25th Congress on Reproductive Biomedicine

20th Congress on Stem Cell Biology & Technology

28-30 August 2024

Tehran - Iran

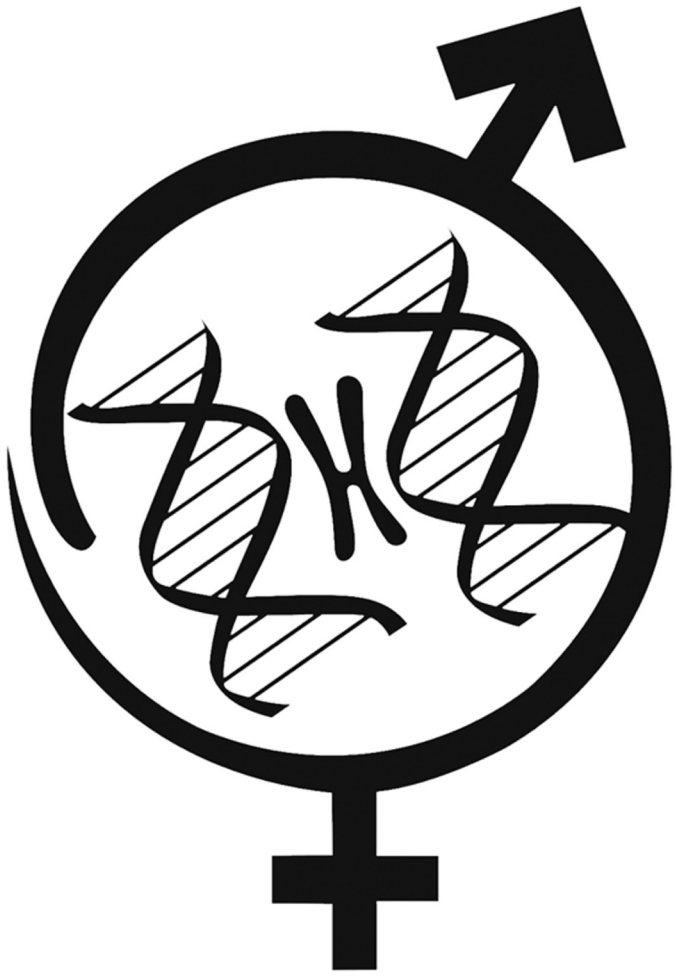
ABSTRACT BOOK

Abstracts of

Royan International Hybrid Twin Congress

25th Congress on Reproductive Biomedicine
28-30 August 2024

19th Seminar on Nursing and Midwifery
28-29 August 2024



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Abstracts of the
25th Hybrid Congress on Reproductive Biomedicine
28-30 Augugst 2024

18th Seminar on Nursing and Midwifery
28-29 August

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Maryam Hafezi

Dear Colleagues,

On behalf of the Organizing Committee, it is my pleasure to invite you to **25th Royan International Congress on Reproductive Biomedicine (28-30 August 2024), Tehran, Iran**.

In **2023**, we were delighted to welcome more than 1500 participants who joined our **24th congress** from more than 14 different countries.

Once again, in **2024**, the Royan International Congress will be officially held in a **"hybrid format"** which combines "in-person" with "online" sessions. However, we will be looking forward to hold the event, this year as **in-person** as possible to be able to prepare for meeting up with the pioneers and well known researchers of the field, **face to face**, to discuss the latest scientific updates in **reproductive health**.

The scientific committee seeks out international experts to hold a comprehensive and useful program, including the male and female infertility, clinical embryology and reproductive genetics. The program consists of state-of-the-art lectures, debates, and oral/poster presentations on issues of interest from the infertility field to facilitate the use of novel methods to better understand the basic underpinnings of the ART and ascertain the best practices for clinical management.

The **25th Royan Congress (28-30 August 2024)** will be guided by the motto **"Let Our Hopes Shape the Future"**.

We are eager to meet you whether in person or on-line soon in the city of thoughts; **Tehran**!

Best regards,
Maryam Hafezi, MD, PhD
Chairperson
25th ROYAN International Congress on Reproductive Biomedicine (2024)

Abstracts of

Royan International Hybrid Twin Congress

25th Congress on Reproductive Biomedicine

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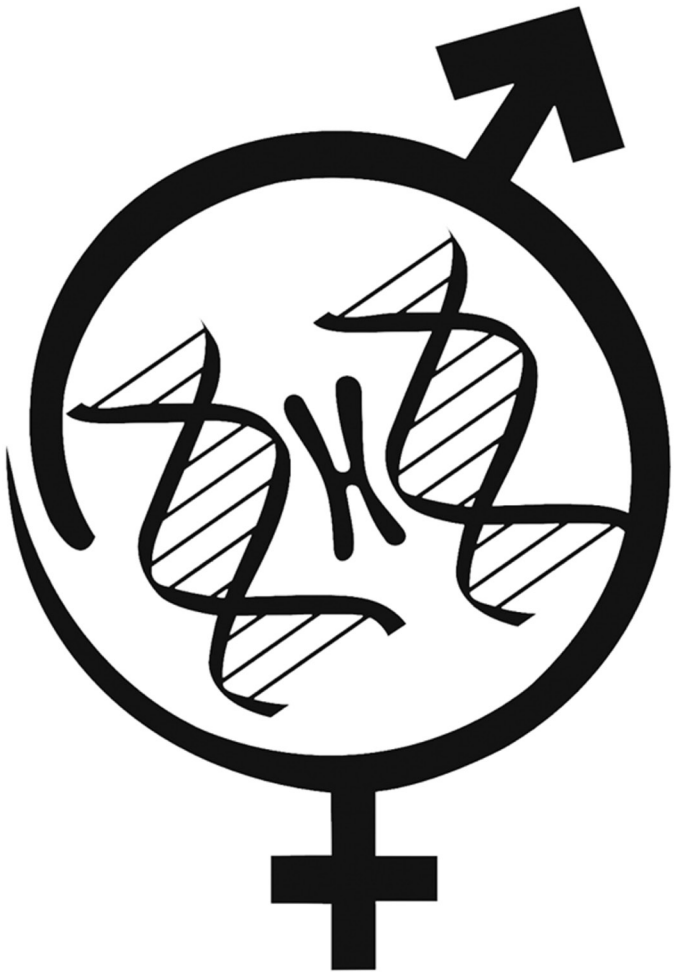

Royan Institute

Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

Invited Speakers

Andrology

I-1: Reassessing Recurrent Implantation Failure Challenges: Unraveling the Misunderstood Phenomenon in Human Fertility

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Despite numerous publications regarding recurrent implantation failure (RIF), there is as yet no universally accepted definition and the number of embryo and the stage of embryo (Day3 or Day5) that one may transfer before labeling a couple with the definition of RIF remains controversial. With the advent of preimplantation genetic testing for aneuploidy (PGT-A) a new horizon has occurred for defining RIF. Based on recent PGT-A analysis result of three successive euploid single embryo transfer, the rate of true recurrent implantation failure is reported to be much lower that previously reported. Therefore, during this presentation we will expand on true rate of recurrent implantation failure and whether there is room for other adjunct treatment or approaches like hysteroscopy or endometrial scratching, immune-therapy and…

I-2: From Noncommunicable Diseases to Male Infertility: Western Diet Patterns

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Noncommunicable diseases (NCDs) account for 74% of global deaths. Overweight and obesity are major risk factors for NCDs like hypertension, hyperlipidemia, type 2 diabetes, cardiovascular disease, metabolic syndrome, high cholesterol, and cancer. In 2022, 2.5 billion adults were overweight, with over 890 million obese. Overweight prevalence ranged from 31% in South-East Asia and Africa to 67% in the Americas. Globally, 16% of adults were obese in 2022, with obesity prevalence doubling from 1990 to 2022. Once an issue in high-income countries, overweight and obesity are now rising in low- and middle-income nations, due to increased consumption of Western diets, including sugar-sweetened, high-fat, AGE, and ultra-processed foods. These dietary changes, along with physical inactivity, contribute to obesity, diabetes, and NCDs.
Overweight and obesity may be linked to male infertility. Infertility is a global concern, with the World Health Organization citing prevalence rates of 3.5% to 16.7% in developed countries and 6.9% to 9.3% in less developed ones, affecting around 186 million people globally. Male infertility can result from abnormalities in semen parameters, such as sperm concentration, motility, and morphology. Dietary factors affect male reproductive function, as demonstrated by experimental and epidemiological studies. However, there is limited evidence on how adherence to various dietary patterns affects semen parameters.
Sugar has become a staple in the Western diet, rising from 15 grams per day at the start of the 20th century to 94 grams per day by the early 21st century. In the U.S., ultra-processed foods now make up 56% of the diet, with 62% of the sugar consumed coming from these foods. Added sugar (i.e., any fructose-containing sweetener; sucrose, high-fructose corn syrup, maple syrup, honey, agave) is present in 74% of items in American grocery stores, as the food industry adds it to boost sales. Globally, sugar consumption tripled from 1960 to 2010, while the world population doubled, highlighting a significant rise in added sugar intake coinciding with the rise of NCDs.
Despite having the same caloric content (4.1 kcal/g), fructose and glucose are metabolized differently. Glucose is essential for energy, and the liver will produce it from amino acids and fatty acids if dietary intake is insufficient (gluconeogenesis). In contrast, fructose, while a source of energy, has no critical biochemical role in eukaryotes. Excessive fructose surpasses the liver's metabolic capacity, leading to fat accumulation, insulin resistance, and NCDs. Physiologically, chronic fructose intake promotes fasting hyperinsulinemia and hypertriglyceridemia, disrupts leptin function, and does not suppress ghrelin, fostering overeating and addiction-like behaviors, unlike glucose, which does not have these effects.
Conclusions: Fructose, a physiological nutrient, can have detrimental metabolic effects when consumed in excess, particularly affecting the liver through hepatic insulin resistance and fat accumulation. Moreover, concerns exist that exogenous or endogenously produced fructose may be metabolized in extrasplanchnic cells, potentially prompted by specific physiological signals or pathological conditions, revealing previously unrecognized functions. Therefore, investigating the roles of exogenous and endogenous fructose in the pathogenesis of NCDs and male infertility can open important novel research perspectives.
Keywords: Noncommunicable Diseases, Male Infertility, Fructose, Insulin Resistance, Hypertriglyceridemia

I-3: Microbiome in the Male Genital Tract

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This presentation investigates the intricate relationship between the microbiome and male reproductive health, with a specific focus on the microbiome-testis axis and its implications for male infertility. The microbiome, which comprises diverse microbial communities within the human body, includes approximately 3.9×10^{13} bacteria—excluding archaea, viruses, fungi, and other eukaryotes—surpassing the estimated 30 trillion human cells. This complex microbial ecosystem plays a pivotal role in various physiological processes. Notably, the genetic repertoire of the microbiome encompasses over 3 million genes, significantly exceeding the 23,000 genes found in the human genome. Advanced next-generation sequencing (NGS) technol-

ogies, such as shotgun metagenomic sequencing, have greatly enhanced our ability to detect fungi, parasites, and DNA viruses with superior resolution and sensitivity compared to traditional amplicon sequencing techniques. Recent research highlights the significant variability in the human metagenome, with only a limited number of genes shared across individuals. The gut microbiome, in particular, has been implicated in modulating immune responses, metabolic functions, and hormonal regulation, all of which are critical for maintaining testicular function and spermatogenesis.
This presentation provides a comprehensive overview of both historical and contemporary methodologies for microbiome analysis, emphasizing the advancements achieved through high-throughput sequencing technologies. Particular attention is given to the role of gut dysbiosis—disruptions in the balance of the microbiome—in inducing systemic inflammation, oxidative stress, and endocrine dysfunctions, which can detrimentally affect sperm quality and overall fertility. The impact of the gut microbiome on sex hormone levels, energy supply for sperm cells, and the integrity of the blood-testis barrier is examined in detail. Novel findings suggest that testicular tissue is not entirely sterile in the physiological sense, as differences in microbial communities have been observed between fertile men and those with azoospermia. The concept of a " Microbiome-Testis Axis & quot; is introduced, proposing that alterations in gut microbiota can directly influence testicular health. Furthermore, this presentation explores the potential of microbiome profiling as a diagnostic tool for male infertility and discusses emerging therapeutic strategies, including the application of probiotics, prebiotics, and fecal microbiota transplants, to restore and maintain reproductive health.

I-4: Autologous Platelet-Rich Plasma (PRP) and Stem Cell Therapy for Male Infertility

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Autologous Platelet-Rich Plasma (PRP) and stem cell therapy are emerging treatments for male infertility, particularly when traditional methods of assisted reproduction have failed. Both therapies are basically aimed at improving spermatogenesis and the quality of sperm by means of regenerative mechanisms PRP therapy involves the extraction of blood from a patient and its consequent processing to concentrate the platelets. Such concentrated plasma is rich in growth factors and cytokines, which have been reported by a few previous studies to play a very key role in tissue regeneration and repair. In cases of male infertility, PRP is believed that may promote improvement in sperm quality and function by promoting healing and blood flow to testicular tissue. Stem-cell therapy for male infertility focuses on the use of various stem cells for the restoration of the damaged testicular tissue to recover spermatogenesis. The types of stem cells under research include mesenchymal stem cells, spermatogonial stem cells, and induced pluripotent stem cells. Such cells may differentiate into sperm-producing cells or secrete factors that promote the process of spermatogenesis.
While both PRP and stem cell therapy offer innovative solutions for treating male infertility, especially in difficult-to-treat cases like severe oligozoospermia and azoospermia, their application remains relatively unexplored. So far, the influence of PRP on spermatogenesis has only been studied in experimental animals. Further research and clinical trials are needed to establish the efficacy and safety of these treatments in various patient populations.

Animal Biotechnology

I-5: Construction of a circRNA–lincRNA–lncRNA–miRNA–mRNA ceRNA Regulatory Network Identifies Genes and Pathways Linked to Goat Fertility

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There is growing interest in the genetic improvement of fertility traits in female goats. With high-throughput genotyping, single-cell RNA sequencing (scRNA-seq) is a powerful tool for measuring gene expression profiles. The primary objective was to investigate comparative transcriptome profiling of granulosa cells (GCs) of high- and low-fertility goats, using scRNA-seq.
Methods: Thirty samples from Ji'ning Gray goats (n = 15 for high fertility and n = 15 for low fertility) were retrieved from publicly available scRNA-seq data. Functional enrichment analysis and a literature mining approach were applied to explore modules and hub genes related to fertility. Then, interactions between types of RNAs identified were predicted, and the ceRNA regulatory network was constructed by integrating these interactions with other gene regulatory networks (GRNs).
Results and discussion: Comparative transcriptomics-related analyses identified 150 differentially expressed genes (DEGs) between high- and low-fertility groups, based on the fold change (≥ 5 and ≤ -5) and false discovery rate (FDR <0.05). Among these genes, 80 were upregulated and 70 were downregulated. In addition, 81 mRNAs, 58 circRNAs, 8 lincRNAs, 19 lncRNAs, and 55 miRNAs were identified by literature mining. Furthermore, we identified 18 hub genes (SMAD1, SMAD2, SMAD3, SMAD4, TIMP1, ERBB2, BMP15, TGFB1, MAPK3, CTNNB1, BMPR2, AMHR2, TGFBR2, BMP4, ESR1, BMPR1B, AR, and TGFB2) involved in goat fertility. Identified biological networks and modules were mainly associated with ovary signature pathways. In addition, KEGG enrichment analysis identified regulating pluripotency of stem cells, cytokine–cytokine receptor interactions, ovarian steroidogenesis, oocyte meiosis, progesterone-mediated oocyte maturation, parathyroid and growth hormone synthesis, cortisol synthesis and secretion, and signaling pathways for prolactin, TGF-beta, Hippo, MAPK, PI3K-Akt, and FoxO. Functional annotation of identified DEGs implicated important biological pathways. These findings provided insights into the genetic basis of fertility in female goats and are an impetus to elucidate molecular ceRNA regulatory networks and functions of DEGs underlying ovarian follicular development.

I-6: Exploring Hub Genes in Bovine Mastitis: Insight from Multi-Omics and Network Analysis

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Bovine mastitis is a common and costly disease which has a considerable effect on the profitability of the production system owing to negative impacts on milk yield and quality, reproductive performance, early culling, animal welfare, and cost of treatment. Specifically designed multi-omics studies can be used to prioritize candidate genes and identify biomarkers and the molecular mechanisms underlying mastitis in dairy cattle. Hence, the present study aimed to explore the genetic basis of bovine mastitis by integrating microarray and RNA-Seq data containing healthy and mastitic samples in comparative transcriptome analysis with the results of published genome-wide association studies (GWAS) using literature mining approach. The Integration of different information sources resulted in the identification of common 33 relevant genes associated with bovine mastitis. Among them, 7 genes such as CXCR1, HCK, IL1RN, MMP9, S100A9, GRO1, and SOCS3 were identified as the hub genes (highly connected genes) for mastitis susceptibility and resistance, which were subjected to protein-protein interactions (PPI) network and gene regulatory network construction. Gene ontology annotation and enrichment analysis revealed 23, 7, and 4 GO terms related to mastitis in the biological process, molecular function, and cellular component categories, respectively. Moreover, the main metabolic-signalling pathways responsible for the regulation of immune response or inflammatory were significantly enriched in cytokine-cytokine receptor interaction, IL-17 signaling pathway, viral protein interaction with cytokine and cytokine receptor, and chemokine signaling pathway. Consequently, the identification of these genes, pathways, and their respective functions could contribute to a better understanding of the genetic and mechanisms regulating mastitis and can be considered a starting point for future studies on bovine mastitis.

Keywords: mastitis; transcriptome analysis; hub genes; multi-omics data; regulatory networks; bovine

I-7: The interplay between early embryo metabolism and mitoepigenetic programming of development"

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In the field of animal reproduction, the environment associated with gametes and embryos refers to the parent's condition as well as conditions surrounding gametes and embryo in vivo or in vitro. This environment is now known to influence not only the functionality of the early embryo but potentially the future phenotype of the offspring. Using transcriptomic and epigenetic molecular analysis, and the bovine model, recent research has shown that both the female and the male metabolic status, for example age, can affect gene expression and gene programming in the embryo. Evidence demonstrates that milking cows losing weight at the time of conception generates compromised embryos and offspring's with a unique metabolic signature. A similar phenomenon has been associated with different culture conditions and the IVF procedure. The general common consequence of these situations is an embryo behaving as "economy" mode where translation, cell division and ATP production is reduced, potentially to adapt to the perceived future environment. New epidemiologic studies show that these changes results in a different phenotype especially with animal created by IVF which shows consequences later in life.

Embryology

I-8: Advances in Developing a Transplantable Engineered Ovary

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Ovarian tissue engineering presents promising advancements in fertility preservation, particularly for prepubertal girls and women requiring immediate cancer treatment. A significant challenge remains for patients with a high risk of ovarian metastasis, where traditionaltransplantation poses substantial risks. Emerging strategies include the in vitro culture of preantral follicles, purging of cancer cells from ovarian tissue, and autotransplantation of isolated preantral follicles. This presentation introduces the concept of a transplantable engineered ovary (TEO) and explores methodologies for reintroducing isolated follicles into patients safely. Key findings include successful protocols for isolating and culturing ovarian cells, and the differentiation of these cells into functional theca cells. Our group has also evaluated various 3D matrices, such as fibrin and PEGylated fibrin, for their capacity to support follicular survival and development. PEGylated fibrin, in particular, has shown promise in mimicking the biomechanical properties of the ovarian extracellular matrix, crucial for follicle viability and growth. We have applied reverse bioengineering techniques to tailor a biomimetic fibrin-based matrix, replicating the elasticity and bioactive properties of ovarian tissue from women at reproductive age. Our results demonstrate that these matrices support the survival and proliferation of granulosa cells, maintaining critical cell-cell communication essential for follicular development. Our findings suggest that transplantable artificial ovaries could restore ovarian function and enable natural conception, providing a significant breakthrough for patients at high risk of ovarian metastasis.

I-9: Optimization of Decellularized of Human Placental Macroporous Scaffolds for Proliferation and differentiation of Spermatogonial Stem Cells

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Background: there is still no suitable treatment for infertility in premature boys with cancer who have lost their spermatogonia. The proliferation and differentiation of neonatal mouse sper-

matogonia cells in the human placental detoxification matrix will be investigated.
Materials and Methods: Human placenta was obtained from cesarean section mothers and treated with different concentrations of triton and sodium dodecyl sulfate for 15 or 30 minutes. Removal of cells from tissues was determined by H&E and DAPI staining and DNA content analysis. The biocompatibility of spermatogonia cells on scaffolds was determined by MTT. Proliferation and differentiation of spermatogonia cells on the scaffold were examined using qRT-PCR for pre- meiotic genes (ID4 , Gfrα1), meiosis (Sycp3) and post-meiosis (Prm1 and acrosin). Flow cytometry for Gfrα1 and Prm1 were cultured after 14 and 35 days respectively.
Result: DAPI and H&E staining and DNA content assay showed that %0.5 SDS and SDS + Triton groups were completely decellularized. MTT test showed a decrease in cell viability in the %0.5 SDS group ($p \leq 0.05$). Gfrα1 gene expression was significantly increased in both two- dimensional and three-dimensional groups after 14 days of culture ($p \leq 0.05$). The three- dimensional group was significantly higher than the two-dimensional group ($p \leq 0.05$). The expression of acrosin and Prm1 genes also increased significantly in the three-dimensional group after 35 days and was higher in the three-dimensional group, which was confirmed by flow cytometry results ($p \leq 0.05$).
Conclusion: The human placenta decellularized scaffold can be a good environment for culturing spermatogonia and provide the way for further studies and advances in infertility.
Keywords: Spermatogonia Stem Cellsm, Extracellular Matrix, Decellularized Placental Tissue, Reproduction.

