors Index

L

M

N

O

P

R

S