I-10: Advanced Bioengineering of Female and Male Germ Cells

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Germ cell development in vivo heavily depends on specific signals that regulate their growth, differentiation, and maturation. Correctly incorporating these signals in vitro—considering their timing and location—is essential. This requires precise control over the types and concentrations of growth factors, hormones, and other biochemical or biophysical cues within the extracellular matrix.
Advanced bioengineering techniques aim to create microenvironments that closely mimic natural conditions for female and male germ cells. By integrating essential signaling cues into these engineered environments, it is possible to support in vitro germ cell growth, improve understanding of their behavior, and explore therapeutic applications. Techniques such as additive manufacturing, nanofabrication, and micro-physiological systems enable the development of biomimetic 3D environments that replicate the conditions necessary for germ cell maturation. These advanced techniques allow for precise fine-tuning of signaling cues in a spatiotemporal manner, which is crucial for maintaining cell functionality and achieving successful differentiation and maturation of germ cells

I-11: The Role of Sperm in ART Success Rate

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Sperm quality plays a crucial role in the success of assisted reproductive technology (ART) outcomes. The integrity of sperm DNA, motility, morphology, and concentration are critical factors that may influence ART outcomes. Recent studies have emphasized the importance of sperm DNA fragmentation (SDF) levels, with higher SDF being associated with lower pregnancy rates and increased miscarriage rates. Comprehensive sperm evaluation is essential for optimizing ART outcomes and improving the chances of successful conception and live birth. In conclusion, the role of sperm in ART outcomes is multifaceted. By understanding the critical role of sperm in ART, reproductive specialists can enhance treatment protocols and support couples on their journey towards parenthood. Keywords: sperm DNA fragmentation, sperm parameters, clinical pregnancy, male factor infertility

I-12: Improved Testicular Organoid Development by Inhibition Of TGF-B Signaling Pathway

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Background: Testicular Organoids (TOs) have attracted great interest as a reliable experimental tissue model in reproductive biology for various studies such as toxicology, drug discovery, and regenerative medicine. The existing studies on generating TOs with testis-specific structure and function are rare and face many challenges. Our previous finding highlighted the fundamental role of transforming growth factor-beta (TGF-β) inhibition in the proliferation of mouse and human spermatogonia. Based on this finding and other evidences of critical role of TGF-β signaling in testis development, we hypothesized that the proliferative effect of TGF-β inhibition on the key cellular components involved in organoid development, would enhance the efficiency of TO development.
Materials and Methods: Testicular cells were isolated from prepubertal mice (14 days old) and encapsulated in a Matrigel-based hydrogel. On day 5 of culture, cells were exposed to two small molecule TGF-β inhibitors (TGF-βi) to reveal whether TGF-β inhibition affects TO formation capacity and functionality. The effect of TGF-β inhibition on TO development was evaluated by histological and hormonal assays and mRNA and protein expression analyses.
Results: The TGF-βi-derived TOs (TiTOs) showed a highly organized structure characterized by the presence of lumen-bearing tubular structures, with spatial expression patterns of tissue-specific markers, and superior steroidogenic activity recapitulating the compartmentalized architecture and physiological function of testicular tissue. The positive effects of TGF-β inhibition were attributed to the promoted cell proliferation, as

demonstrated by up-regulation of CDKs, down-regulation of proliferation inhibitor genes (CDKi) and upregulation of proliferation genes as well as increased number of Ddx4+/PCNA+ cells in the TiTOs.
Conclusion: We achieved well-organized TOs with seminiferous tubule-like structures and improved features resembling characteristics of testicular tissue. This new insight would initiate novel steps forward to develop more effective methods for generation of TO models. Moreover, the small molecule TGF-β inhibitors may find interest as promising drug candidates to address male infertility issues.
Keywords: Testis Development, Testicular Organoid, Transforming Growth Factor Beta Signaling Pathway, Small Molecule / Male Infertility

Ethics

I-13: Ethical Analysis of Providing Assisted Reproductive Technologies in Iran

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Assisted reproductive technologies (ART) in the Iranian context present complex ethical challenges, further intensified by the high prevalence of primary infertility in the region. This presentation will analyze ART services with a focus on the rights of children to know their genetic parents, the potential for discrimination, the right to access healthcare, and the best interests of the resulting child. It will also explore issues surrounding the commodification of human reproductive abilities and the risks of exploitation within the context of ART.
In addition, the presentation will critically examine policy-making in this field, emphasizing the need for adherence to procedural values such as participation, inclusion, accountability, and transparency. The discussion will advocate for clearer policies on contentious issues like sex selection, germ-line therapeutic editing, human enhancement, polygenic scores in reproduction, and the use of artificial intelligence for embryo selection.
By addressing the ethical dimensions and the high prevalence of infertility, this analysis seeks to contribute to responsible policy development and the protection of vulnerable individuals within the reproductive process in Iran.

I-14: Gender Dysphoria and Fertility Preservation as a Sexual and Reproductive Health Right

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Sexual health is fundamental to the overall health and well-being of individuals, couples and families, and to the social and economic development of communities and countries. Sexual health, when viewed affirmatively, requires a positive and respectful approach to sexuality and sexual relationships, as well as the possibility of having pleasurable and safe sexual experiences, free of coercion, discrimination and violence. The ability of men and women to achieve sexual health and well-being depends on their: Access to comprehensive, good-quality information about sex and sexuality; knowledge about the risks they may face and their vulnerability to adverse consequences of unprotected sexual activity; ability to access sexual health care; living in an environment that affirms and promotes sexual health (WHO). With a right-based view of sexual health, people known as transgender and gender diverse (TGD) or having gender dysphoria, like other people in society, have the right to experience childbearing.
Hormonal interventions and surgical procedures that are performed to manage gender dysphoria, often have serious effects on a person's fertility in the future. It is thus critical to discuss infertility risk and fertility preservation (FP) options with transgender individuals and their families prior to initiating any of these treatments and to continue these conversations on an ongoing basis thereafter and, of course, it is important that the possibility of benefiting from these services is also provided for them. Whereas the use of embryos, mature oocytes, and sperm have all proven to be efficacious when employed within clinical treatments, cryopreserved gonadal tissues would require either future retransplantation aimed at obtaining fully functional gametes or the application of laboratory methods for culture, which are still under development in basic science research settings (Practice Committee of the American Society for Reproductive Medicine, 2019).
Keywords: Sexual Health, Gender Dysphoria, Transgender and Gender Diverse, Fertility Preservation, Sexual and Reproductive Health Rights (SRHR)

Female Infertility

I-15: New Updates on Endometriosis

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I-16: HIV & Infertility

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HIV and fertility have been challenging issues worldwide. HIV can increase the risk of infertility through several factors. Since sperm and ovules do not harbor the HIV virus, their storage is not a concern, and disinfecting instruments is straightforward because the virus has a lipid envelope and is very sensitive to disinfectants. In Iran, we face significant ethical and legal challenges in this regard. Recent studies highlight the importance of maintaining an undetectable HIV viral load and the role of potent antiretrovirals in preventing HIV transmission via sexual

routes. Managing serodiscordant couples (where one partner is HIV-positive and the other is not) who wish to conceive has become less challenging in recent years. Options to prevent HIV acquisition in newborns include intrauterine insemination (IUI), pre-exposure prophylaxis (PrEP), and adherence to antiretroviral treatment. When the mother is HIV-positive and the partner is negative, intrauterine insemination and ensuring a sustained undetectable viral load are effective ways to prevent mother-to-child transmission of HIV. Recent studies suggest that maintaining an undetectable viral load alone is also highly effective. If the male partner is HIV-positive, pre-exposure prophylaxis alongside antiretrovirals is an effective method to prevent transmission. As mentioned, antiretroviral treatment alone can be an effective way to control transmission. This presentation will discuss all these aspects.

I-17: Possible effects of hepatitis B infection on infertility treatment outcomes

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Infertility is one of the most important health problems in world, where more than 100 million couples of reproductive age suffer from this condition. Various factors affect the functioning of the human reproductive system, the most important of which are: physical diseases, psychological state, smoking, drinking, environmental pollution, and viral infection. Hepatitis B virus is one of the most important infectious agents that threaten human health. According to the reports of the World Health Organization, there are more than three hundred million people infected with this virus in the world. In Iran, the prevalence of HBV infection in recent years is estimated to be less than 2%, about 1.9% in males and 1.5% in females.
There have been many studies on the effects of hepatitis B on male and female reproductive systems, and the results of these studies have been different and sometimes contradictory.
In this lecture, I will talk about the possible effects of hepatitis B on natural pregnancy outcomes, as well as infertility and the consequences of infertility treatment.
The answers to the following questions will be discussed in this lecture and panel by reviewing the literature:
- Is HBV infection:
- Associated with increased risk of female tubal infertility?
- Related to longer durations of infertility?
- Related to lower rates of implantation among patients undergoing in vitro fertilization treatment?

I-18: Embryological Aspects in the Management of Endometriosis or Adenomyosis (Oocyte, Embryo, and Endometrial Receptivity

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I-19: Different Strategies to Overcome RSA & RIF According to New Guidelines

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RIF describes the scenario in which the transfer of embryos considered to be viable has failed to result in a positive pregnancy test sufficiently often in a specific patient to warrant consideration of further investigations and/or interventions.
Focusing on couples that would be able to achieve a pregnancy through ART implies that a standardized range of investigations (the 'fertility workup') will have already been completed before the treatment process starts and that patients are deemed suitable for ART and for carrying a pregnancy.
Standard fertility workup in female and male patients that needs before RIF investigation including; Medical history, physical examination, pelvic 2D ultrasound for detection of structural abnormalities, where needed for additional imaging, assessment for ovulatory function through a menstrual calendar and laboratory testing, AMH or other ovarian reserve testing, semen analysis.
Applying the recommended definition of RIF in clinical practice is related to age and status of euploidy embryos.
If RIF is suspected in the couple, following investigation are recommended; re-assessment of lifestyle factor, endometrial thickness, assessment of APA and APS in case of risk factor.
Can be considered; Karyotyping (both parents), 3D Us hysteroscopy, endometrial function testing, chronic endometritis testing, assessment of thyroid function, progesterone levels (late follicular, mid-luteal)
Not recommended; Vit D testing, microbiome profiling, peripheral NK cell testing, Uterine NK cell testing, Uterine T lymphocytes assessment, Assessment of blood cytokine level, Assessment of HLA-C compatibility, assessment of mDNA content, Sperm DNA fragmentation/Fish analysis.

I-20: Rejuvenation in the Ovary

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The use of PRP in reconstructive medicine has been one of the interesting topics in the treatment of patients with thin endometrium, recurrent implantation failure and poor responders and spermotogenesis. The aim of this article is to review the studies conducted so far on this topic in Hamadan Iran.
In the last research, we compared the results of combined injection of PRP with gonadotropin (150IU FSH &75IU LH) and its comparison with PRP alone.
Keywords: Marzie Farimani, Roghayeh Anvari Aliabad, Mohadese Moeini

I-21: Adenomyosis and Endometriosis: New Insights on The Pathogenesis and Treatments

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Endometriosis and adenomyosis are chronic conditions characterised by endometrial-like tissue outside the uterus and within the myometrium, respectively. These conditions result in debilitating symptoms such as pelvic pain, dysmenorrhea, dyspareunia, and infertility, severely impacting patients' quality of life. While surgical intervention has traditionally been a cornerstone of management, hormonal therapies play a crucial role in symptom control and disease management. First-line conventional hormonal treatments include combined oral contraceptives and progestins (including medroxyprogesterone acetate, norethindrone acetate, and dienogest). Second line-therapies include gonadotropin-releasing hormone (GnRH) agonists (such as leuprolide acetate and goserelin acetate), which suppress ovarian function by desensitising GnRH receptors, leading to hypoestrogenism and amenorrhea. While effective in relieving symptoms, their long-term use is limited by hypoestrogenic side effects such as hot flashes, vaginal dryness, and bone mineral density loss. Recently, GnRH antagonists have been introduced in the treatment of endometriosis and adenomyosis. GnRH antagonists (such as elagolix) inhibit pituitary GnRH receptors, rapidly suppressing gonadotropin secretion and ovarian estrogen production. Unlike GnRH agonists, they cause less initial hormone flare and offer the flexibility of dose adjustment, potentially minimising side effects. Aromatase inhibitors such as letrozole and anastrozole block the conversion of androgens to estrogens, reducing local estrogen production within endometriotic lesions. While primarily used in assisted reproductive technologies, their role in the long-term management of endometriosis and adenomyosis is under investigation. Biologic agents targeting pro-inflammatory cytokines and angiogenic factors implicated in the pathogenesis of endometriosis and adenomyosis are being explored as potential therapeutic options. These include anti-TNF-α agents, anti- VEGF antibodies, and immunomodulatory agents. In conclusion, hormonal therapy remains a cornerstone in the management of endometriosis and adenomyosis, offering symptomatic relief and disease control. Recent advancements in hormonal treatment options, including GnRH antagonists, aromatase inhibitors, and biologic therapies, hold promise for improving outcomes and addressing the unmet needs of patients with these debilitating conditions. However, further research is needed to optimise treatment strategies, minimise side effects, and improve long-term outcomes.

I-22: Endometrial PRP

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In vitro fertilization (IVF) and embryo transfer are the most effective assisted reproductive technologies (ART) for treating infertility . Successful embryo implantation depends on perfect connection between embryonic development and good endometrial receptivity. Therefore, increasing endometrial receptivity has become a important concern of reproduction research. The major indicator of endometrial receptivity is endometrial thickness, and a successful pregnancy greatly depends on an adequate endometrial thickness. Thin endometrium occurs in 1.5%–9.1% of cases, active involvement of local immune cells at the implantation site impairs endometrium receptivity. various immunomodulatory therapies have been investigated. PRP is typically obtained through a blood draw and processed to concentrate into the uterus. The treatment is often administered before embryo transfer or at times that align with preparatory phases for IVF. Studies suggest promising outcomes, but more research is needed to establish standardized protocols and long term efficacy.

I-23: Advanced Methods to Select Embryos for Transfer

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I-24: How to Standardize PRP and International Protocol

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I-25: Intraovarian Injection of Platelet-Rich Plasma (PRP) in the treatment of Patients with Poor Ovarian Reserve and Poor Ovarian insufficiency

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Results of meta-analysis showed that after PRP treatment there was a significant improvement in ovarian hormone levels and ovarian reserve markers, and also significant improvements in hormonal profiles and pregnancy outcomes.
Results indicated that PRP treatment led to a significant increase in AMH level, improved follicular development, and higher rates of oocyte retrieval and embryo quality in patients with poor ovarian reserve (POR).
The treatment protocol involved multi-point injections of PRP into bilateral ovaries, with different preparation methods and concentrations which led
to different outcomes. While PRP is generally considered safe, there are potential but negligible complications for intra-ovarian injections including bleeding, infection, and injury to surrounding structures.
There are some concerns regarding microbial growth in PRP samples and the potentially harmful effects of high concentrations of hematopoietic cells on embryos, therefore its application should be with caution in clinical practice.
There is a need for conducting well-organized, randomized controlled trials to validate the efficacy and safety of PRP, and also standardize treatment protocols.
Further research is needed to explore the mechanism action of PRP in the ovary, its effects on the Hippo pathway, and its optimal concentration.

I-26: Interaction Between Endometriosis and Bacteria in Infertile Women in Iran

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Background: Endometriosis is a common disorder that affects 20-50% of infertile women. Disease correlates with the loading of lactobacilli and changes in the number of gram negative and gram-positive bacteria. Objectives: This article aims to investigate the interaction between endometriosis and some bacteria
Materials and Methods: One hundred women between 18 and 40 years of age referred to the IVF department of Arash Women's Hospital in Tehran were studied. Fifty of them were diagnosed with endometriosis and the rest were referred for investigation or freezing of their gametes or embryos. Specimens were collected from endometrial tissue and cervix using swab. They were used for cultures and real time PCR to quantify Lactobacillus.
Results: Seventeen different gram positive and gram negative bacteria and 3 yeasts were isolated from women with and without endometriosis. The highest prevalence was related to Enterococcus faecalis, followed by Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, E. faecium, Proteus mirabilis, Edwardsiella tarda and Citrobacter spp. A significant relationship was identified between the increase of Enterococcus spp, members of Enterobacteriaceae family and the decrease in the number of Lactobacillus in endometriosis ($P<0.05$), which is consistent with previous studies. Staphylococcus aureus was isolated from the cervix of 3 women with endometriosis. Cervical and endometrial bacteria were very similar.

I-27: Evaluating Intervention in Infertile Women

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I-28: HPV and Infertility

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HPV (Human Papilloma Viruse) is a non envelope DNA virus. More than 100 types of HPV have identified. About 30-40 types are anogenital ones .It is the most common sexually transmitted infection in the world. Most often it is asymptomatic .But it can manifest as genital warts or cancers. Genital warts is a disease of young people . High risk HPV 16 and 18 types account for majority of worldwide cervical cancers and low risk HPV 6 and 11 are most often associated with external anogenital warts. These two types are responsible for >90% of genital warts. 70% of new HPV infections spontaneously clear within one year, and as many as 91% clear within 2 years. Major Routes of HPV Transmission is sexual.
There are many articles about correlation between HPV infection and infertility. HPV seems to affect both men and women. The virus can bind to the head of a spermatozoon and reduce sperm motility in men and may reduce the endometrial implantation of trophoblastic cells in women. Some studies show that HPV infection can effect on sperm count and motility and decrease count of sperm cell and decrease motility capability of these cells. Several studies show a decreased pregnancy rate for intrauterine insemination and in vitro fertilization in women with HPV compared to controls, while other studies show no correlation. HPV has also been linked with preterm rupture of membranes, spontaneous preterm birth, and a potentially increased rate of early pregnancy loss. Nowadays, 2,4 and 9 valents HPV vaccines are available. It recommended for both men and women at the age of 9-26 years old. However, it can use until the age of 45 in some situations. HPV vaccines are not recommended for use in pregnant women. If a woman is found to be pregnant after initiating the vaccination series, the remainder of the 3-dose series should be delayed until completion of pregnancy. If a vaccine dose has been administered during pregnancy, no intervention is needed.

Genetics

I-29: Autoimmune Disease-Related Hub Genes in Infertility

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Infertility is a multifactorial condition influenced by genetic, immunological, andenvironmental factors. Autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), and Multiple Sclerosis (MS) are known contributors to infertility through mechanisms such as chronic inflammation and autoantibody production. This presentation reviews published bioinformatics data to identify and characterize hub genes that are common between autoimmune diseases and infertility, specifically focusing on antisperm antibodies and endometriosis. We systematically reviewed bioinformatics studies that analyzed gene expression data from patients with autoimmune disorders (SLE, RA, MS) and infertility conditions (antisperm antibodies, endometriosis). Our aim was to identify key hub genes within the gene regulatory networks of these conditions, which serve as central nodes and have significant roles in disease pathogenesis. From our review, we identified several hub genes that are commonly dysregulated in both autoimmune diseases and infertility. STAT3, a gene involved in immune regulation and inflammation, was consistently highlighted as a critical hub in the reviewed studies. 1Similarly, FOXP3 and IL6, genes essential for immune homeostasis and inflammatory responses, were identified as significant contributors to both autoimmune conditions and infertility. These findings suggest that the identified hub genes play pivotal roles in the pathophysiology of both autoimmune diseases and infertility, interfering with key reproductive processes. By targeting these hub genes, novel diagnostic biomarkers and therapeutic interventions could be developed to modulate immune responses and improve reproductive outcomes for affected individuals.

I-30: Genomic Medicine and Emerging Technologies for A Better Understanding of RIF and RSA

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Infertility affects about 18% of reproductive-age couples in developed countries. Various factors, including genetic, immunological, endocrine, and anatomical abnormalities, impact a woman woman's ability to conceive. With more couples opting for in vitro fertilization (IVF), new infertility endophenotypes with presumed genetic causes have emerged. Advances in genomic research, especially whole exome sequencing, have enable the identification of novel genes whose pathogenic variants are linked to IVF infertility endophenotypes like maturation arrest, fertilization failure and Preimplantation embryonic lethality. Despite these advances, human reproduction remains inefficient, with many embryos failing to implant due to chromosomal aberrations such as aneuploidies, typically inherited from the female gamete. Even with preimplantation genetic testing for aneuploidies (PGT-A), some euploid embryos fail to succeed, as PGT cannot detect all genetic abnormalities. New approaches, such as extending embryo culture to day 14 and using whole genome sequencing and omics technologies, aim to uncover genetic causes of embryonic arrest and implantation failure. Genome editing tools also help study lethal genes identified through prenatal diagnosis in the preimplantation stage, elucidating pathways associated with successful implantation. These advancements provide insights into unexplained infertility, leading to better treatment strategies and personalized management. However, current studies mainly focus on prenatal and post-natal diagnosis, with preimplantation and IVF research facing limitations like small sample sizes, lack of ethnic diversity, and a focus on consanguineous mating scenarios. Addressing these limitations is crucial for a comprehensive understanding of infertility and for developing inclusive and robust diagnostic and treatment strategies.

I-31: Identification of Candidate Genes for Uterine Leiomyoma by Family-Based Exome Sequencing

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Background: Uterine leiomyomas (ULs), benign solid tumors arising from uterine myometrium, are the most common pelvic tumors among females of reproductive age. Despite the universal prevalence of ULs and its huge impact on women's lives, the exact etiology and pathophysiologic mechanisms have not been fully understood. Numerous studies indicate that genetic factors play a crucial role in ULs development.
The present study aim was identifying the genetic causes of ULs in a consanguineous Iranian family with a history of ULs
Materials and Methods: For this purpose, Whole-exome sequencing (WES) was performed on five members of a consanguineous Iranian family with ULs followed by bioinformatics analyses. Moreover, targeted Sanger sequencing was applied on 32 sporadic non-related patients with ULs. The pathogenicity along with structural and functional effects of the candidate variant were assessed by MutPred2 and HOPE servers. I-TASSER and UCSF Chimera were also used for modeling and visualizing the predicted variant, respectively.
Results: After analyzing the results of WES in this family, a likely pathogenic missense variant in DLX3 gene (c.263A>G; p.Y88C) identified on transactivation (TA) domain, co-segregated with the phenotype and consistent with autosomal dominant inheritance. All patients in this family were heterozygous for this mutation. The identified variant was not found in any of the 32 individuals. MutPred2 predicted the pathogenicity of this mutation by both phosphorylation and sulfation loss as actionable hypotheses. HOPE project revealed the identified variant residue is smaller and more hydrophobic comparing to the wild-type residue.
Conclusion: This is the earliest WES study reporting the first mutation in DLX3 gene associated with ULs pathogenicity in Iranian population highlighting the effectiveness of WES as a strong diagnostic method. However, further functional studies on this variant are needed to confirm the potential pathogenicity of this mutation. Further genetic studies can identify other genes related to ULs in order to shed more light on the etiology and improve the management of these patients. The discovered mutation was submitted to ClinVar under SCV002028354.
Keywords: Uterine leiomyoma/ Whole-exome sequencing/ Homeobox genes/ DLX3

I-32: A Simultaneous Next-Generation Sequencing Approach to The Diagnosis of Couple Infertility

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Over the past three decades, researchers have made significant efforts to recognize genes responsible for human infertility phenotypes. In recent years, an expanding number of genes associated with male and female infertility have been distinguished. The genetics of infertility is no longer limited to the analysis of karyotypes or particular genes, and it is now possible to analyze a few dozen infertility genes at the same time.
In Iranian community, we have a high rate of consanguinity (40-60%) and different genetic background. Therefore, highthrouput sequencing can be useful in better concept of infertility evaluation.
Next generation sequencing has been used for over a decade to detect underlying genetic causes of human disease and, more recently, to genetically diagnose the causes of male and female infertility. These new diagnostic tools have the potential to solve a significant proportion of idiopathic infertility.
Whole-exome sequencing (WES) analysis permits the identification of clinically significant DNA variants causing human diseases, including infertility. A significant number of genetic variations that cause disorders of sex development, oocyte maturation defects, fertilization failure, embryonic arrest, and preimplantation embryonic lethality show Mendelian inheritance patterns. Many important genes were described in hypothalamus - pituitary- gonadal axis and other apoptotic, angiogenesis, immunity related genes.

Understanding and prioritizing pathogenic DNA changes found in genes related to fertility, both in IVF patients and gamete donors would improve diagnostic and clinical management

of the patients. Also, these data can be used in pre-implantation genetic testing for polygenic disorder (PGT-P). This test selects different embryos across score construction methods with randomness for increasing the success rate of pregnancy. Furthermore, other new technical efforts, consisting of long read sequencing, whole genome sequencing and Trio- WES can be improved the outcome of pregnancy.

Imaging

I-33: Interventional Treatment of Endometriosis

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Interventional radiology shows promises in the field of women's health, particularly in pelvic interventions. This presentation discusses the latest advancements in interventional radiology techniques for pelvic conditions affecting women including adenomyosis, abdominal wall endometriosis and uterine leiomyoma. Extraperitoneal endometriosis involving the abdominal wall may be treated by percutaneous thermal ablation, such as cryoablation, whereas uterine leiomyoma and adenomyosis can be managed either using percutaneous thermal ablation or using uterine artery embolization. Continued research and development in interventional radiology will further enhance the minimally-invasive interventions available for women's health, improving outcomes and quality of life for this large patient population of women.

Oral Presentation

Andrology

O-1: Autophagy Modulation in 3D Cultured Rat Testicular Fragments Influenced The Spermatogenesis Capacity

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Background: Tissue engineering modalities are alternative approaches to restoring the function of the reproduction system using different techniques. This study aimed to address the possible effect of autophagy modulation on the dynamic growth of rat whole testicular cell culture incorporated within the alginate-gelatin hydrogel after 7 days .
Materials and Methods: Rat testicular fragment-embedded alginate-gelatin hydrogels were incubated in a DEME/F12 culture medium containing retinoic acid, testosterone, and FSH. To modulate the autophagy, 20 μM metformin and/or 15 μM hydroxychloroquine were added to the cell culture medium. Autophagy-related factors, Beclin-1, LC3, and P62, were monitored using western blotting. The survival rate was studied using the MTT. Morphological and histological analyses were performed using hematoxylin-Eosin staining and the number of $OCT3/4^+$ cells was studied using immunofluorescence staining. The activity of enzymes such as SOD, GPx, and levels of TAC were also monitored.
Results: Incorporation of testicular fragments increased the survival rate compared to the plastic surface group ($P<0.05$). Metformin stimulated the autophagy response coincided with the induction of Beclin-1, total LC3 and LC3-II/I ratio increase, and reduction of P62 compared to the hydroxychloroquine-treated group ($P<0.05$). Histological examination showed that autophagy stimulation/inhibition led to changes in the differentiation capacity of seminiferous tubule progenitors towards mature cell types such as spermatocytes and spermatids. A statistically significant reduction was found in the number of $OCT3/4^+$ spermatogonial stem cells in both groups that received metformin and hydroxychloroquine. Metformin increased the activity of SOD in cultured rat testicular fragments, indicating the activation of anti-oxidant mechanisms.
Conclusion: Taken together, autophagy modulation can affect spermatogenesis in rat testicular fragments embedded in an alginate-gelatin substrate. It seems that modulation of autophagy can be used as a suitable modality for affecting spermatogenesis in men with non-obstructive azoospermia.
Keywords: Rat Testicular Fragments, 3D Culture System, Alginate-Gelatin, Autophagy, Spermatogenesis

O-2: Sperm Production from Neonatal Mouse Testicular Tissue Using Plasma Rich in Growth Factors

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Background: spermatogenesis using Knockout serum replacement (KSR) does indeed have some limitations, including its ineffectiveness for all strains of mice and other species. Therefore, developing a suitable media for different strains and species is of high importance. This study investigates the potential use of plasma rich in growth factors (PRGF) as a serum substitute in the media for neonatal NMRI mouse testicular tissue.
Materials and Methods: Testicular tissue fragments were cultured using the gas-liquid interphase method on agarose gel for 42 days. The experimental group's media was composed of α-MEM enriched with 5% PRGF (optimal concentration), while the control group contained α-MEM with 10% KSR. The integrity of seminiferous tubules was assessed using a scoring system (1-4, worst to best). Immunofluorescence assays were performed using primary antibodies against PLZF, SYCP-3, and ACRBP to identify spermatogonial stem cells, spermatocytes, and sperm-like cells, respectively. Proliferation (Ki67), pro-apoptotic (Bax), and anti-apoptotic (Bcl-2) markers were also evaluated.
Results: After 42-day culture, tissues were mechanically dissociated and flagellated sperm was observed into suspension in 3 out of the 4 samples cultured with 5% PRGF. Histological examinations indicated differentiation of germ cells up to the elongated spermatid. The percentage of tubules with good and best preserved integrity scores (3-4) was significantly higher in the 5% PRGF compared to 10% KSR. Furthermore, the 5% PRGF media promoted a higher mean number of PLZF+, SYCP3+, ACRBP+, and Ki67+ cells per tubule, indicating enhanced spermatogenesis and cellular proliferation. The mean fluorescence intensity of Bax was significantly higher in the KSR group, while Bcl-2 was higher in the 5% PRGF group, although not statistically significant.
Conclusion: This study demonstrates that a media containing 5% PRGF can induce complete spermatogenesis in NMRI mice, offering a promising alternative to KSR-supplemented media.
Keywords: Testicular Organ Culture, PRGF, spermatogenesis

O-3: Hyaluronic Acid - Alginate Hydrogel for The Transdifferentiation of Testis Cells into Erythrocyte and Hepatocyte-Like Cells; A Practice Within An Effective Agent Choice

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Background: Spermatogonia stem cells (SSCs) exhibit pluripotency, enabling them to undergo differentiation into many cell lineages, including neurons, glia, endothelial cells, and hepatocytes when cultured . Although the specific mechanisms are not yet fully understood, it has been observed that biopolymer agents, such as hyaluronic acid (HA) and alginate (Alg), have the potential to induce transdifferentiation of SSCs. The current work aimed to examine the process of spermatogenesis and the conversion of mouse testicular cells into hepatocytes and erythrocyte-like cells utilizing the HA-Alg hydrogel.
Materials and Methods: After being extracted from the testes of a 5-day postpartum mouse (5 DPP), the testicular cells were separated into two enzymatic stages and then put into a composite hydrogel containing 0.5% HA and 1% alginate. On days 14 and 28 of culture, the colonies' growth, the cells' viability, and their histology were assessed.
Results: Despite observing significant cell proliferation on day 14 and the development of circular-shaped organoids on day 28, it was noted that the organoids generated in the HA-Alg medium tended to maintain their circular morphology on day 28. Notably, the testicular cells transformed into cell types resembling erythrocytes and hepatocytes. The hepatocyte-like cells exhibited the presence of glycogen and lipid deposits, indicating their hepatocyte-like characteristics. Interestingly, immunostaining analysis revealed the secretion of albumin and the presence of VEGFR on day 14. However, on day 28, albumin expression was not detected, while the expression of Sox9 (a marker for hepatocytes), Vegf, CD34, and C-kit (markers for erythrocytes) showed increased levels in the gene expression evaluation.
Conclusion: The present findings indicated that HA-Alg could be a potent and effective agent for the transdifferentiation of testis cells into erythrocyte and hepatocyte-like cells, as recent studies have confirmed the transformation of SSCs into hepatocyte cells during culture.
Keywords: 3D Culture, Hyaluronic Acid, Liver Organoids, Mouse Testicular Cell

Embryology

O-4: "Off-On" Signal Fluorescent Biosensor for Mir-20a-5p Detection as A Potential Non-Invasive Diagnostic Biomarker of Male Infertility

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Background: Male infertility is a heterogeneous disease that can occur due to spermatogenesis defects. Recently, alterations in miRNAs expression profile in semen have been linked to damaged spermatogenesis, suggesting miRNAs could be used as potential infertility biomarkers. The reliable identification of aberrant expression of miRNA is still a difficulty today, especially when using a quick, easy, and portable detection technique.
Materials and Methods: Our detection strategy was based on immobilizing dye-labeled single-stranded DNA (Cy5 dye-labeled-ssDNA) to Graphen Oxides (GO) that detect target miR-20a-5p. For this purpose, in the first step, adsorption of Cy5-labeled single-stranded DNA (ssDNA) on GO leads to fluorescence quenching of Cy5. Next, by adding its complementary DNA (cDNA), a double-stranded DNA (dsDNA) was formed, resulting in recovering the fluorescence of Cy5 by desorbing and releasing from GO
Results: Before/after the hybridization of miRNA-20a, the changes in fluorescence intensities were studied. The response of fluorescence emission intensities was observed to be linearly ranged with the increase of the miR-20a concentration from 10-9 to 3.2 × 10-6 M. The resulting fluorescence sensor showed a limit of the detection of 1.12×10−9 M.
Conclusion: The practical application value of the GO-based biosensor was confirmed by the detection of the miR-20a-5p biomarker, in clinical plasma samples, suggesting that the proposed sensing platform is promising for the early detection of non-invasive male infertility.
Keywords: Nano biosensor, MicroRNA, Male Infertility

O-5: Evaluation of Oocyte Maturation Genes in The Follicular Fluid of Endometriosis Patients Referred to Royan Institute

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Background: Endometriosis (EM) is an inflammatory disease characterized by endometrial tissue lesions outside the uterus. EM causes infertility and pelvic pain and has an adverse effect on ovarian physiology. Decreased oocyte quality is a possible mechanism in EM-related infertility. Since it is not possible to directly evaluate oocyte quality in patients with EM, so follicular fluid (FF) is used as an indirect method to evaluate oocyte quality, because the altered levels of molecules in FF are closely related to oocyte quality and follicular growth. Therefore, this study aims to investigate the expression of genes related to oocyte quality and maturation including growth differentiation factor 9 (*GDF9*), bone morphogenetic protein 15 (*BMP15*), and interleukin-6 (*IL-6*) in FF in women with EM.
Materials and Methods: FF was obtained from 10 women with moderate to severe EM (EM group) and 10 women without EM (control group). In addition, no significant difference was observed in factors such as age, body mass index (BMI), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and anti-mullerian hormone (AMH) levels that affect oocyte quality. Then, the expression level of *GDF9*, *BMP15*, and *IL-6* genes in FF was investigated by qRT-PCR
Results: No significant difference was observed in the expression of GDF9 (P=0.2246) and BMP15 (P=0.7959) between the control and the EM group. However, *IL-6* showed a significant increase in the expression in EM compared to the control group (P= 0.0185).
Conclusion: High expression of *IL-6* genes in EM patients may be related to the inflammatory processes and makes it a good biomarker for the diagnosis of EM-related infertility. It seems that there is a need to confirm this data with more patient samples to conclude whether there is a difference between EM

patients compared to healthy control women in terms of gene expression of *GDF9*, and *BMP15*.
Keywords: Endometriosis, Follicular fluid, GDF9, BMP15, IL-6

O-6: Predicting The Ploidy Status of Blastocysts: Potential Application of Blastocoel Fluid Gel Electrophoresis Band Intensity in Embryo Selection

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Background: Selecting embryos for transfer is essential in assisted reproduction. It seems that the presence of blastocoel fluid DNA (BF-DNA) in the cavity of preimplantation human blastocysts occurs as a response to common preimplantation chromosomal abnormalities
Materials and Methods: This correlational study included, 40 blastocysts that were classified into two categories based on patients' records for array comparative genomic hybridization (a-CGH) conducted following trophectoderm (TE) biopsy analysis: the Normal/Segmental/Survivable Aneuploidy (N/SA=14), comprised embryos with normal karyotypes or segmental/ single aneuploidies that are compatible with viability and the Other Aneuploidies (OA=26), consisted of embryos with double/multiple aneuploidies that ultimately lead to embryo demise. Biopsies of blastocoel fluid were undertaken for whole genome amplification (WGA), followed by observation of band intensity of the amplification product and quantification of BF-DNA levels.
Results: We observed that the band intensity of the amplification product was affected by the embryos' aneuploidy status ($P<0.05$). There was a correlation between the band intensity with the amount of BF-DNA and the complexity of aneuploidy ($P<0.001$ and $P<0.05$ respectively). Nonetheless, there was no statistically significant correlation between BF-DNA quantity and ploidy status. The amount of DNA was higher in the OA group compared to the N/SA group, this increase did not reach statistical significance ($P=0.2$).
Conclusion: Our findings show that observing the intensity of the bands on an agarose gel is potentially applicable to predict if embryos are healthy or not. This method is cheaper, more feasible and less invasive compared to trophectoderm biopsy and preimplantation genetic testing for aneuploidy (PGT-A), making it useful for deciding which embryos are more suitable for transfer, however for such embryo selection, like PGT-A, prenatal testing is recommended.
Keywords: Blastocoel Fluid, Aneuploidy, Whole Genome Amplification

O-7: Evaluation of The Effect of Lecithin and Nanolecithin in Repairing Membrane Damage, Maintaining Membrane Integrity and Improving Human Sperm Function in The Freeze-Thawing Process

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Background: Sperm cryopreservation is an effective method to preserve fertility in men. Treatments such as chemotherapy and radiotherapy as well as some surgeries may damage the reproductive organs and impair sperm function. Sperm cryopreservation can be a suitable solution for fertility preservation in these cases. However, sperm cryopreservation is associated with sperm damage. One of the most important factors that is affected by cryopreservation is sperm plasma membrane. According to animal and human studies, lecithin reduces sperm cryo-damage by replacing membrane lipids and coating it. In this study, lecithin and nano-lecithin are used to reduce membrane damage during sperm freeze-thaw process.
Materials and Methods: In the first phase, from three lecithin concentrations of 0.5, 1 and 2% and lecithin nanoparticles and particles made by sonicator with sizes of 50 to 100 nm, 100 to 200 nm and more than 200 nm, used to make sperm freezing medium (n=30). The size of nanoparticles was confirmed by dynamic light scattering and transmission electron microscopy. After thawing, sperm parameters including motility, viability, mitochondrial membrane potential (MMP), lipid peroxidation (MDA) and DNA fragmentation were evaluated. In the second phase, the amount of acrosomal reaction was evaluated by PSA-FITC in the group with the best and worst results in the first phase. Also, the attachment and interactions between lecithin nano-particles and spermatozoa membrane and the difference in attachment in the front and back of the head, were detected using Dil labelling in the group with the best and worst results in the first phase. Using field emission scanning electron microscope (FESEM), the surface structure of the sperm membrane and the lecithin binding sites in it, were investigated in the group with the best results in the first phase.
Results: The group with the size of 50-100 nm with a concentration of 1% showed a significant increase in viability compared to other groups after thawing, and the amount of DNA fragmentation and MDA in this group was significantly reduced. Motility in all groups had a significant decrease compared to before freezing, and lower concentrations and smaller particle sizes had better results than other groups. MMP was significantly decreased in all the groups compared to before freezing, and no significant differences were observed between different groups. Using lecithin nanoparticles with a size of 50-100 nm and a concentration of 1%, the acrosomal reaction showed a significant decrease compared to lecithin with a concentration of 2%. the investigation of DiI-labeled nanoparticles and the features of the plasma membrane determined with FESEM, the binding and influx of lecithin nanoparticles through the sperm membrane was observed. The binding was mostly on the head of sperm and in the post-acrosomal regions.
Conclusion: Lecithin nanoparticles, due to their small size and high surface-to-volume ratio can effectively bind to the sperm

membrane protecting it from damage during freeze-thawing process resulting in improved sperm viability.
Keywords: Sperm Cryopreservation, Lecithin, Nano-Lecithin, Cryoprotectant Agents, Plasma Membrane

O-8: Bovine Embryo Sexing Using Spent Embryo Culture Medium Fatty Acid Profiles

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Background: There is a difference in metabolism between male and female embryos before implantation and gonad development and it is due to the X and Y chromosomes and their gene expression. As the industrial world is moving towards the artificial selection, sexed-embryos for economic reasons, scientists are proposing to use non-invasive methods and finding biomarker(s) to achieve the goal. These differences can vary from consuming glucose to depletion and accumulation of specific amino acids in the SECM.
Materials and Methods: Bovine oocytes were aspirated, matured and fertilized in standard condition and cultured for 3 days, until cleavage stage. Then 19 hatched zygotes were transferred to single drops of culture media and incubated for 7 days. After blastocyst formation, embryos and their SECM were collected separately and frozen. Embryos were used for PCR and SECMs were used for detection of FA profiles.
Results: In the chromatogram of the culture medium the percentage of dihomo-gamma-linolenic acid (C20: 3n6) were highest in male SECM (0.3, 0.60 ± 0.28 and 14.21 ± 1.99 % for basal culture media sample, female SECM and male SECM, respectively; mean ± standard division) Deschuras index for C16 fatty acids increased in the culture medium of female embryos (0.10 vs. 0.18%). In the culture medium of female embryos, the desaturase index for C16 fatty acids increased (0.10 vs. 0.18%); this index for C18 fatty acid was almost unchanged in male and female cultures.
Conclusion: C20:3-n6 fatty acid in SECM seems to be a suitable biomarker for non-invasive sexing in male embryos of bovine embryos.
Keywords: Bovine, IVF Culture Medium, Lipidomics, Non-Invasive Methods, Sex Selection

Female

O-9: Prediction of IVF Success Based on Machine Learning Approaches: A Development and Validation Study

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Background: Fertilization (IVF), a breakthrough in infertility treatment, has a success rate of 30-40%, slightly higher than healthy couples' rates. However, it also has complications and high costs, posing burdens for infertile couples. The complexity of decision-making in medicine further complicates IVF adoption. To properly predict IVF success, machine learning (ML) can be used to create prediction models based on contributing factors. This study aimed to select the optimal predictive model for determining IVF success by conducting a comparison study of multiple classifiers.
Materials and Methods: The data of 812 couples undergoing IVF at Royesh clinics, Helal-e-Iran hospital were used in this study. Five well-known classifiers: Random Forest, Artificial Neural Network (ANN), Support Vector Machine (SVM), Recursive Partitioning and Regression Trees (RPART), and AdaBoost were compared to select the most robust predictive model. Furthermore, Genetic Algorithm was applied as the feature selection method. The feature selection was carried out applying Genetic Algorithm (GA).
Results: Adaboost and Random Forest outperform the other classifiers, with the areas under the ROC curve (AUC) of 89.80 and 87.40%, respectively. Also, ten features were identified as the most common contributing factors to IVF success.
Conclusion: The study highlights the effectiveness of ensemble learning methods like AdaBoost and Random Forest in predicting IVF outcomes. These models can provide a promising tool for IVF practitioners, allowing for more exact treatment planning.
Keywords: Fertilization, Assisted Reproductive Techniques, Machine Learning, Predictive Model, Clinical Decision-Making

O-10: Intrauterine Insemination before Transfer of Frozen-Thawed Embryo in Women Over 40 Years Old with Recurrent Implantation Failure: A Randomized Prospective Study

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Background: To evaluate whether a combination of IUI and frozen-thawed embryo transfer (FT-ET) with mild stimulation would improve the pregnancy rate (PR)in women over 40 with RIF, considering these women did not have any chances to repeat ivf process , if an egg released with mild stimulation , they don't miss their chance for a natural pregnancy.
Materials and Methods: 95 women (over 40) with RIF were assigned into two groups. The study group was composed of 45 women who received mild stimulation followed by IUI and FT-ET. The control group was composed of 50 women who received mild ovulation followed by FT-ET.
Results: There were No statistically significant difference between the groups for the grading of thawed embryos, mother age and number of embryos transferred. We excluded the wom-

en over 44 and male factor cases from our study. In the study group, the PR per ET were 24.44% (11 of 45). In the control group, PR per ET were 16% (8 of 5o). we found no significant difference between two groups, but our study groups have higher pregnancy rate per ET, maybe greater number of analysis increase the chances that a significant difference will be found among groups.
Conclusion: In older women with RIF, the PR may be improved by combining IUI and FT-ET with mild stimulation. In addition this method makes patients have the chance of natural pregnancy,Male partner's seminal fluid appears to be important in preparing the female immune respond to support embryo implantation. in other hand IUI catheter scratching can improve PR in study group.
Keywords: Frozen-Thawed Embryo Transfer, Intrauterine insemination, Recurrent Implantation Failure, Mild Stimulation

O-11: The Expression Levels of Autophagy Genes and Their Relationship with Apoptosis in Women with Thin Endometrium Undergoing IVF Compared to Those with Normal Pregnancy History

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Background: Endometrial atrophy (EA) is characterized by thinning of the endometrium (<5 mm), with unclear underlying causes. Autophagy, essential for uterine function and reproductive physiology, has been associated with EA. This study investigated expression levels of autophagy-related genes (*ATG5*, *ATG7*, *LC3B*, *Beclin1*) and FOXO transcription factors (FOXO1, FOXO3a, FOXO4, FOXO6) in women with thin endometrium compared to healthy pregnant women.
Materials and Methods: Real-Time PCR measured gene expression levels in 40 patients with thin endometrium and 40 healthy pregnant women. Expression levels of autophagy genes and FOXO transcription factors were compared between groups.
Results: Analysis revealed significant differences between patient and healthy pregnant groups. Patients with thin endometrium exhibited elevated expression levels of ATG5, ATG7, LC3B, Beclin1, FOXO1, FOXO3a, FOXO4, and FOXO6. Pathway enrichment analysis showed FOXO transcription factors' involvement in the FOXO signaling pathway, critical for apoptosis, cell cycle regulation, and oxidative stress response. Autophagy-related genes ATG7 and ATG5 were found to participate in the autophagy pathway, including mitophagy, clearing damaged mitochondria.
Conclusion: Dysregulation of autophagy genes and FOXO transcription factors may contribute to endometrial atrophy. The findings suggest potential involvement of the FOXO signaling pathway and autophagy-related pathways in cellular processes associated with endometrial thinning. Further research is needed to elucidate underlying mechanisms, offering prospects for targeted therapeutic interventions.
Keywords: Endometrial atrophy, Autophagy, Apoptosis,

O-12: Uterine Fluid Derived Exosomes Enhance Endometrial Receptivity by Upregulating Leukemia Inhibitory Factor and Downregulating Mucin-16 Genes in Endometrial Cells

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Background: Endometrial exosomes carry bioactive agents to uterine epithelial cells and trophectoderm to promote implantation. This study investigates the probable molecular mechanisms by which exosomes improve endometrial receptivity.
Materials and Methods: Exosomes were isolated from uterine fluid by Gradient ultracentrifugation and characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM), and western blotting. Endometrial Ishikawa cell line were treated with isolated exosomes and implantation assay was performed to evaluate the effect of exosomes on the receptivity potential of endometrial cells. Finally, the expression of several endometrial receptivity markers was evaluated by real-time PCR.
Results: DLS graph and TEM imaging showed that the isolated exosomes had a cup-shaped or spherical morphology with a mean size of 91.8 nm and zeta potential of -9.75 mV. Relatively strong immunoblotting bands for exosome-specific protein markers (CD-9 and CD-81) confirmed the isolation of exosomes. implantation assay revealed that treatment of endometrial cells by uterine exosomes enhances the receptivity potential of endometrial cells 1.5 ± 0.5 folds relative to the control group. Gene expression analysis showed that treatment of endometrial cells with uterine-derived exosomes results in upregulation and downregulation of leukemia inhibitory factor (LIF) and Mucin-16, respectively; however, the expression of Trophinin and insulin-like growth factor-binding protein 1 (IGFBP1) was not affected.
Conclusion: These findings confirmed the vital role of exosomes in endometrial receptivity and showed that regulation of LIF and Mucin-16 expression is one of the probable mechanisms by which exosomes affect endometrial receptivity.
Keywords: Exosomes , Endometrium, Receptivity

Genetics

O-13: Effect of Paternal Trans Fatty Acids Diet on Gene Expression and Epigenetic Pattern of Androgen Receptor and Steroidogenic Acute Regulatory Protein in Rat Offsprings Testis Tissue

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Background: Paternal malnutrition can impact sperm epigenome, affecting fetal development and offspring health. Trans and saturated fatty acids have adverse effects on sperm function

and male fertility which can be passed down through generations so, obviously genes affected. In the current study Androgen receptor (AR) and Steroidogenic Acute Regulatory Protein (StAR) were considered. AR is a key factor in promoting male traits during embryonic development and StAR facilitates cholesterol transfer through Testosterone synthesis.
Materials and Methods: Expression of AR and StAR genes evaluated quantitatively by real-time PCR in testis tissues of 30 offspring rats which their fathers were fed in 4 different diets: control (c), trans fatty acid (CTH), vitamin E (E), and a combination of vitamin E and trans fatty acid (ETH). Then incorporation levels of gene activation/repression epigenetic marks of H3K9ac/H3K9me2 as well as DNA methylation mark of MeCP2 were assessed using chromatin immunoprecipitation.
Results: High fatty acid diets were associated with decreased expression of AR and StAR genes in offspring testicular tissue. Also, epigenetic changes of studied genes were in alignment with expression results. The effect of fatty acid on expression and epigenetics was slightly compensated by vitamins.
Conclusion: The results of this study showed the importance of parental nutrition in epigenetic inherited changes in the genome and transferring it to the offspring in such a way that can affect the offspring's infertility. And vitamin E partially mitigates the harmful effects of fatty acids.
Keywords: Epigenetics, Ar, Star, Trans Fatty Acid, Male Fertility

O-14: Blastocoel Fluid DNA Quantification as A Potentially Alternative for Viable Human Embryo Selection

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Background: Embryo selection in assisted reproductive technology (ART) centers are mostly based on morphological assessment. The presence of cell-free DNA (cfDNA), reported to be collected in approximately 90% of the BF samples during the freezing process. It is believed that aneuploid embryos release cell-free DNA into the blastocoel cavity through the apoptotic mechanism.
Materials and Methods: This study included 29 cryopreserved human blastocysts donated by 20 couples undergoing preimplantation genetic testing for aneuploidy (PGT-A) due to secondary infertility and recurrent miscarriage. They were classified into the Segmental/Survivable Aneuploidy (SA), Double Aneuploidy (DA) and Multiple Aneuploidy (MA) groups based on comparative genomic hybridization microarray (array-CGH) by Trophectoderm (TE) biopsy. Following BFs aspiration, whole genome amplification of the BFs was performed. The amount of BF-DNA was measured by Qubit. Apoptotic activity in blastocysts was assessed for TNFRSF10B, CASP2, BAX, and CASP3 genes using Real-Time quantitative PCR.
Results: BF-DNA was detected in all 29 blastocoel fluids. A significant BF DNA increase was noted in MA vs. SA group ($P<0.05$). The levels of BF-DNA were notably higher in the DA group compared to the SA group and in the MA group compared to the DA group, however statistical analysis did not reveal significant correlations ($P=0.17$ and $P=0.38$, respectively). Overexpression of TNFRSF10B, CASP2, and CASP3 apoptotic genes was observed in MA and DA groups vs. SA group, while BAX gene was downregulated. A significant positive correlation existed between BF-DNA concentration and TNFRSF10B, CASP2 and CASP3 genes ($P<0.001$, $P<0.05$, $P<0.001$, respectively) with an inverse correlation to BAX gene alterations($P<0.05$).
Conclusion: The results of this study suggest the possibility of survivable embryos selection through the scoring of embryos based on the amount of BF-DNA released into their blastocoel cavity. It would potentially sound to be a cost-effective alternative for embryo selection rather than morphological assessment and costly PGT-A however for such embryo selection, like PGT-A, prenatal testing is recommended.
Keywords: Blastocoel Fluid, Aneuploidy, Apoptosis, Gene Expression

Poster Presentation

Andrology

P-1: Association of Ceramides and Sphingosine Levels With Sperm Quality in Infertile Men

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Background: Ceramides serve as pivotal intracellular messengers, intricately involved in regulating cell growth, differentiation, and programmed cell death. These lipid molecules, derived from sphingomyelin within cell membranes through the action of sphingomyelinases, are activated by various stressors, notably oxidative stress. Concurrently, sphingosine, a precursor to sphingosine-1-phosphate (S1P), modulates diverse cellular processes such as proliferation, growth, and differentiation. This study delves into an examination of these markers, utilizing standard chromatographic techniques, particularly focusing on sphingosine and ceramide subtypes (including C14, C16, C18, and C20 ceramides), isolated from samples representing normozoospermia and oligozoospermia.
Materials and Methods: Fresh sperm samples (n=20) were obtained from couples undergoing fertility treatments at the Alzahra Educational and Remedial Center (IVF center) in Rasht. These samples were meticulously collected and subjected to analysis in accordance with World Health Organization criteria, subsequently categorized into normozoospermia (control group) and oligozoospermia. Notably, patients had not undergone any hormonal, chemotherapeutic, or radiotherapeutic interventions prior to sample collection. The levels of sphingosine and ceramide subtypes (C14, C16, C18, and C20) were quantified using high-performance liquid chromatography (HPLC).
Results: The results of our analysis reveal significant disparities in ceramide levels between oligozoospermia and normozoospermia groups. Specifically, we observed a marked increase in the levels of C14 ($P<0.05$), C18 ($P<0.01$), and C20 ($P<0.05$) ceramides in oligozoospermia samples compared to those with normozoospermia. This suggests a potential dysregulation in ceramide metabolism associated with oligozoospermia. Furthermore, our investigation unveiled a notable elevation in sphingosine levels among individuals diagnosed with oligozoospermia in comparison to those with normozoospermia ($P<0.05$). This finding underscores the significance of sphingolipid metabolism in male fertility and implicates sphingosine as a potential biomarker or mediator in the pathophysiology of oligozoospermia
Conclusion: Taken together, these results shed light on the intricate interplay between sphingolipid metabolism and sperm quality, providing valuable insights into the molecular mechanisms underlying male infertility. Further studies are warranted to elucidate the precise role of ceramides and sphingosine in sperm function and to explore their potential as therapeutic targets for the management of oligozoospermia.
Keywords: Ceramide, Sphingosine, Oligozoospermia, Male Infertility

P-2: The Ameliorative Effects of Probiotics on Sperm Quality, Oxidative Stress, Sex Hormone, Testicular Structure after Ischemia/Reperfusion Injury following Testicular Torsion/Detorsion.

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Background: Testicular torsion is a common urological emergency and a significant cause of genital injury in males, highlighting the crucial need for early treatment to prevent potential damage to the testicles and infertility. This research aims to assess how probiotics affect ischemia/reperfusion injury following testicular torsion/detorsion (T/D).
Materials and Methods: Thirty-five male rats divided into five groups: sham, T/D group, and three groups of T/D+LRe and T/D+LRm and T/D+LRe+ LRm , involving T/D rats receiving Lactobacillus reuteri and Lactobacillus Rhamnosus probiotics alone or in combination. Testicular torsion was induced for one hour by rotating the left testis 720 degrees clockwise. After 60 days of reperfusion, the testis was removed, and assessments included sperm quality, hormonal levels, histological changes and biochemical markers such as the malondialdehyde (MDA) and reduced glutathione (GSH) levels, and antioxidant enzyme activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT).
Results: Results revealed a significant increase in MDA levels and a decrease in GSH levels after T/D compared to the sham group ($P<0.001$). Following testicular T/D, GPx, CAT, and SOD activities were decreased, while probiotic administration particularly Lactobacillus Rhamnosus significantly increased GSH levels and GPx and CAT activities. Additionally, probiotic administration decreased MDA levels in testis tissue compared to the T/D group. Histopathological evaluations indicated severe testicular damage after T/D, which was mitigated by probiotic of Lactobacillus Rhamnosus administration.
Conclusion: The study suggests that probiotics particularly Lactobacillus Rhamnosus has a beneficial impact on ischemia/reperfusion injury in the rat model of testicular T/D, likely due to its anti oxidative properties.
Keywords: Testicular Torsions/Detorsion, Probiotics, Lactobacillus Rhamnosus

P-3: Systematic Review of Artificial Intelligence Technologies in Semen Analysis and The Selection of Sperm

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Background: Male infertility represents a notable factor contributing to fertility impairment. Artificial intelligence technologies play crucial roles in the treatment of infertility by enabling precise diagnosis in semen analysis and the selection of sperm. The objective of this study is to conduct systematic reviews of artificial intelligence technologies applied to semen analysis and the selection of sperm.

Materials and Methods: The methodology was based on systematic reviews and meta-analyses to examine the most relevant studies on artificial intelligence tools utilizing machine learning and deep learning models. A total number of 78 articles, with a predominant focus on description, diagnosis, analysis, and prediction, spanning from 2013 to April 4, 2024 were reviewed.
Results: In this article, we first reviewed various artificial intelligence technologies in andrology, as well as diagnostic and therapeutic algorithms useful in the diagnosis and treatment of infertile couples. Next, we reviewed studies evaluating sperm morphology using artificial intelligence techniques. Finally, we reviewed studies using artificial intelligence methods for semen analysis and the selection of sperm, in addition to studies using artificial neural networks and deep learning to assess seminal quality.
Conclusion: The results suggest that artificial intelligence approaches, including machine learning, artificial neural networks, and deep learning, could revolutionize automated evaluation, analysis, and selection of sperm.
Keywords: Artificial Intelligence, Semen Analysis, Selection Sperm, Andrology

P-4: The Effect of Hydro Alcoholic Extract of Carrot Seed on Sperm Parameters, Testosterone Level and Catsper1,2 Expression in Adult Male Wistar Type I Diabetic Rats

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Background: Infertility affects approximately one in five couples of childbearing age. Diabetes can lead to endocrine disorders, testosterone levels as well as impaired sperm parameters. The present study investigated the effect of diabetes and the subsequent therapeutic effect of carrot seed extract on reproductive parameters of adult male streptozotocin (STZ)-induced diabetic rats.
Materials and Methods: A single intraperitoneal dose of STZ (50 mg/kg) was administered to male rats in order to induce diabetes. Rats in the diabetic and control groups received 400 mg/Kg of carrot extract daily orally (gavage) for four weeks. Blood samples were collected to study the serum level of testosterone, sperm samples were also collected to measure sperm parameters and perform immunohistochemical study of catsper1,2 expression.
Results: Catsper1,2 expression, testosterone levels and sperm parameters were significantly reduced in the diabetic control group compared to the normal control group. There were significant differences between the two groups in terms of the general abnormalities of sperm head and tail morphology. On the other hand, treatment with carrot seed hydroalcoholic extract increased Catsper1,2 expression and reduced abnormalities in the sperm parameters and as well as modulated testosterone levels.
Conclusion: The results of the present study show that induction of diabetes caused changes in Catsper1,2 protein expression and impaired the morphology of sperm, sperm parameters as well as testosterone level and treatment with carrot seed extract improved the disorders symptoms.
Keywords: Infertility, Catsper1&2, Sperm Parameters, Diabetes, Carrot Seed

P-5: Effect of Treatment with Hydrogen-Rich Water on Spermatogenesis in High-Fat Diet Rats

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Background: Obesity, a complex chronic condition, rose significantly in recent decades due to numerous factors. Obesity can affect spermatogenesis via several mechanisms such as increased sperm intracellular concentrations, superoxide concentrations, and oxidative sperm DNA damage. Hydrogen-rich water (HRW) is believed to have therapeutic antioxidant properties that can neutralize harmful free radicals in the human body. Therefore, in this study, the potential of HRW was assessed to prevent sperm dysfunction in high-fat diet (HFD) -induced obesity in rats.
Materials and Methods: Thirty male Wistar Albino rats were divided into three groups. 1) control: fed with a normal diet, 2) Obese: fed with HFD (45%), 3) HFD + HRW: fed with a high-fat diet and received HRW. HRW was administered orally (1.5 mmol/L) every day. After 16 weeks, blood and tissue samples (testis and epididymis) were taken for biochemical and histopathological evaluations. Malondialdehyde (MDA), as oxidative stress, superoxide dismutase (SOD) and total thiol groups, as antioxidative markers were also measured in testis and epididymal tissues.
Results: Results showed that high -fat diet primarily increased food intake, body weight, lee index which was significantly reduced in HRW-treated group. Histological studies showed that testis weight, sperm count and sertoli and spermatogonia cells are significantly lower in obese group which were improved by HRW treatment. Moreover, HRW treatment improved luminal diameter of seminiferous tubules, epididymal epithelia height and increased tissue SOD and total thiol group and reduced MDA level in testis.
Conclusion: Administration of HRW can improve spermatogenesis in obese animals by improving histological and oxidative/antioxidative balance in testis and epididymis suggesting its potential as a protective agent against diet-induced reproductive dysfunction.
Keywords: Obesity, High Fat Diet, Hydrogen-Rich Water, Spermatogenesis

P-6: Successful Live Birth in A Couple with Severe Teratozoospermia : A Case Report

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Background: Currently it has been reported that sperm morphology is a poor prognostic factor in predicting fertility ability, although it is not correct in severe teratozoospermia. In this case report study, the outcome of one intra-cytoplasmic sperm

injection (ICSI) cycle of an infertile couple with severe teratozoospermia is explained.
Materials and Methods: A couple with a 11-year history of infertility and one failed ICSI cycle referred to our infertility clinic. Semen analysis showed astenoteratozoospermia (concentration: 100 million/ml; motility: 0% and normal morphology: 0%). 99% of sperm cells were only membrane without any nucleus and 1% had nucleus with broken neck connecting together with a membrane . The couple were candidate for embryo donation. We suggest to the couple referred for karyotyping and Y chromosome evaluation before beginning the treatment. But the couple did not accept because of cost. So, the couple subjected to the ICSI cycle despairingly. Ovarian stimulation and oocyte–cumulus complexes aspiration were performed.
Results: Collectively, three oocytes were collected (1 MI and 2 MII). Mature oocytes were used for microinjection with abnormal sperm cells with nucleus and broken neck. Next day, one 2pn was observed and after 3 other days embryo culture, 1 compact embryo was transferred to the uterine cavity. After 14 days, beta HCG was positive and trans-vaginal sonography in seventh week showed a live fetus in the uterus with a good position. After 38 weeks, a healthy girl with weight of 2200 gr was delivered.
Conclusion: Live birth can be acquired with severe forms of sperm morphology. We suggest at least one ICSI cycle in patients with severe teratozoospermia before applying other treatments.
Keywords: Teratozoospermia, Live Birth, Sperm Morphology

P-7: Protective Effects of Melatonin on Human Sperm Parameters during Cryopreservation in Asthenozoospermic Men

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Background: Oxidative stress and reactive oxygen species (ROS) production are the important reasons for decreased sperm function during the cryopreservation process. Using of antioxidants can improve the cryo-survival of sperm act as a protection approach. Moreover, equilibration time is an important factor that could effect on the quality of thawed sperm. The aim of this study was to investigate the effects of Melatonin as an antioxidant on the quantitative parameters of asthenozoospermic men after freeze-thaw.
Materials and Methods: Thirty semen samples were collected from asthenozoospermic patients who had been referred to the infertility treatment center of Amir-AL-Momenin Infertility Treatment Center, Arak city in 2023-2024. Each sample was divided into 3 groups: control (fresh), freeze (treated with cryoprotectant alone), and freeze+ melatonin (treated with cryo-protectant+1 mM Melatonin solution). In the freezing groups, samples were cryopreserved with human sperm freezing medium and rapid freezing method. In each sample, sperm mobility according to WHO criteria, sperm viability using eosin-nigrosin staining and sperm morphology using the Diff Quick kit were assessed. Data were analyzed statistically using the Repeated Measure Analysis method and Bonferroni post-hoc test.
Results: Sperm motility, viability and morphology significantly decreased in the Freeze group compared to the control group ($P<0.001$). Whereas, in the Freeze+ Melatonin group a significant increase was observed in these parameters compared to the Freeze group ($P<0.001$).
Conclusion: Our results showed that Melatonin as antioxidants in medium doses improved the sperm parameters in the asthenozoospermic men after freeze-thawing.
Keywords: Asthenozoospermia, Cryopreservation, Melatonin, Sperm Parameters

P-8: Methanolic Extract of Iranian Oak Impede The Ferroptosis in Testis of Type II Diabetic Rats

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Background: Diabetes can have a significant impact on the male reproductive system and disrupt the spermatogenesis in the testes. It is well indicated that metabolic disorders can induce ferroptosis, a novel type of programmed cell death. Although our previous study showed that Iranian oak (Quercus brantii) can have a proper effect on the control of diabetes complications, the mechanisms of its effects on the reproductive system of diabetic men are not well known.
Materials and Methods: Twenty adult male Wistar rats were divided into 4 groups, including control (con), extract (Ex), diabetic (Dia), and diabetic + extract (Dia+Ex). Type II diabetes was induced by a high-fat diet and a low dose of streptozotocin (35 mg/kg) in diabetic animals. One week after streptozotocin injection, the Ex and Dia+Ex groups received 100 mg/kg/day of total methanolic extract of Q. brantii by oral gavage for 40 consecutive days. Finally, animals were euthanized, and left testes were fixed in 10% neutral buffered formalin for histological and stereological studies, and right testes were frozen in liquid nitrogen for evaluation of ferrostatin-1 expression by real-time PCR. Data were analyzed by one-way ANOVA and Tukey's post hoc test.
Results: The results of this study showed that the induction of type II diabetes significantly decreased body weight, testes weight, and gonadosomatic index, as well as the total volume of the germinal epithelium and the total number of Leydig, Sertoli, and spermatogenic cells compared to the control group, and led to severe structural destruction. Also, it was found that there was an elevation of ferroptosis in the testicular tissues of diabetic rats. Our results indicated that Q. brantii can improve the histological architecture of the testis and reduce ferroptosis significantly compared to the Dia groups.
Conclusion: It can be concluded that the use of a methanolic extract of Iranian oak in patients with type II diabetes may be a promising therapeutic target to improve spermatogenesis by preventing ferroptosis.
Keywords: Iranian Oak, Quercus Brantii, Ferroptosis, Testis, Diabetes

P-9: Methanolic Extract of Iranian Oak Alleviate Sperm DNA Fragmentation in Type II Diabetic Rats by Modulation of Oxidative Stress in Epididymis

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Background: Epidemiological evidence shows diabetic men have a higher risk of infertility compared to healthy men, and oxidative stress is accepted to be the main contributing factor. Our previous study showed that Iranian oak (Quercus brantii) has high antioxidant and anti-diabetic properties. This study was designed to evaluate the effect of a methanolic extract of Iranian oak (MEIO) on the oxidative stress indices of epididymis and sperm DNA fragmentation in type II diabetic rats.
Materials and Methods: Twenty adult male Wistar rats were divided into 4 groups, including control (con), extract (Ex), diabetic (Dia), and diabetic + extract (Dia+Ex). Type II diabetes was induced by a high-fat diet and a low dose of streptozotocin (35 mg/kg). One week after streptozotocin injection, the Ex and Dia+Ex groups received 100 mg/kg/day of total MEIO by oral gavage for 40 consecutive days. At the end of the experiment, animals were euthanized, and their left epididymis was removed, dissected in Ham's F10, and incubated at 37°C. Total count, motility, viability, sperm deformity index (SDI), tratozoospermia (TZI), and sperm DNA fragmentation index (SDFI) were assessed. Right epididymis were rapidly homogenized and stored at -80 ºC to measure glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (TAC). Data were analyzed by SPSS using a one-way ANOVA test and Tukey's post-hoc.
Results: Our results showed that the induction of type II diabetes significantly decreased the total number, motility, and viability of sperm, as well as the GPx, SOD, and TAC of the epididymis. SDI, TZI, and SDFI of the sperm and MDA of the epididymis in diabetic rats were significantly higher than those in the control group. Results indicated that MEIO can modulate oxidative stress, improve quantity and quality indices of sperm, reduce SDFI significantly compared to the Dia group, and bring it to the control level.
Conclusion: It can be concluded that the administration of a MEIO can be considered a suitable protective strategy for improving infertility or subfertility complications in type II diabetic males.
Keywords: Iranian Oak ,Quercus Brantii, Sperm, Oxidative Stress, Diabetes

P-10: Varicocele-Associated DNA Hypomethylation: Role of Aberrant TET2 Expression

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Background: Varicocele, a condition characterized by abnormal dilation of the pampiniform venous plexus in the spermatic cord, is associated with epigenetic modifications such as DNA methylation. Abnormal DNA methylation can cause male infertility by leading to abnormal sperm parameters, this has been observed in men with varicocele too. This study explores global DNA methylation status in testicular spermatogenic cells of varicocele-induced rats. It evaluates semen quality and focuses on key epigenetic markers, including 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC), along with mRNA and protein levels of ten-eleven translocation (TET) methylcytosine dioxygenases 1-3.
Materials and Methods: This experimental study involved 24 mature male Wistar rats, with 8 rats allocated to each group: control, sham, and varicocele. Sperm quality was evaluated, and DNA methylation patterns of testicular spermatogenic cells were analyzed utilizing reverse transcription-polymerase chain reaction (RT-PCR), western blot, and immunofluorescence techniques.
Results: In varicocele-induced rats, sperm quality and chromatin/DNA integrity decreased, and lipid peroxidation increased versus controls. During spermatogenesis, 5-mC and 5-hmC epigenetic marks and TET1-3 mRNA/proteins were expressed. 5-mC was present in all testicular cells, while 5-hmC was exclusive to spermatogonia and a few spermatids. The Varicocele group displayed diminished 5-mC signal, prominent 5-hmC signal, increased TET2 mRNA/protein expression, and intense TET1-3 fluorescent signals in testicular cells, contrasting with the faint signals observed in the control group.
Conclusion: Our findings demonstrate the upregulation of the TET2 enzyme in testicular tissues of varicocele cases was associated with increased levels of 5-hmC and DNA hypomethylation. Hence, they could serve as potential biomarkers for varicocele-associated male infertility.
Keywords: DNA Methylation, Male Infertility, Sperm, Varicocele, 5-Methylcytosine

P-11: The Effect of Rho Kinase Inhibitor (Y27632) on Histopathological Parameters of Testicular Tissue of Azoospermic Mouse

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Background: Thanks to progress in cancer treatments, the chance of patients survivability have been increased which causes an increase in the demand for fertility preservation. In nearly all of the cancer treatment protocol, induction of the apoptosis in highly proliferative cells such as the spermatogonial stem cells have been reported. Y27632 is a specific inhibitor of Rho associated protein kinase (ROCK) which involved in suppression of apoptosis in vast variety of stem cells in culture conditions. Therefore, we aimed to evaluate the effects of Y27632 on the histopathological changes of busulfan treated male mice.
Materials and Methods: Eighteen C57BL6 mice were divided into three groups. Control (without busulfan and Y27632), vehicle (busulfan 40 mg/kg + normal saline) and treatment (busulfan + Y27632 5mg/kg). Histopathological changes of testes tissue including, diameter and thickness of seminiferous tubules, spermatogenesis index (SPI), and tubular differentiation index (TDI) were evaluated after treatment.
Results: The diameter and thickness of the seminiferous tubules in treatment and vehicle groups were significantly lower compared to control group. However, these parameters were superior in Y27632 treated group compared to vehicle ($P<0.05$). Moreover, TDI and SPI were decreased significantly in vehicle

group compared to the control. While, treatment of mice with Y27632 indicated similar TDI and SPI with control group.
Conclusion: Our results indicated that, treatment of busulfan treated mice with Y27632 showed an improvement in thickness and diameters of seminiferous tubules as well as spermatogenesis.
Keywords: Azoospermic, Busulfan, Y27632, ROCK

P-12: The Effects of Hydrogen-Rich Water on Male Fertility and Spermatogenesis: A Novel Approach to Enhancing Reproductive Health

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Background: Infertility is a significant global public health issue that impacts millions of couples, particularly those residing in low- and middle-income countries. It is a pervasive health concern that requires comprehensive research and advanced therapeutic approaches, as approximately 50 million couples worldwide face challenges conceiving. Infertility affects both men and women and can be caused by various factors such as hormonal imbalances, physical abnormalities, genetic defects, and lifestyle choices. Male infertility accounts for 40% of infertility cases. Molecular hydrogen is known to have potent antioxidant effects and is showing promise as a therapy for diseases caused by oxidative stress. This study aims to summarize the literature on spermatogenesis and assess the influence of hydrogen-rich water on reproductive health and spermatogenesis.
Materials and Methods: We searched databases in PubMed, Scopus, EMBASE, Web of Knowledge, and MeSH for articles regarding the use of antioxidants, specifically hydrogen molecules, hydrogen-rich water, and enhanced spermatogenesis from 2014 to 2024. Out of 2,700 articles, we found 80 studies that met our criteria.
Results: Hydrogen molecules, due to their antioxidant activity, can selectively affect the most potent oxidant, making it an effective treatment option for infertility caused by oxidative stress. Studies have shown that hydrogen-rich water can reduce inflammatory factors caused by testicle damage and disturbances in spermatogenesis.
Conclusion: Molecular hydrogen using as hudrogen-rich water has antioxidative and anti-inflammatory effects and can be considered as a good candidate for improving spermatogenesis especially in some diseases including obesity, diabetes and spermatogenesis.
Keywords: Infertility, Spermatogenesis, Molecular Hydrogen, Oxidative Stress

P-13: Prevalence of Diabetes Risk in Infertile Men

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Background: Infertility is a widespread health issue globally, affecting over 186 million people worldwide. In Iran, the prevalence of infertility is estimated to be 7.88%. Male factors are responsible for infertility in roughly one-third of couples. Research suggests that infertility may be linked to certain metabolic abnormalities. This study aimed to investigate the prevalence of diabetes among infertile men.
Materials and Methods: This study is a cross-sectional analysis of the Tehran Lipid and Glucose Study (TLGS), which involved 98 male participants. Logistic regression was used to assess the relationship between diabetes risk and infertility in men.
Results: The overall prevalence of diabetic risk among infertile men was found to be 20%. The distribution of participants by age was as follows: <40 years (21.4%), 40-50 years (52%), and > 50 years (26.5%), with no significant correlation ($P > 0.05$). The education level of participants was predominantly in the 6-12-year range (77.3%) and above 12 years (22.7%), also showing no significant correlation ($P > 0.05$). The smoking history of participants was 45%, which was not significantly associated with diabetic risk ($P > 0.05$). Additionally, 48% of participants were overweight and 21.4% were obese, but these factors were not significantly linked to diabetic risk ($P > 0.05$). In the fully adjusted model, the odds ratio (OR) for diabetes mellitus (DM) was 1.04 (95% CI, 0.51-2.09), indicating no significant association ($P > 0.05$).
Conclusion: This study indicates a prevalence 20% of diabetic risk among infertile men. However, the analysis did not reveal any significant correlations between age groups, education levels, smoking history, overweight or obesity status, and the risk of diabetes in this population. Additionally, the fully adjusted model did not demonstrate a significant association between diabetes mellitus and male infertility. These results suggest that while diabetic risk is notable among infertile men, factors like age, education, smoking habits, and weight status may not be strong predictors of this risk within the study cohort. Further research is recommended to explore additional factors that could contribute to diabetic risk in infertile males.
Keywords: Infertility, Men, Diabetes Mellitus, Prevalence

P-14: The Effect of Selenium on Sperm Parameters in Mice Following Treatment with Cyclophosphamide

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Background: Cyclophosphamide, a widely used anticancer drug, carries significant pharmacological benefits but also induces oxidative stress in tissues, particularly affecting the testis and sperm parameters. This study aimed to explore selenium impact on the sperm parameters in adult mice undergoing cyclophosphamide treatment.
Materials and Methods: We randomly divided 36 adult NMRI male mice into four groups: control, cyclophosphamide (100 mg/kg/bw/week), selenium (1 mg/kg/bw/day), and cyclophosphamide + selenium, treating them for 35 days. After dissecting the mice, we cut the caudal region of the testicular epididymis in ham's F 10 culture medium to collect ejected sperm for evaluating sperm parameters. Data were analyzed using one-way ANOVA and Tukey's test, and the means were considered significantly different at $P<0.05$.
Results: The group treated with cyclophosphamide exhibited a significant decrease in the mean sperm count, percentage of motility, viability, sperm membrane integrity, and the normal

sperm morphology compared to the control group (P<0.001). The cyclophosphamide + selenium group showed a significant increase in the mentioned parameters compared to the cyclophosphamide group (P<0.001). The means of the sperm parameters in the selenium group were the same as the control group when compared to those (P>0.05)
Conclusion: Our findings indicate that selenium, acting as a potent antioxidant, can reduce cyclophosphamide's negative impact on sperm parameters by alleviating oxidative stress.
Keywords: Cyclophosphamide, Selenium, Sperm Parameters, Mice

P-15: Deciphering The Role of DOT1L in Spermatogenesis: Insights from Single-Cell RNA Sequencing

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Background: Spermatozoa exhibit a distinct genomic organization characterized by chromatin that is largely devoid of histones and instead comprises protamines. This unique composition offers enhanced compaction and safeguards the paternal genome until fertilization. The critical transition from histones to protamines, essential for producing functional sperm, occurs in spermatids. Our study highlights the role of the H3K79-methyltransferase disruptor of telomeric silencing-1 (DOT1L) in this process. DOT1L facilitates spermatid chromatin remodeling, aiding in the reorganization and compaction of the spermatozoon genome. It also alters chromatin before histone removal, affecting genes linked to flagellum development and apoptosis during spermatid differentiation. Consequences of disrupted DOT1L activity include less compact sperm heads and reduced motility, leading to compromised fertility.
Materials and Methods: We utilized single-cell RNA sequencing (GEO access number: GSE216907 and their platform: Illumina HiSeq 2000) to analyze tissue and organ microenvironments at a molecular and single-cell level. For this purpose, 5 adult testicular samples with normal spermatogenesis and 7 samples from individuals with non-obstructive azoospermia (NOA) were examined to assess DOT1L's role in spermatogenesis. This technology revealed that DOT1L expression is lower in spermatogonia, spermatocytes, and sperm.
Results: Furthermore, our analysis of the protein-protein interaction database indicated that DOT1L interacts with Histone H2B type 1-J (H2BC11), Histone H2B type 1-K (H2BC12), Histone H3.1 (H3C12), and Protein AF-10 (MLLT10). These interactions suggest that DOT1L and these proteins play a collective role in the differentiation of spermatocytes into sperm.
Conclusion: Our research indicates that the identified genes and their associated hub proteins are likely key factors in understanding the pathophysiology of germ cell abnormalities and genomic integrity in mitosis and meiosis. These genetic and protein interactions may provide critical insights into the underlying mechanisms driving these conditions, potentially leading to more targeted and effective treatments for infertility related to germ cell dysfunction.
Keywords: Spermatozoa, Protamines, Methyltransferase, Single-Cell RNA Sequencing, spermatogenesis

P-16: The Association of Mass and Individual Sperm Motility with Abnormal Sperm Morphology

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Background: Sperm motility is believed to be one of the most important parameters in evaluating the fertilizing ability of ejaculated sperm, and fertilization rates of human oocytes have been shown to correlate closely with sperm motility. Both sperm motility parameters and percent normal morphology are significant factors in predicting fertilization and pregnancy rates. The purpose of this study was to determine the relationship between the sperm motility and abnormal sperm morphology.
Materials and Methods: Testis samples were obtained from 45 rams. The viability and abnormal morphology parameters of the cauda epididymal sperm were assessed by means of the Eosin-Nigrosin stain method. The viability and sperm abnormalities were assessed by counting 300 sperm cells in a microscope at 1000× magnification, using immersion oil. The cauda epididymal sperm motility was assessed in a light microscopy at 400× magnification at 37ºC. A computer-assisted sperm motility analysis (CASA) was used to analyse sperm motility.
Results: According to the statistical analysis, significant correlations were found between coiled principal piece and end piece of tail and mass motility (P<0.01) and mass motility and individual motility (P<0.01). Also, significant positive correlation existed between live sperm and detached head (P<0.01), coiled principal piece and end piece of tail and coiled midpiece of tail (P<0.01), slender head and macro cephalic (P<0.01), pyriform head and twin head (P<0.05).
Conclusion: Observation of individual and mass motility and estimation of the percentage of progressively motile sperm will provide information about sperm membrane integrity, as well as the morphologic integrity of spermatozoa. In conclusion, in this study mass motility correlated significantly with coiled principal piece and end piece of tail and individual motility.
Keywords: Sperm Motility, Abnormal Sperm, Morphology, Ram, Epididymal

P-17: Effect of Different Doses of L-Carnitine on Spermatogenesis and Sperm Parameters in NMRI Mice

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Background: Infertility is the inability to achieve pregnancy after a year of regular, unprotected intercourse, affecting about 15% of couples, with male factors contributing to 50% of these cases. While semen analysis in male infertility might seem normal or show slight issues, conception can still be elusive. Currently, antioxidant therapy, designed to combat oxidative stress,

has gained popularity as a treatment, but its effectiveness is debatable. Therefore, the purpose of this study was to investigate the effects of different doses of L-carnitine on spermatogenesis and sperm parameters in healthy NMRI mice.
Materials and Methods: To achieve this purpose, 9 male NMRI mice were randomly divided into three groups (n=3). The experimental groups were as follows: (1) Control: no L-carnitine (LC) supplementation, (2) Low dose: 20 mg/kg of LC, and (3) High dose: 500 mg/kg of LC. After 4 weeks, the mice were sacrificed, epididymal spermatozoa were evaluated for sperm parameters, and histopathological examinations were conducted on testicular tissue. Statistical analysis used one-way ANOVA and LSD post hoc test, with a significance level set at $P < 0.05$.
Results: The current results indicated that the low and high doses of LC led to a significant decrease in sperm progressive motility ($P < 0.05$) compared to controls. The groups showed no significant differences in total sperm motility, concentration, and abnormal morphology. In the meantime, administering low and high doses of LC did not significantly change Johnsen's testicular histopathological score.
Conclusion: Overall, adding LC to the drinking water of mice for 4 weeks could affect sperm motility. Given LC's high capacity for chelating calcium—possibly affecting calcium-dependent processes like sperm motility—and its potential to disrupt peroxisomal β-oxidation of fatty acids, which could potentially influence sperm motility by affecting cellular energy homeostasis, caution is advised when using LC, especially in high doses. However, these underlying mechanisms need further detailed investigation.
Keywords: Male Infertility, Sperm Chromatin Integrity, L- Carnitine, Supplementations, Mouse

P-18: No Title Leveraging Artificial Intelligence to Uncover Impact of Medicines in Male Infertility Treatments: Systematic Review

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Background: Artificial intelligence (AI) has played a pivotal role in advancing the field of male infertility treatment by predicting and identifying the therapeutic impacts of drugs. The aim of this study is to utilize AI to identify the effects of various drugs on sperm parameters to improve the treatment of infertility in men.
Materials and Methods: This review was conducted following PRISMA-SR checklist The PICO strategy was utilized for this purpose. The main words included: “male infertility” AND “hormone therapy” OR “drugs” AND “artificial intelligence”. Articles from the Scopus and PubMed databases between 2014 and 2024 were derived and screened.
Results: We initially identified 199 articles. 24 duplicate articles were removed. Finally, 7 articles that followed inclusion and exclusion criteria were entered in our study. AI were examined on 5 main sperm parameters including sperm DNA fragmentation (SDF), sperm motility, sperm morphology, sperm concentration and sperm vitality. Treatment response of different drugs such as superoxide dismutase (SOD), L-carnitine and L-acetylcarnitin and anastrozole were predicted by AI methods and identified predictors such as estrogen, FSH, type of male infertility and etc
Conclusion: Despite the advancements in understanding the impact of medications on sperm quality and fertility, there are limitations that necessitate further research. The role of AI in male infertility treatment presents promising prospects for future advancements in enhancing male fertility.
Keywords: Male Infertility, Artificial Intelligence, Predictors, Drugs

P-19: Vitamin E Treatment Reduces Reperfusion-Induced Oxidative Stress and Apoptosis in Testicular Torsion/Reperfusion-Induced Condition in Rats

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Background: Testicular torsion (TT) is a well-known urological condition, causing testicular damages. Testicular detorsion (TD) is the only possible treatment to reverse TT-induced ischemia, however, TD-induced reperfusion injuries may also affect testis. Therefore, various antioxidants are being tested to inhibit reperfusion injuries. Vitamin E is a well-known antioxidant agent to improve infertility conditions such as varicocele condition and recent meta-analysis studies in line have proven the beneficial impacts. This study tried to investigate Vitamin E potential effects after a one-hour experimental TT and TD by examining the testicular antioxidant capacity and intrinsic apoptosis-related proteins.
Materials and Methods: Forty-eight male Wistar rats were randomly divided into control and experimental groups. Thirty-two rats had a 720-degree TT surgery, for one hour. Eight animals following TT (TT group) and eight animals one hour following TD (TT/TD group) were euthanized. Sixteen rats received normal saline (TD/NS group) and Vitamin E (100 mg/kg; TD/E100 group) for one-week post-surgery. For controls, sixteen rats were used as control and sham-operated (Sham-1; 8 rats/group). Antioxidant capacity and lipid peroxidation (MDA, TAC, Catalase, GPx), histopathological, and Immunohistochemistry (Bcl-2, Bax, Caspase3) analyses were performed.
Results: The TAC, Catalase, GPx, and Bcl-2 levels were decreased in TT, TD, and TD/NS groups compared to control and sham groups, while MDA, Bax and Caspase-3 levels were increased. Moreover, spermatogenesis was arrested and Johnsen's score was reduced in TT, TD, and TD/NS groups compared to the Control and Sham-2 groups. Vitamin E treatment increased TAC, Catalase, GPx, Johnsen score, Bcl-2 positive cells number, and diminished MDA, Bax, and Caspase-3 levels in TD/E100 group compared to TT, TD, and TD/NS groups.
Conclusion: It can be concluded that Vitamin E (100 mg/kg) administration post-TD can positively improves TT/TD recovery by up-regulating antioxidant enzymes activity in the testis,

and inhibiting mitochondria-dependent apoptosis in germ cells. Thus, an increased germ cells survival rate in the testis leads to an improved Johnsen score.
Keywords: Vitamin E, Testicular Torsion, Testicular Detorsion, Apoptosis, Rat

P-20: The Effect of Theobromine on Sperm Parameters in Cryopreserved Samples from Asthenozoospermic Men

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Background: Sperm cryopreservation, a key technique in assisted reproductive technology, often results in significant damage to sperm quality. Adding antioxidants to the cryopreservation medium can help mitigate these adverse effects. Theobromine, a plant alkaloid derived from methylxanthine, is widely used and known for its antioxidant properties, which positively affect sperm parameters. This study investigated the effect of theobromine supplementation on sperm parameters during the cryopreservation of semen samples from asthenozoospermic men.
Materials and Methods: Semen samples were obtained from 30 asthenozoospermic men. Each sample was then divided into three groups: control (fresh), freeze (treated with cryoprotectant alone), and freeze + theobromine (treated with cryoprotectant and 10 mmol/L theobromine). The samples in the freezing groups were cryopreserved using a human sperm freezing medium and a rapid freezing method. For each sample, sperm motility was assessed according to World Health Organization (WHO) criteria using light microscopy. Viability was evaluated using eosin-nigrosin staining, and sperm morphology was examined using the Diff-Quick kit. Data were statistically analyzed using the Repeated Measures Analysis method.
Results: Mean sperm motility, viability, and normal morphology significantly decreased in the Freeze group compared to the Control group ($P<0.05$). In the Freeze + Theobromine group, a significant increase in sperm motility and viability was observed compared to the Freeze group ($P <0.05$). However, there was no significant difference in normal morphology between the Freeze + Theobromine group and the Freeze group ($P>0.05$).
Conclusion: Our data indicate that theobromine reduces the adverse effects of cryopreservation on sperm motility and viability in asthenozoospermic men, but it has no impact on normal morphology.
Keywords: Asthenozoospermia, Cryopreservation, Theobromine, Sperm Parameters

P-21: Effects of Methane Rich Saline on Sperm Parameters in Wistar Rats Exposed to Lead

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Background: Lead is a heavy metal with numerous applications due to its high malleability, low melting point, and high resistance to oxidation. Lead poisoning results in various general injuries across different age groups. The reproductive system is among the target organs affected by lead. Lead poisoning is a major contributor to infertility. Lead impacts the hypothalamus-pituitary-testicular axis, lowering the sex hormones LH, FSH, and disrupting the functions of Sertoli and Leydig cells. This disruption leads to decreased testosterone production, causing testicular atrophy and disturbances in sperm production (reduced sperm production and increased abnormal sperm count). Accumulation of lead in the reproductive system increases oxidative factors, resulting in elevated DNA damage, membrane lipid peroxidation, decreased sperm axoneme phosphorylation, and issues with sperm DNA condensation, leading to the formation of sperm with abnormal morphologies. Methane can mitigate the damage caused by lead poisoning to sperm by exerting an anti-inflammatory and antioxidant effect.
Materials and Methods: Thirty-five adult male Wistar rats, around 8 weeks old and weighing approximately 180-220 grams, were randomly allocated into five groups of seven rats each. The groups included a sham group receiving intraperitoneal normal saline, a positive control group receiving intraperitoneal methane-enriched saline at 5 ml/kg, a negative control group receiving intraperitoneal lead at 10 mg/kg/IP, a lower dose treatment group receiving intraperitoneal lead at 10 mg/kg/IP and methane-enriched saline at 2 ml/kg, and a maximum dose treatment group receiving intraperitoneal lead at 10 mg/kg/IP and methane-enriched saline at 5 ml/kg. Twenty hours post final injection, following anesthesia and blood collection, the right epididymis tail was isolated and preserved in Ham's F10 solution for sperm analysis, including assessment of movement, number, vitality (eosin staining), and morphology (Papanicolaou staining) under microscopic examination.
Results: Sperm parameters in the lead group significantly decreased compared to other groups. The percentage of sperm parameters in the treated groups showed a notable increase compared to the lead group. Additionally, sperm parameters in the MRS group significantly increased compared to all other groups ($P<0.05$).
Conclusion: Lead reduces sperm parameters, while MRS not only enhances sperm parameters but also retrieve the toxic effects of lead on sperm parameters.
Keywords: Lead, MRS, Sperm Parameters, Wistar Rat

P-22: Varicocele Increased Heat Shock Protein 90a is Associated with Apoptosis Index in Testis; An Experimental Study

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Background: Varicocele is associated with a progressive decrease in male fertile potential, but the main underlying mecha-

nism is yet to be determined. This experimental study was carried out to investigate the area-dependent effects of varicocele on heat shock protein 90a (HSP90a) and its association with the apoptosis index in the testis.
Materials and Methods: Twenty mature male Wistar rats (200 ± 25 g) were randomly divided (n=10/group) into four months sham (sham-4) and four months varicocele (VCL-4) groups. Animals were euthanized by an overdose of thiopental and testicular tissues were dissected out and used for immunohistochemical and the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assays. HSP-90a protein level and apoptosis index (API) were analyzed in the central and subcapsular areas of testicular tissues. Results are presented as mean ± SD. Groups were compared using One-way ANOVA (Tukey's HSD post-hoc) Significance was set at 5%.
Results: Increased levels of HSP-90a and API (%) were observed in VCL-4 group when compared to sham-4 group. The HSP-90a expression pattern did not show any significant difference in the central and subcapsular areas of varicocele and sham groups. However, API evaluation demonstrated a higher level of apoptosis in the subcapsular section compared to the central portion of VCL-4 testis, while no significant difference was observed in the control group. Moreover, statistical analyses demonstrated a positive correlation between HSP90a protein level and API in both areas.
Conclusion: Experimental varicocele leads to decreased semen quality, sperm functional integrity, and spermatogenesis arrest. To our knowledge, this is the first study demonstrating a direct association between HSP90a and API increase in the varicocele-induced condition. Moreover, API has been demonstrated to be altered in an area-dependent manner in varicocele-induced rats. However, HSP90a seems to be homogenously altered in the varicocele testis.
Keywords: Varicocele, Testis, Rat, HSP90a, Apoptosis

P-23: Lutein Mitigates Adverse Effects of Diabetes and High Fat Diet on Male Reproduction Health by Modulating Mitochondrial Dynamics in Sperm

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Background: Diabetes mellitus type 1 (D1) and high-fat diet (HF) are well known to, adversely affect male reproductive health, including sperm function. Mitochondrial function is of importance within sperm, impacting its motility and capacitation, a crucial determinant of male reproductive success. This study explored the significance of mitochondrial dynamics during D1 and HF, and the possible protective effect of Lutein, an antioxidant carotenoid.
Materials and Methods: Thirty-six adult male Wistar rats were divided into five groups (6 per group): control, D1 (Streptozotocin-injected, ip), HF+D1 (received high-fat diet, and Streptozotocin), HF+D1+Lutein 25 (25 mg/kg, orally), and HF+D1+Lutein 50 (50 mg/kg, orally). HF groups received HF for 60 days followed by D1 induction and Lutein supplementation for 48 days. Epididymal sperm was collected to assess count and motility. Sperm viability was evaluated using Eosin-nigrosin staining. Mitochondrial biogenesis and dynamics were evaluated by examining the PGC1a, TFAM, Mfn1/2, OPA1, Fis1, Drp1 expression using qRT-PCR. Data were analyzed using one-way ANOVA and Bonferroni post-hoc tests, with $P<0.05$ considered significant.
Results: D1 and D1+HF significantly ($P<0.05$) disrupted sperm count, motility, and viability. These conditions significantly ($P<0.05$) decreased PGC1a, TFAM, Mfn1/2, OPA1, while increasing Fis1 and Drp1 expression compared to control group. Lutein supplementation at both doses (25 and 50 mg/kg) remarkably ($P<0.05$) ameliorated sperm motility and viability, and increased PGC1a, TFAM, Mfn1/2 and OPA1. No significant differences were observed between the effects of the two lutein doses. The rats received Lutein 50 exhibited significantly ($P<0.05$) reduced Fis1 and Drp1 expression compared to Lutein 25 animals.
Conclusion: Lutein effectively mitigates the negative effects of diabetes and HF on sperm parameters by regulating mitochondrial biogenesis and function. Both 25 mg/kg and 50 mg/kg doses of Lutein improve sperm quality and mitochondrial gene expression, underscoring its therapeutic potential in preserving male reproductive health under diabetic and high-fat diet conditions.
Keywords: Lutein, Diabetes, High-fat Diet, Mitochondrial Biogenesis, Sperm

P-24: Antioxidant Role of Aqueous Extract of Ashwagandha on Testis and Epididymis of Male Rabbits Treated with Lead Acetate

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Background: Lead acetate is one of the most common environmental pollutants that damages reproductive organs through the induction of oxidative stress. This study aimed to evaluate the protective effect of the extract of ashwagandha, with anti-inflammatory and potent antioxidant properties, on oxidative stress indicators and adverse effects induced by lead acetate in male rabbits.
Materials and Methods: The 15 male rabbits were divided into 3 groups: 1) control group (treated with distilled water); 2) lead acetate group (treated with lead acetate (150 mg/kg WB); 3) ashwagandha + lead acetate group (treated with ashwagandha (250 mg/kg BW) and 150 mg/kg BW lead acetate). After 30 days, the histology of the epididymis, and testis and sperm concentration were examined. In addition, glutathione (GSH) and catalase (CAT) activity and also lipid peroxidation (malondialdehyde: MDA) were assessed in the blood samples in these three groups.
Results: In the lead acetate group, a significant decrease ($p\leq0.05$) in the GSH and CAT activity, the diameter of seminiferous tubules and epididymis, thickness of the gremial layer and sperm concentration, and a significant increase ($P\leq0.001$) in MDA levels were observed compared with the control group. In the ashwagandha + lead acetate group, ashwagandha could reverse these negative effects and increase GSH and CAT activity, the diameter of seminiferous tubules and epididymis, thickness of the gremial layer and sperm concentration, and decrease MDA levels compared with the lead acetate group.
Conclusion: Lead acetate induces adverse effects on reproduc-

tive organs and the aqueous extract of ashwagandha could reverse oxidative stress, and reduce damages induced on testis and epididymis.
Keywords: Ashwagandha, Lead Acetate, Rabbit Testis and Epididymis, Antioxidants Enzymes

P-25: The Effect of Hydroalcoholic Extract of Foeniculum Vulgare on Testicular Characteristics in Adult Mice

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Background: Foeniculum vulgare (fennel) with a long history of usage in folkloric and science based herbal medicine is dioeciously and has therapeutic applications. This study was aimed to assess the effect of hydroalcoholic extract of fennel on the testes of adult mice.
Materials and Methods: Pregnant NMRI mice were randomly divided into 3 groups: extract-treated groups received 500 and 1000 mg/kg/day fennel extract (FE), and the control group (CTL) received no treatment. The treatments started from pregnancy day 1 and continued until PND 56 (adult mice). Body and right testis weights and testis dimensions were recorded. Hematoxylin and eosin stained ovary sections were prepared to calculate the proportion of tubule diameter, tubule area, epithelium thickness. The number of spermatogonia, primary spermatocytes and spermatids within the seminiferous tubules were manually counted (8 section in each testis, n=8).
Results: The result showed that showed that no significant changes between the groups in the body and testes weights and testis dimensions. The number of spermatogonia, primary spermatocytes and spermatids after administration of fennel decreased significantly as compared with the control group ($P < 0.05$). Furthermore, decrease in the thickness of the epithelium, tubule diameter and tubule area were observed in the experimental groups ($P<0.05$).
Conclusion: Hydro-alcoholic fennel seed extract at these doses could reduce reproductivity and has anti-fertility activity in male rats.
Keywords: Foeniculum Vulgare , Testes, Mice

P-26: Effect of Biotin and Folic Acid on Motility and Malondialdehyde Levels in The Oligospermia Men Before and After Cryopreservation

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Background: Sperm cryopreservation is a technique used in laboratories and fertility centers to assist individuals with fertility issues. Cryopreservation can increase the production of reactive oxygen species (ROS), affecting the quality of the sperm and increased the lipid peroxidation(LPO) level of sperm cell, enhancing reduced motility. This study aimed to evaluate the effect of biotin and folic acid on motility and the concentration of malondialdehyde (MDA) levels in the oligospermia men before and after cryopreservation.
Materials and Methods: Specimens were obtained from 30 oligospermia men, aged between 25 to 45 years. Each sample were divided into five groups: fresh group, freezing group without antioxidant, freezing group with biotin (10 nM), freezing group with folic acid (50 nM), and freezing group with a combination of biotin (10 nM) and folic acid (50 nM). Samples were evaluated for motility before and after freezing using computer-aided sperm analysis software. The concentration of MDA was measured using a spectrophotometer at a wavelength of 535nm in each group.
Results: The average absorption of MDA levels in the groups before cryopreservation (0.46 ± 0.1) was lower than other groups after cryopreservation, which was significantly different ($P<0.05$). The average MDA concentration in the biotin + folic acid group (0.82 ± 0.11) was lower than other groups after cryopreservation ($P<0.05$). The average percentage of sperms with total motility in the cryopreserved group treated with biotin and folic acid was (22.7 ± 6.2), which was more than the control group, which was significantly different ($P<0.05$).
Conclusion: The biotin and folic acid antioxidants combination in the sperm freezing medium reduced the MDA concentration and LPO level after cryopreservation. Thereby, increasing the sperm motility and may play a positive role in the sperm function by maintaining fertility.
Keywords: Cryopreservation, Biotin, Folic Acid, Oligospermia, Malondialdehyde

P-27: Investigating The Correlation Between Mercury, Arsenic, and Zinc Levels in Semen and Blood Serum of Infertile Men

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Background: Heavy metals like arsenic (As) and mercury (Hg) can harm male reproductive function, with unclear mechanisms. Zinc (Zn) is an essential element for reproduction but can be affected by heavy metal contamination, impacting sperm motility and DNA damage. This study examines the correlation between male infertility and Zn, Hg, and As levels in semen and blood serum.
Materials and Methods: In this study, 50 fertile men (control group) and 50 infertile men, aged 20 to 60, were examined. Semen parameters were analyzed. Inductively coupled plasma mass spectrometry (ICP-MS) was used to measure Zn, Hg, and as levels in semen and serum samples. Data were analyzed using SPSS version 24 software, with a significance level set at 0.05 for statistical tests.
Results: This study revealed that the age and body mass index (BMI) distributions were not significantly different between the

two groups. However, sperm count and total motility showed significant differences, with p-values of 0.002 and 0.001, respectively. No significant difference was observed in the volume, liquefaction time, and pH of the semen samples. A significantly higher serum Hg levels were observed in infertile men as compared to fertile men (P=0.001), however, no difference was found in semen Hg levels (P=0.88). Although serum Zn levels were significantly higher in the control group (P=0.029), no difference was noted in semen Zn levels between the two groups (P=0.233). Semen As levels were significantly higher in infertile men (P=0.004), but not in serum samples (P=0.082).
Conclusion: The results of this study suggest that heavy metals present in serum or semen samples may have an impact on male fertility.
Keywords: Male Infertility, Heavy Metals, Arsenic, Mercury, Zinc

P-28: Detection of Toll-Like Receptors 7/8 (TLR7/8) in Human Spermatozoa

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Background: Toll-like receptors (TLRs) are a subset of the innate immune system. They are part of Pattern-recognition-receptors (PRRs) that recognize and respond to Pathogen-associated molecular patterns (PAMPs). Studies have shown that TLR1-10 genes are expressed in all male reproductive organs and sperm. Observations in mouse, bull, and goat have shown that TLR7/8 is expressed only in X chromosome-bearing sperm and activation of TLR7/8 alters sperm motility. Of course, more studies are needed in this field. This study aimed to identify and detect TLR7/8 in human sperm to pave the way for further research and studies.
Materials and Methods: 27 normal sperm samples were collected from spare samples of patients who attended the clinical laboratory of the Royan Institute. Each sample was divided into two aliquots. One for Western analysis and the other for immunofluorescence analysis. After western analysis, 3 samples were selected to detect the localization with immunofluorescence analysis.
Results: All 27 samples in the Western analysis showed the cleaved form of TLR7 but the expression of TLR8 was lower than TLR7; hence we only examine the TLR7 localization. By immunofluorescence analysis, TLR7+ signals were strongly observed in the neck and a little expression was detected in the tails of almost half of the sperms. These results are in accordance with previous studies in other animals.
Conclusion: TLR7 was observed in half of the patient's sperms. Some sperms may not express TLR7 and according to previous studies, this could be due to the fact that X-chromosome-bearing sperms express this receptor while Y-chromosome-bearing sperms do not. These findings can be used for further research in immunology and even sex selection.
Keywords: Toll-Like Receptors, Sperm, Western Blot, Immunofluorescence

P-29: Evaluation of C. G2783A Variation of *BRDT* Gene and Protein Expression in Infertile Men with Abnormal Sperm Morphology

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Background: One of the known causes of infertility is teratozoospermia, which refers to defects in sperm morphology. Recent studies have identified BRDT (bromodomain-associated testis), a key transcriptional regulator in meiotic and post-meiotic cells, as a potential cause of infertility. The *BRDT* gene is specific to the testis and is expressed in spermatocytes, round spermatids, elongated sperm and mature sperm. A reported case within a family revealed a homozygous mutation (c.G2783A, p.G928D) in the *BRDT* gene, leading to the production of acephalic sperm.
Materials and Methods: In this study involved the examination of 60 patients referred to Royan, utilizing polymerase chain reaction (PCR) and sequencing of exon 19 of the *BRDT* gene. The patient group consisted of 60 individuals with teratozoospermia, characterized by acephalic sperm, globozoospermia, and short-tailed sperm. Additionally, six samples from this group, along with two controls, were selected for further analysis using the western blot method. Furthermore, an Immunocytochemistry test was performed on three samples from the teratozoospermia group and one control.
Results: The results of our study, based on the PCR test and sequencing, demonstrated that the c.G2783A mutation in exon 19 of the BRDT gene had no impact on the production of acephalic and short-tailed sperm, as well as globozoospermia, across all 60 samples. Furthermore, no mutations were observed in this exon. Additionally, the evaluation of BRDT protein expression through Immunocytochemistry analysis and western blot analysis did not indicate any significant differences in the location or level of *BRDT* protein expression among the studied groups.
Conclusion: Our study showed that the c. G2783A mutation had no significant impact on the production of acephalic sperm, nor on the incidence of globozoospermia or short tail in our study subjects.
Keywords: BRDT, Teratozoospermia, Acephalic Syndrome, Globozoospermia, Short-Tailed

P-30: Assessment of Sperm Function in Men with Varicocele: A comparative Study with Fertile Controls

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Background: Varicocele, marked by abnormal vein enlarge-

ment in the spermatic cord, is associated with heightened sperm oxidative stress and DNA damage. Given the crucial role of sperm DNA health in natural and assisted conception, this study aims to assess sperm parameters and chromatin status, including DNA integrity and protamine deficiency, in infertile individuals with varicocele compared to fertile men.
Materials and Methods: Semen samples were obtained from 300 men with varicocele and 150 fertile men at the Isfahan Fertility and Infertility Center, with written informed consent. Sperm parameters [World Health Organization (2010) guidelines], DNA damage (TUNEL test), and protamine deficiency (Chromomycin A3 staining) were assessed in these individuals. Descriptive analysis characterized the study parameters, while an independent t-test compared parameters between groups. Pearson correlation coefficients were used to explore relationships among the study parameters.
Results: The varicocele group exhibited significantly higher mean of DNA damage, and sperm protamine deficiency as well as lower quality of sperm parameters compared to the fertile group ($P<0.05$).
Conclusion: This study involving infertile individuals with varicocele and fertile controls underscores the importance of sperm parameters and chromatin condensation in fertility. The findings can advance both diagnosis and treatment strategies for male infertility associated with varicocele and contribute to future meta-analyses.
Keywords: Varicocele, Sperm Parameters, Protamine Deficiency, DNA Damage

P-31: The Effects of Folic Acid on Testicular Toxicity and Spermatogenesis Indices of Adult Male Mice Treated with 5-Fluorouracil

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Background: 5-Fluorouracil (5-FU) is one of the anti-metabolite drugs which, in addition to its chemotherapy properties, may also disturb the cell metabolism and the survival of normal cells, which is the basis of developmental and reproductive toxicities. This study was conducted in order to investigate the antioxidant effect of folic acid on testis structure and spermatogenesis process of mice treated with 5-fluorouracil.
Materials and Methods: 24 adult male NMRI mice were divided into 4 groups (n=6), control 5-fluorouracil (15 mg/kg, 5 consecutive days), folic acid (1 mg/kg, daily) and 5-fluorouracil + folic acid. After 35 days of treatment, testicular tissue parameters by stereological methods and testosterone levels were estimated. The data were analyzed by One-Way ANOVA and Tukey's test and considered significant at the level of ($P<0.05$).
Results: A significant decrease in the weight and volume of the testis, the volume, diameter and height of the germinal epithelium of the seminiferous tubules and the level of testosterone hormone and a significant increase in the volume of the interstitial tissue were observed in the 5-fluorouracil group compared to the control group ($P<0.01$). The number of germ cells and spermatogenesis indices in the group treated with 5-fluorouracil showed a significant decrease compared to the control group ($P<0.001$). In the simultaneous treatment group, folic acid partially reversed these parameters and made them close to the control group.
Conclusion: Folic acid as an antioxidant can improve tissue damage caused by 5-fluorouracil and prevent testicular tissue destruction by inhibiting free radicals.
Keywords: 5-Fluorouracil, Folic Acid, Testis, Spermatogenesis, Mice

P-32: Development of Microfluidic Chip Architecture for Sperm Quality Analysis

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Background: Approximately 15% of married couples around the world face the problem of infertility, and in half of the cases this is due to the male factor. Conventional sperm sorting methods bypass natural barriers existed *in vivo*. Microfluidic systems for sperm sorting are a new way to provide cell sorting similar to *in vivo* conditions.
Materials and Methods: The development of microfluidic systems for sperm sorting includes the following steps: choose a simulation method, develop a printing method, develop a microfluidic chip design, conduct simulations, and validate the chip.
Results: Among the simulation methods, the optimal one is laminar flow. It gives results close to real experimental ones. Despite this, Stokes flow can also be used to estimate how much larger the velocity might be in a real experiment. The most successful printing method with using LCD printing is micromolding. To avoid silicone inhibition, it is necessary to rinse the mold for 30 minutes in isopropyl alcohol and additionally expose for 30 minutes under UV. Chip design is usually developed in accordance to one of the main sorting mechanisms. In addition, there are several limitations for chip design, arising from sperm cells and flow properties. First, there must be a flow in the sperm reservoir so the sperm can enter the sorting part of the chip itself. Secondly, it is necessary to avoid sharp corners in the design, as this contributes to the formation of bubbles in the system. Third, branching the flow into several channels promotes the formation of bubbles if these flows connect in another part of the channel.
Conclusion: Based on all these data, a chip was developed for sperm sorting based on rheotaxis. This work was financially supported by the Russian Science Foundation, grant No. 24-45-20007
Keywords: Microfluidics, Sperm Sorting, Microfluidic Chip Manufacturing, Computional Fluid Dynamics

P-33: Mir-423-3P and Mir-24A Regulated Motility and Apoptosis Index in Ashtenozoospermic and Normozoospermic Bulls Semen After Freeze-Thaw

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Background: We aimed to evaluate the expression of motility and apoptosis related miRNA in asthenozoospermic (AS) and normozoospermic (NS) post-thawed Holstein bull semen
Materials and Methods: For evaluation sperm parameters between two groups of study, semen samples after collection procedure, were diluted and then frozen. After thawing process, semen samples in two groups of study were analyzed by using CASA system for sperm kinematic and phosphatidyl externalization for apoptosis status. two miRNAs related to apoptosis and motility were evaluated with qRT-PCR
Results: In functional and flow cytometric parameters, AS group was significantly better that NS group. There were significant differences between the two groups of study regarding miRNA-related expression (miR-243-3p and miR-24a). miR-243-3p had negative correlation with progressive motility and late apoptotic cell in two groups of study (r=-5621, r=-0.7321, P<0.05). Also, miR-243-3p, and miR-24a were significant positive correlation with live cells in two group of study (r=0.7192, r=0.4296 and P<0.05).
Conclusion: The results of the present study showed in two groups of study, in most of the kinematic parameters and functional index, the results and efficiency of normozoospermic group was better than asthenozoospermic group. Regarding miRNA expression associated with apoptosis and motility (miR-243-3p and miR-24a) were noteworthy correlation with apoptosis status.
Keywords: Asthenozoospermic, Normorzoospermic, MiRNAs, Apoptosis

P-34: Protective Effects of Royal Jelly on Ferroptosis –Induced Testicular Damage in Adult Rats Following Experimental Heat Stress Induction

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Background: Heat stress reduces key parameters such as sperm density and motility and alters sperm morphology. Ferroptosis is a non-apoptotic, iron-dependent form of programmed cell death characterized by the accumulation of iron-dependent lipid peroxides. Royal Jelly (RJ), secreted by the hypopharyngeal and mandibular glands of worker honeybees between the sixth and twelfth days of life, is a rich source of vitamins, including riboflavin, thiamine, niacin, folic acid, biotin, pyridoxine, and smaller amounts of vitamins C, D, A, and E. RJ is known to alleviate premenstrual symptoms, osteoporosis, and improve hormonal balance and fertility in both men and women by enhancing the quality of eggs and sperm. This study evaluates the effects of RJ on mechanisms of ferroptosis in adult male rats subjected to heat stress.
Materials and Methods: This study involved 32 healthy adult male Wistar rats, divided into eight groups: control, control + RJ, 37°C heat stress, 37°C heat stress + RJ, 40°C heat stress, 40°C heat stress + RJ, 42°C heat stress, and 42°C heat stress + RJ. The rats were exposed to heat stress in warm water baths at 37, 40, and 42°C for 20 minutes daily over a 42-day period (6 weeks). After heat exposure, RJ was administered orally by gavage. Following the experimental period, the left testis was extracted for histological examination and fixed in 15% formalin, while the right testis was stored at -70°C for molecular analysis.
Results: Histological examination revealed that the control and control + RJ groups exhibited normal testicular morphology with active spermatogenesis. Heat stress induced significant, temperature-dependent structural changes in the seminiferous tubules and disrupted spermatogenesis. However, RJ administration markedly reduced these histopathological changes in the 37°C + RJ, 40°C + RJ, and 42°C + RJ groups.
Conclusion: These findings suggest that RJ may have a protective effect against heat-induced testicular damage and could enhance sperm quality by mitigating heat stress-related injuries. The study provides promising insights into the potential therapeutic role of RJ in combating heat stress-induced testicular damage and improving reproductive health.
Keywords: Heat Stress, Ferroptosis, Royal Jelly, Testicular Damage, Spermatogenesis

P-35: Effect of Different Doses of Zinc Gluconate on Spermatogenesis and Sperm Parameters in NMRI Mice

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Background: The long-term effects of oxidative stress can lead to detrimental effects on male fertility, including DNA damage, impaired sperm quality, and hormonal imbalances. Antioxidant therapy has gained popularity as a treatment, but the optimal approach is not well-established, and excessive use may be harmful. Therefore, the purpose of this study was to investigate the effects of different doses of zinc gluconate on spermatogenesis and sperm parameters in healthy NMRI mice.
Materials and Methods: To achieve this purpose, 9 male NMRI mice were randomly divided into three groups (N=3). The experimental groups were as follows: (1) Control: no zinc gluconate (ZG) supplementation, (2) Low dose: 1 mg/kg of ZG, and (3) High dose: 9 mg/kg of ZG. After 4 weeks, the mice were sacrificed, epididymal spermatozoa were evaluated for sperm parameters, and histopathological examinations were conducted on testicular tissue. Statistical analysis used one-way ANOVA and LSD post hoc test, with a significance level set at P<0.05.
Results: The findings revealed that administering a high dose of ZG resulted in a significant reduction in sperm progressive motility (P<0.05) compared to the control and low-dose groups. There were no significant differences in sperm concentration or abnormal morphology among the groups. Interestingly, sperm total motility was significantly higher in the low-dose group compared to the controls (P<0.05). Furthermore, neither low nor high doses of ZG caused a significant change in Johnsen's testicular histopathological score
Conclusion: The study found that administering high-dose ZG to mice through drinking water for four weeks led to a decline in sperm motility. Zinc seems to interact with sulfhydryl groups and disulfide bonds in sperm, particularly in the tail, indicating

its role in motility. This suggests that high zinc levels could harm these key structures, impairing sperm movement. However, the exact mechanisms remain unclear, necessitating additional research to understand how excess zinc impacts sperm function

Keywords: Male Infertility, Sperm Chromatin Integrity, Zinc Gluconate, Supplementations, Mouse

Animal Biotechnology

P-36: Immature Mouse Oocytes Demonstrate Enhanced Maturation, Fertilization Potential, and Embryo Development Outcomes Following Treatment with Platelet-Rich Plasma

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Background: This study aimed to investigate the effects of platelet-rich plasma (PRP) supplementation in the Maturation (IVM) medium on various parameters including Morphometric Characteristics, Apoptotic Gene Expression, Fertilization rate and embryo development of Immature Mouse Oocytes quality and maturation.
Materials and Methods: Germinal vesicle (GV) stage oocytes with cumulus cells were collected from mature female BALB/c mice and divided into control and experimental groups. In the experimental group, GV oocytes were cultured in an IVM medium supplemented with 5% PRP, while the control group oocytes were cultured without PRP. Several parameters were assessed, including the proportion of GV, MI, MII, and degenerated oocytes, Zona pellucida (ZP) thickness, Perivitelline space size (PVS), mature oocyte diameter, expression of apoptosis-related genes, and subsequent development of matured oocytes.
Results: The PRP-supplemented group showed significant improvements in numerous parameters compared to the control group, including a higher proportion of MII oocytes, fertilized oocytes, cleavage, and blastocyst embryos. Additionally, ZP thickness was significantly lower in the PRP group ($P<0.05$). The expression of apoptosis-related genes Bax and caspase-3 was significantly downregulated, while the apoptosis inhibitor Bcl2l1 was upregulated in the PRP group compared to the control group ($P<0.05$).
Conclusion: Supplementing the IVM culture media with PRP led to enhanced oocyte maturation, fertilization, and early embryonic development. This suggests that PRP can positively influence oocyte quality and maturation efficiency during IVM, thereby offering potential improvements for fertility treatments.
Keywords: Platelet-Rich Plasma, Fertilization, Immature Follicles, Mouse, Apoptosis

P-37: The Protective Impacts of Spirulina on Reproductive Toxicity Induced by Cisplatin in Male Rats

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Background: Cisplatin is a chemotherapy drug that is widely used for treating various types of cancers. Oxidative stress may be a cause of cisplatin toxicity in male reproductive organs and, therefore, antioxidants might reduce the distractive effects of oxidative stress induced by cisplatin. The present study aimed to investigate the efficacy of Spirulina platensis, a microalga rich in antioxidant compounds, against cisplatin-induced oxidative damage.
Materials and Methods: In this study, 30 male rats were divided into 3 groups: 1) control group (rats which received normal saline); 2) cisplatin group (rats which received cisplatin, 5mg/kg BW); and 3) spirulina+ cisplatin group (rats which received injections of cisplatin (5mg/kg BW) + 5% spirulina in the fed diet). After one month, the levels of reproductive hormones [Testosterone (T), Follicle-Stimulating Hormone (FSH), and Luteinizing Hormone (LH)] and antioxidant enzymes (glutathione: GSH, superoxide dismutase: SOD), and lipid peroxidation (malondialdehyde: MDA level) in the blood samples and also epididymal sperm parameters were tested in these groups.
Results: Our results showed that FSH, LH, T, GSH, and SOD levels, as well as sperm count, motility, viability, and normal morphology, decreased, and the MDA amount increased in the cisplatin group compared with the control group. In the spirulina + cisplatin group, spirulina could compensate adverse effects of cisplatin compared with the cisplatin group.
Conclusion: Spirulina with its antioxidant properties was able to reverse the negative effects of cisplatin and improve parameters involved in the reproduction.
Keywords: Spirulina, Cisplatin, Sperm parameters

P-38: Assessment of Streptozotocin-Induced Diabetes Effects on The One-Carbon Cycle and Sperm Functionality in Mice

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Background: Men with diabetes face an elevated risk of infertility, often characterized by indicators of oxidative damage and reduced methylation in sperm, indicative of a deficiency in the one-carbon cycle (1CC). To delve deeper into this phenomenon, our study sought to explore the impact of diabetes on

the one-carbon cycle using mouse models of streptozotocin-induced diabetes, encompassing both type 1 and type 2 diabetes.
Materials and Methods: In this experimental study, we divided 50 male mice, aged eight weeks, into four groups: sham, control, type 1 diabetes mMellitus (DM1), and DM2. The DM1 group underwent an eight-week regimen of a Normal Diet (ND), followed by five consecutive days of intraperitoneal streptozotocin (STZ) injections at a dosage of 50 mg/kg body weight. Conversely, the DM2 group was subjected to an eight-week high-fat diet (HFD), succeeded by a single intraperitoneal injection of STZ at a higher dosage of 100 mg/kg. After twelve weeks, all mice were euthanized for parameter assessment. Notably, the sham group received citrate buffer injections as the solvent for STZ.
Results: Both types of diabetic animals exhibited severe impairment in spermatogenesis, characterized by heightened DNA damage (P=0.000), reduced chromatin methylation (percent: P=0.019; intensity: P=0.001), and compromised maturation (P=0.000). Additionally, disruptions in the one-carbon cycle (1CC) were evident, marked by elevated homocysteine levels (P=0.000) and diminished availability of carbon units [methionine (P=0.000), serine (P=0.088), folate (P=0.016), B12 (P=0.025)] required for methylation processes.
Conclusion: We've noted a distinct impairment of the one-carbon cycle (1CC) in diabetic individuals' testes, likely due to insufficient intracellular glucose and reduced carbon unit supply. Addressing these issues through interventions enhancing glucose uptake into sperm cells and providing extra methyl donors could potentially improve fertility in diabetic patients, pending further clinical validation.
Keywords: Sperm function, Chromatin, Diabetes, Glucose, Methylations

P-39: Investigating The Effects of Hydrogen Sulfide on Sperm Parameters and Testicular Histology in Rats Fed A High-Fat Diet

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Background: A high-fat diet is a well-known risk factor for male infertility and reproductive dysfunction. Overconsumption of fat can impair male reproductive function. Hydrogen sulfide (H2S) has beneficial effects involved in regulating various biological processes. To investigate the potential protective effects of sodium hydrosulfide (NaHS), an H2S donor, on sperm quality and testicular histomorphometry in a high-fat diet (HFD) rat model.
Materials and Methods: Male Wistar rats were divided into 6 groups (n=6/group): control, control + 3 mg/kg/day NaHS, control + 5 mg/kg/day NaHS, HFD, HFD + 3 mg/kg/day NaHS, HFD + 5 mg/kg/day NaHS. After 11 weeks on their respective diets, NaHS was administered daily via intraperitoneal injection for 4 weeks. Sperm parameters (count, motility, vitality, morphology) and testicular histomorphometry (spermatogenic cells, Sertoli cells, Leydig cells, seminiferous tubule diameter) were evaluated.
Results: The HFD group exhibited significantly reduced sperm parameters (P<0.05) and impaired testicular histomorphometry (P<0.01) versus control. NaHS supplementation dose-dependently improved sperm count, motility, vitality, morphology (P<0.05), and markers of testicular histomorphometry like spermatogenic cells and seminiferous tubule diameter (P<0.01), except Sertoli and Leydig cells.
Conclusion: Exogenous H2S administration via NaHS mitigated HFD-induced alterations in sperm quality and testicular histomorphometry, suggesting a protective role against diet-induced male infertility.
Keywords: Hydrogen Sulfide, Sperm Parameters, Testicular Histology, High-Fat Diet, Male Infertility

P-40: Investigating The Difference in Rumen Fluid pH and Volatile Fatty Acid in Estrus and Anestrus Sheep of Grey Shirazi in Breeding Season

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Background: In this study, the main goal is to investigate the changes in the rumen pH and volatile fatty acid (VFA) during the breeding season in the estrous period for estrus and anestrous Grey Shirazi ewes.
Materials and Methods: To investigate pH and VFA changes in rumen fluid during the estrous cycle of estrus and anestrus animals, two groups of 10 estrus anestrus animals were formed, and rumen fluid samples were taken from every four days. In order to check the pH, we collected rumen fluid samples every day four day based on the cycle of each group and measured its pH using a pH meter. This measurement was between 3-4 hours after feeding by animals of each groups. Volatile fatty acids VFAs were measured from the collected samples at the beginning and end of the estrous cycle by using gas chromatography, the sample after defrosting the rumen fluid at 4 degrees Celsius and preparing for analysis in gas chromatography, about 800 microliters of it was used.
Results: Changes in pH showed that, on the first day or time 1, its value was higher in the cyclic group than in the acyclic group, and after four days it decreased significantly, which was not significantly different at this time (2) with time 4. In the results related to VFA, it was also observed that the amount of acetate decreased on day 1, which will help the pH conditions of the rumen and reduce it, and propionate is important in providing microbial protein and nutritional nutrient.
Conclusion: Overall, in the study, the results of pH and VFA obtained from the rumen fluid in the estrus group showed that the significant difference on certain days with the anestrus group.
Keywords: Rumen Fluid, pH, Estrus, VFA

P-41: Assessing The Impact of Advanced Glycation End Products on Sperm Health in C57BL/6 Mice

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Background: Advanced glycation end products (AGEs) are prevalent in metabolic disorders like diabetes, obesity, and infertility-related conditions, where they exert adverse effects on cellular and tissue health. To better understand how AGEs affect both sperm structure and function, our research used mouse models exposed to tailored diets that promote AGE accumulation.
Materials and Methods: In this experiment, we divided two groups of 5-week-old C57BL/6 mice: one fed a control diet and the other an AGE-enriched diet. After 13 weeks, we assessed various parameters, such as Fasting Blood Glucose (FBS) and sperm structure and function. Additionally, we examined testicular superoxide dismutase levels, malondialdehyde content, total antioxidant capacity, Johnson score, and the presence of RAGE and Carboxymethyl Lysine (CML) proteins.
Results: After 13 weeks, we observed significant differences between AGE and control groups. AGE group showed an increase in FBS levels compared with the control group ($P<0.005$). With regard to sperm parameters, AGE group showed lower mean values and a higher percentage of sperm abnormalities, including nuclear histone retention, chromatin deficiencies, DNA fragmentation, increased membrane lipid peroxidation, compared to the control group ($P<0.005$). In addition, AGE group showed a significant reduction in total testicular antioxidant capacity and a lower Johnson score compared to the control group ($P<0.005$). Mean levels of testicular superoxide dismutase did not differ significantly between the two groups ($P>0.005$). However, the AGE group had the highest mean level of testicular malondialdehyde content, as well as higher accumulation of RAGE and CML proteins compared to the control group ($P<0.005$).
Conclusion: AGEs have negative effects on male reproductive health, causing metabolic problems, sperm abnormalities and oxidative stress, highlighting the role these compounds can play in male infertility, particularly in the case of metabolic disorders.
Keywords: Advanced Glycosylation End Products, Carboxymethyl Lysine, Receptor of Advanced Glycation End Products, Sperm Parameters, Sperm Function

P-42: Investigating Effect of Diclofenac Sodium on Serum Level of Total Antioxidant Capacity in Rat Models of Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is the most common hormonal disorder in women of reproductive age worldwide. Oxidative stress has been found to be closely related to the onset and progression of PCOS, leading to chronic low-grade inflammation in these individuals. Diclofenac sodium (DIC) is a non-steroidal anti-inflammatory drug. Since there is not enough information to understand the effect of diclofenac on reducing inflammation in PCOS, the aim of this study is to determine the effect of sodium diclofenac on the level of antioxidant and anti-inflammatory factors in the ovarian tissue of PCOS rats.
Materials and Methods: In this study, 25 adult female Wistar rats with an approximate weight of 180 to 200 grams were examined. In order to induce PCOS in animals, estradiol valerate (EV) was used. Rats were randomly divided into five experimental groups (5 in each group) as follows: Group 1 (control; received olive oil), Group 2: EV (0.3 mg/kg) and saline, Group 3, 4 and 5 received diclofenac with doses of 2.5, 5 and 10 mg/kg, respectively. Also, animals received a single dose of EV and were treated with diclofenac for one week after 28 days. Then, the antioxidant status was evaluated in rats with PCOS and the healthy group.
Results: The total antioxidant capacity (TAC) in the groups treated with different doses of diclofenac significantly increased compared to the PCOS control group ($P<0.05$).
Conclusion: The results show that the exposure of groups with PCOS to different doses of diclofenac can affect the reduction of inflammation. Although this was an animal study, the clinical results should be discussed again.
Keywords: Polycystic Ovary Syndrome, Total Antioxidant Capacity, Antioxidant, Rat

P-43: Navigating Spermatogenesis: Investigating Transsulfuration Pathway in Vitamin D Deficient Mice

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Background: Vitamin D deficiency (VDD) is a significant health issue, associated with heightened oxidative stress (OS) across several clinical contexts, including obesity and male infertility. The transsulfuration pathway plays a crucial role in preserving redox balance by generating antioxidants like Glutathione (GSH) and H2S and regulating homocysteine levels. Disruptions in this pathway can escalate OS levels and have been linked to various health conditions, including male infertility. Hence, this study aimed to evaluate the impact of VDD on sperm functionality, the enzymes governing the transsulfuration pathway, and the expression of HO-1 in a mouse model.
Materials and Methods: In this study, sixteen male C57 mice were randomly allocated to either the control or VDD groups for 14 weeks. Following this duration, sperm quality parameters were assessed, and the expression levels of Cystathionine Beta-Synthase (CBS), cystathionine gamma-lyase (CSE), and heme oxygenase-1 (HO-1) in testicular spermatogenic cells were analyzed using Reverse Transcription-Polymerase Chain Reaction (RT-PCR), western blot, and immunofluorescence techniques.
Results: The VDD group exhibited increased body weight, reduced sperm quality, testicular damage, and decreased testosterone levels compared to controls. VDD elevated serum homocysteine, vitamin B12, and sperm oxidative stress markers.

In testicular tissue, CBS and CSE proteins were downregulated, while HO-1 was upregulated at mRNA and protein levels.
Conclusion: In a VDD mouse model, testosterone and spermatogenesis were impaired via OS mechanisms independent of vitamin D's classical actions, indicating a specific disruption in the alternative transsulfuration pathway.
Keywords: Spermatogenesis, Vitamin D Deficiency, Transsulfuration Pathway, Mouse, Oxidative Stress

P-44: High DNA Stainability: A Reliable Indicator of Sperm Nuclear Integrity?

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Background: The sperm chromatin structure assay (SCSA®) identifies both the DNA fragmentation index (DFI) and high DNA stainability (HDS), which reflects sperm nuclear compaction. However, the significance and utility of HDS remain unclear. To address this, spermatozoa from 397 infertile men underwent SCSA®, Transferase dUTP Nick End Labeling (TUNEL), and chromomycin A3 (CMA3) tests, with 100 men additionally undergoing Aniline Blue (AB) and Toluidine Blue (TB) staining. This study aims to determine the relevance and reliability of HDS.
Materials and Methods: Semen samples from 397 infertile men underwent analysis using the SCSA®, TUNEL, and CMA3 tests. Additionally, a smaller subset (N = 100) underwent AB and TB staining, in addition to the SCSA®, TUNEL, and CMA3 tests. All male patients (n = 397, mean age = 36.78 years) participating in the study provided signed consent forms. We used SPSS software (version 22; Chicago, IL, USA) for analysis. Descriptive statistics presented means ± SD, while Pearson correlation and ANOVA tests determined relationships and differences ($p < 0.05$).
Results: HDS seems to lack reliability as an indicator of nuclear immaturity, given its weak correlation with CMA3, AB, and TB stains. The low association between HDS and sperm DNA fragmentation (TUNEL and SCSA®), as well as DNA condensation (CMA3, AB, and TB) tests, suggests a potential decoupling of these parameters. In contrast to DFI and TUNEL, HDS has not demonstrated correlation with typical clinical scenarios of male infertility such as asthenozoospermia, teratozoospermia, or astheno-teratozoospermia.
Conclusion: HDS shows weak correlations with tests assessing sperm nucleus maturity. This study represents the first comparison of SCSA®, TUNEL, AB, TB, and CMA3 assays on identical samples, revealing their strengths, weaknesses, and the need for careful interpretation.
Keywords: High DNA Stainability, Sperm Nuclear Integrity, Sperm DNA Fragmentation, Sperm DNA Condensation

P-45: The Impact of Various Sperm Preparation Media on Cryopreserved Buffalo Spermatozoa

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Background: The domestic buffalo is vital for the dairy and meat industries but faces reproductive challenges that affect fertility. These challenges include low semen volume, inadequate buffering capacity leading to a slightly acidic pH, seasonal variations affecting semen quality, and reduced viability and fertility after cryopreservation. Addressing the post-freezing sperm quality decline is crucial, necessitating a nutrient medium to improve sperm viability. Our study aims to compare and choose the best nutrient medium to improve sperm parameters using the swim-up technique.
Materials and Methods: The study compared five different media: human tubal fluid (HTF), Tyrode's albumin lactate pruvate (TALP), HAM'S F10, INRA, and Multi-Wash on their ability to improve the quality of cryopreserved buffalo sperm. The samples were incubated with the media to perform the swim-up technique. After that, the media top layer was collected and their parameters such as (concentration, motility, and progressive motility) were evaluated by a computer-assisted sperm analysis (CASA) system.
Results: HTF medium significantly improved sperm concentration and motility over the other media following swim-up. TALP medium also showed improvement in sperm concentration and motility after HTF but was significantly better only when compared to Multi-Wash. HAM'S F10 and INRA media had similar effects, they showed improvement in both mentioned parameters, but it was non-significant, Multi-Wash was identified as the least effective. No significant relationship was observed between different media in terms of progressive motility.
Conclusion: HTF medium is the most effective for the swim-up process, marking a significant contribution to improving buffalo reproductive health and potentially increasing fertility rates through assisted reproductive technologies.
Keywords: Swim-Up Technique, Buffalo Sperm, HTF

P-46: Investigating The Effect of One Month Fasting on The Signaling Pathway of Insulin Resistance on Rat Model of Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a hormonal and endocrine disorder and is among the most prevalent causes of infertility in women of reproductive age. Insulin resistance is a common occurrence in women with PCOS and plays a significant pathophysiological role in the metabolic and reproductive complications associated with the condition. This insulin resistance is typically attributed to excess visceral fat tissue and pro-inflammatory mechanisms. This study aimed to investigate the effects of one month of fasting on the insulin resistance signaling pathway in a mouse model of PCOS.
Materials and Methods: Female Sprague-Dawley rats were randomly assigned to three groups. The control group received a standard diet for 16 weeks, along with daily gavage of car-

boxymethyl cellulose (CMC) from the eighth week for 21 days. The PCOS group was fed a high-fat diet (HFD) for 16 weeks, and additionally underwent PCOS induction with letrozole (dissolved in CMC) by gavage from the eighth week for 21 days. The fasting group experienced the same conditions as the PCOS group for 16 weeks, with the exception that starting from the end of the eighth week, they underwent fasting for 12 hours during both day and night for 30 days. Following the fasting period, the animals were euthanized with ketamine and xylazine, and ovarian tissue samples were collected to examine histological characteristics and changes in the expression of specific genes.
Results: The results of our study demonstrated that fasting led to improvements in the metabolic indices of insulin resistance. Furthermore, fasting induced a significant increase in the expression of genes involved in the insulin resistance signaling pathway, including Pi3k, Akt, Pten, Pdk1, Glut4, and Irs1.
Conclusion: These findings suggest that fasting could serve as a potential therapeutic solution for modifying the metabolic syndrome associated with PCOS. Further studies are warranted to comprehensively evaluate the therapeutic efficacy of fasting in managing PCOS-related complications.
Keywords: Polycystic Ovary Syndrome, Insulin Resistance Signaling Pathway, Fasting

P-47: Follicle-Like Structure Generation from Sheep Theca Stem Cells

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Background: Considering the significant increase in infertility rates, as well as the continuous growth and advancements of treatment methods and fertility assistance in recent years, treatments based on the use of stem cells are promising strategies in this direction. In line with human studies, Adib showed that sheep theca stem cells can differentiate into germ cell-like cells. This study explores the differentiation of sheep theca stem cells into germ cell-like cells and forming follicle-like structures through a novel centrifugation technique.
Materials and Methods: Initially, sheep theca stem cells were induced to differentiate into germ cell-like cells using a differentiated medium. Following differentiation, the germ cell-like cells were collected and combined with theca stem cells, and phytohemagglutinin was added to promote cell cohesion. The suspension was centrifuged, and the tube was then rotated 180° and centrifuged again. The cell pellet is now called a Reaggregated follicle (RF). This combination was then cultured in SAGE culture media for one week to promote the development of RF.
Results: The resulting RF structures demonstrated promising potential for further reproductive biology and biotechnology applications.
Conclusion: The findings of this study provide a foundation for future research into ovarian tissue engineering and the generation of functional ovarian structures from stem cells.
Keywords: Reaggregated Follicle, Theca Stem Cell, Germ Cell-Like Cells

P-48: Evaluation of Isolated Heart Function ىollowing Ischemia-Reperfusion Induction in Polycystic Ovary Syndrome Model Rats: Role of Endoplasmic Reticulum Stress

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, which seems to increase the risk of heart disorders. In this study, we aim to investigate the response of cardiac muscle to ischemia-reperfusion injury and evaluate the possible role of endoplasmic reticulum stress (ERS) in this response in rats with PCOS.
Materials and Methods: 40 female Wistar rats were randomly divided into five groups of 8, including control (CTR), PCOS, cardiac Ischemia-Reperfusion (IR), polycystic ovary + cardiac ischemia-reperfusion (PCOS + IR), polycystic ovary + cardiac ischemia-reperfusion + ERS inhibitor (PCOS + IR + SLB).The PCOS model was induced through letrozole gavage, and then the heart was isolated and transferred to the Langendorff apparatuse. Ischemia was induced by 30 minutes ligation of left anterior descending artery. Reperfusion fallowed by remove of ligation for 60 minutes. The size of myocardial infarction using TTC staining, cardiac tissue changes with the help of H&E Staining, the amount of GRP78 and ATF4 proteins (indicators of ERS) and caspase 8 (apoptosis marker) in cardiac tissue by using western blot method were measured.
Results: The size of the infarct area ($P<0.01$) increased significantly in the PCOS+IR compared to the IR. The levels of GRP78 and ATF4 and caspase 8 in cardiac tissue also showed a significant increase in the PCOS ($P<0.01$) and PCOS+IR ($P<0.001$) compared to the control. Administering salubrinal in PCOS+IR+SLB could significantly reduce the infarct size ($P<0.001$), the expression of GRP78 and ATF4 and caspase 8 ($P<0.01$) compared to the PCOS+IR.
Conclusion: The present study showed that the induction of PCOS by developing ERS aggravated the damage caused by ischemia-reperfusion in isolated rat cardiac tissue which indicates a greater susceptibility to heart damage in patients with PCOS.
Keywords: Polycystic Ovary Syndrome, Ischemia-Reperfusion, Endoplasmic Reticulum Stress

P-49: Investigating The Effects of Reishi on Testicular Tissue of Infertile Patients Caused by Induction of Human Breast Cancer in An Animal Model

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Background: Breast cancer is the most common type of cancer, especially among women around the world, which leads to death, annually. It also affects a significant percentage of men. A complication in men suffering from this disease is pathological damage to the testicles. Reishi (Ganoderma Lucidum) is an important candidate for affecting the mentioned problems. This study examines the effects of this mushroom in the form of complementary medicine on mammary tumors and testicular tissues.
Materials and Methods: In this study, 12 male nude mice were used, which were randomly divided into three equal groups: G1 (healthy mice), G2 (tumor bearing mice without therapeutic interventions) and G3 (tumor bearing mice treated with Reishi). Human breast cancer cell line MCF-7 was cultured and 5 million cells were injected subcutaneously in the left flank of each mouse. After the appearance of tumor masses, a one-month treatment period was started. 100λ aqueous extract of Ganoderma was gavage to the mice of G2 and G3, daily. All mice were kept under standard conditions. Finally, the tumor tissues as well as the testicular tissue of the mice were examined by a pathologist in terms of histopathology.
Results: The weight and volume of tumors in group three showed a significant decrease. The pathology slides of testicular tissues in groups one and three showed normal structure, but in group two, testicular atrophy was evident with high intensity.
Conclusion: The results of this study showed that the aqueous extract of Ganoderma (AshianGanoTeb Biopharmaceutical Company) has a good therapeutic effect on breast tumors. Also, this extract can treat infertile patients with atrophic testes. For this reason, Ganoderma can be considered as a good complementary medicine for the treatment of both breast cancer and its infertility complications.
Keywords: Infertility, Ganoderma Lucidum, Reproductive System, Breast Cancer

P-50: Omega-3 Ameliorative Effects Inhibit Varicocele-Induced Inflammation Condition in Rats

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Background: Varicocele is associated to a progressive decrease in male fertile potential, but antioxidant/anti-inflammatory therapy has been highlighted as the safe approach for possibly improving varicocele condition. This experimental study was carried out to investigate the Omega-3 effects of varicocele condition in rats, focusing on varicocele-induced inflammation condition.
Materials and Methods: Eighteen mature male Wistar rats (200 ± 25 g) were purchased and after 2 weeks of adaptation, rats were randomly divided (n=7/group) into control sham (Control), Varicocele (VCL-2), and varicocele-treated with Omega-3 (600 mg/kg; VCL-2+OMG3). Following two months, the animals were euthanized, left testicules were dissected out and stored in -80OC for furthermore evaluations. IL-6, 1β, and 10, TNF-α, NOX-2 and 4, MCP-1, and HIF1a protein levels were assessed using western blotting technique. Relative density of protein expression was calculated using Image Lab software and β-actin protein expression was considered as the standard.
Results: The results demonstrated that IL-6, 1β, and 10, TNF-α, MCP-1, and HIF1a proteins expression were increased significantly ($P<0.05$) in VCL-2 group compared to Control group. However, no statistically significant difference ($P<0.05$) was observed in case of NOX-2 and 4 between three groups. On the other hand, OMG3 treatment for 60 days could significantly reduce IL-6, 1β, and 10, MCP-1, and HIF1a.
Conclusion: Pro-inflammatory cytokines increase in varicocele-induced rats testis demonstrated that experimental varicocele can lead to inflammatory condition in testis. Reduced pro-inflammatory cytokine reduction in Omega-3 treated animals demonstrated the ameliorative impacts of this therapy, and Omega-3 treatment can be used as a possible treatment of varicocele. However, it should be mentioned that to use this agent at the clinical level, more clinical trials are needed.
Keywords: Varicocele, Rat, Omega-3, Inflammation

P-51: Effects of Methane Rich Saline on Sperm Parameters in Wistar Rats Exposed to Lead

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Background: Lead is a heavy metal with numerous applications due to its features. Lead poisoning results in various general injuries. The reproductive system is among the target organs affected by lead. Lead impacts the hypothalamus-pituitary-testicular axis, lowering the sex hormones luteinising hormone (LH), follicle-stimulating hormone (FSH), and disrupting the functions of Sertoli and Leydig cells. This disruption decreased testosterone production, causing testicular atrophy and disturbances in spermatogenesis. Methane can mitigate the damage caused by lead poisoning to sperm by exerting an anti-inflammatory and antioxidant effect.
Materials and Methods: 35 adult Wistar rats (aged 8 weeks) weighing approximately 180-220 grams, were randomly allocated into five groups of seven. sham group receiving intraperitoneal normal saline, methane group receiving intraperitoneal MRS at 5 ml/kg, lead group receiving intraperitoneal lead at 10 mg/kg/IP, a lower dose treatment group receiving intraperitoneal lead at 10 mg/kg/IP and MRS at 2 ml/kg, and a maximum dose treatment group receiving intraperitoneal lead at 10 mg/kg/IP and MRS at 5 ml/kg. Twenty hours post final injection, following anesthesia and blood collection, the right epididymis tail was isolated and preserved in Ham's F10 solution for sperm analysis, including assessment of movement, number, vitality (eosin staining), and morphology (Papanicolaou staining) under microscopic examination.
Results: Sperm parameters in the lead group significantly de-

creased compared to other groups. The percentage of sperm parameters in the treated groups showed a notable increase compared to the lead group. Additionally, sperm parameters in the MRS group significantly increased compared to all other groups (P<0.05).
Conclusion: Lead reduces sperm parameters, while MRS not only enhances sperm parameters but also retrieve the toxic effects of lead on sperm parameters.
Keywords: Lead, MRS, Sperm Parameters, Wistar Rat

P-52: Effects of Autologous Conditioned Serum on Mouse Spermatogenesis

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Background: spermatogenesis requires a specialized media to support the complex process of sperm development and maturation. Introduced culture media, such as knockout Serum Replacement (KSR), have several restrictions such as low efficiency compared to *in vivo* conditions and ineffectiveness for all mice strains and other species. The aim of this study is to evaluate the effects of Autologous Conditioned serum (ACS) in supporting mouse spermatogenesis.
Materials and Methods: Immature testicular tissue of NMRI mice were cultured on agarose gel in two media including α-MEM enriched with 5% ACS and 10%ACS for 42 days. The spermatogenic tubules integrity was assessed applying a scoring system (1-4, worst to best). Immunofluorescence tests were carried out with primary antibodies targeting PLZF, SYCP-3, ACRBP, SOX9, and STAR to distinguish between spermatogonial stem cells, spermatocytes, sperm-like cells, Sertoli cells, and Leydig cells, respectively. Additionally, Ki67 and caspase 3 markers were analyzed to examine proliferating and apoptotic cells, respectively.
Results: After culturing tissues for 42 days and mechanically dissociating them, round spermatid-like cells were observed in fragments cultured in 5% ACS. Morphological evaluation revealed that spermatogonial stem cells differentiated up to the round spermatid in 5% ACS. The 5% ACS media also preserved significantly higher percentage of tubules based on the integrity scoring (3-4) compared to the 10% ACS. Moreover, quantitative analysis showed a significant increase in the number of PLZF+, SYCP3+, ACRBP+, SOX9+, and Ki67+ cells per tubules in 5% ACS compared to 10% ACS. The average number caspase3+ per tubule was significantly higher in the 10% ACS than in the 5% ACS. However, there was no significant difference in the number of STAR+ cells per tubule between the two media.
Conclusion: This study showed that media supplemented with 5% ACS compared to 10% ACS can more effectively induce spermatogenesis up to round spermatid stage in NMRI mice.
Keywords: Testicular Organ Culture, ACS, Spermatogenesis, Testicular Tissue

P-53: Investigating The Effect of Tetrathiomolybdate on Glutathione Peroxidase Enzyme Activity in Polycystic Ovary Syndrome in Wistar Rats

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Background: Polycystic ovary syndrome (PCOS) causes disruption of sex hormones in the ovaries of women of reproductive age. Many factors contribute to the development of PCOS; Research has shown that the main cause of polycystic ovary syndrome is oxidative stress (OS) produced by mitochondria and chronic inflammation. Tetrathiomolybdate (TTM) is a substance that inhibits the pathway of oxidative stress. The aim of this study is to investigate the effect of TTM on glutathione peroxidase (GPx) enzyme activity in polycystic ovary syndrome in PCOS model of Wistar rats.
Materials and Methods: In this study, estradiol valerate (EV) was used to induce PCOS. 25 adult female Wistar rats with an approximate weight of 150 to 185 grams were used. Rats were randomly divided into five experimental groups (5 rats in each group) as follows: group 1: control (received olive oil), Group 2: EV 0.3 mg/kg and saline, group TA, TB and TC were treated with TTM with doses of 5, 10, 20 mg/kg, respectively. In order to induce PCOS, the animals received a single dose of EV After 28 days, they were treated subcutaneously with TTM for 14 days. Then, GPx enzyme activity status was evaluated in rat with PCOS and healthy group by ELISA method.
Results: The level of GPx enzyme activity in the groups treated with TTM and also in different doses of TTM increased significantly (P<0.05) compared to the PCOS control group.
Conclusion: TTM is effective in controlling PCOS by inhibiting oxidative stress and reducing inflammation, and in the future it can be used as for the treatment of this disease, although it needs more research and study.
Keywords: Polycystic Ovary Syndrome, Oxidative Stress, Tetrathiomolybdate, Glutathione Peroxidase, Inflammation

P-54: Balancing Hormones in Polycystic Ovary Syndrome: Examining The Effects of Nutrition Bio-Shield Supplement in A Rat Model Study

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age, characterized by various clinical and metabolic disturbances. This study aimed to evaluate the impact of a comprehensive nutritional supplement called Nutrition Bio-shield Superfood (NBS) on serum hormone levels in an animal model of PCOS.
Materials and Methods: Rats with PCOS induced by letrozole were divided into five groups, each consisting of five rats. Three groups received different doses of NBS superfood for a

period of 21 days, while two control groups (disease and healthy controls) did not receive any supplementation. Serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, progesterone, and estradiol were measured using radioimmunoassay.
Results: Treatment with NBS significantly decreased serum levels of LH, FSH, and testosterone in rats with PCOS ($P<0.05$). Additionally, it significantly increased levels of progesterone and estradiol ($P<0.05$). Among the three different doses of NBS, the dose of 50 mg/kg exhibited the most notable effect in improving the serum hormone profile. These findings suggest that NBS, particularly at a dose of 50 mg/kg, effectively restores hormonal balance and holds potential therapeutic benefits for PCOS.
Conclusion: Considering the complexities in managing PCOS, including metabolic abnormalities, delayed diagnosis, and suboptimal treatment regimens, these findings indicate that NBS, particularly at this optimal dose, can effectively correct the hormonal dysregulation characteristic of the condition. Consequently, NBS shows significant promise as a complementary therapeutic agent for managing PCOS, addressing the critical need for more effective and personalized treatment options amidst the multifaceted challenges of PCOS management.
Keywords: Polycystic Ovary Syndrome, Dietary Supplements, Endocrine Disorders

P-55: A Systematic Review of Alpha-Lipoic Acid's Role in Sperm Function in Rodent Models of Male Infertility

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Background: Infertility affects 10-15% of couples worldwide, with male factors contributing to half of the cases. Semen's oxidative stress plays a significant role, contributing to 30-80% of cases of male infertility. Studies suggest antioxidants may improve sperm quality and male fertility by protecting cells from oxidative damage. This systematic review explores the protective role of Alpha-Lipoic Acid (ALA), a multifaceted antioxidant, in rodent models for male infertility.
Materials and Methods: We searched four databases for papers discussing the impact of ALA treatment on male infertility in animal models. Up to December 2022, we identified 11,787 articles related to the protective effects of ALA. After evaluating the studies for relevance and assessing the risk of bias (CRD42022341370), we narrowed the list to 23 studies that explored the effects of ALA on sperm function in rodents.
Results: Of these 23 studies, 15 suggested that ALA could improve sperm parameters, while six indicated that ALA treatment significantly reduced sperm DNA damage. Seventeen papers highlighted ALA's antioxidant properties, and four noted its anti-inflammatory effects. Additionally, 13 studies suggested that ALA could modulate androgenesis, while 18 showed that ALA could restore normal testicular architecture. Lastly, two studies demonstrated that ALA was also effective in restoring reproductive performance.
Conclusion: This systematic review reveals that ALA has protective effects in rodent models of male infertility, helping restore spermatogenesis and steroidogenesis, and maintaining redox and immune balance. Both low (<50 mg/kg) and high doses (≥50 mg/kg) showed benefits, but a high risk of bias and limited study quality prevented definitive recommendations. More rigorous, placebo-controlled clinical trials are needed to identify optimal dosage and duration.
Keywords: Alpha-Lipoic Acid, Male Infertility, Rodent Models, Systematic Review

P-56: Impact of Boswellic Acid on The Regeneration of Beta Cells in Diabetic Zebrafish

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Background: Diabetes is a serious disease characterized by a failure to secrete sufficient insulin. An alternative approache for the treatment of diabetes is the regeneration of beta cells. According to the history of Iran's traditional medicine in the treatment of diabetes, it is possible that the boswellic acid plant extract is effective in are very similar. The function of zebrafish organs are remarkably similar to those of humans. This study assessed the impact of boswellic acid on the regeneration of beta cells in diabetic zebrafish.
Materials and Methods: The assessment of boswellic acid was performed in Tg (ins:kaede-NTR) transgenic zebrafish larvae in seven doses (5, 4, 3, 1.95, 1.5, 1.0, and 0.5 µg/ml). The control groups comprised of NT and NC (untreated and treated transgenic larvae with metronidazole, respectively), as well as NECA (a molecule that promotes the beta cell proliferation). The analysis of data from larvae that regenerated beta cells were conducted using Prism and ImageJ programs. The impact of optimal dose (3 µg/ml) on PDX1 and insulin levels was determined using the Polymerase Chain Reaction (PCR) method.
Results: The result of treatment of zebrafish larvae with boswellic acid showed that beta cells regenerated by zebrafish pancreas increased significantly in the NECA and NT compared to NC group ($P=0.040$).
Conclusion: The findings of the present study revealed the high ability of boswellic acid extract the regeneration of primary Tg beta cells (ins:Kaede-NTR) in the zebrafish model; hence, the extract of this compound could be evaluated in higher animal models, as well as cellular models.
Keywords: Beta Cells, Boswellic Acid, Diabetes Mellitus, Restoration, Zebrafish

P-57: Melatonin Restored Sex-Related Hormones, But Not Ovarian Follicular Function, in Menopausal Rats

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Background: Menopause occurs because of biological aging and is identified by the loss of follicular function and alteration of systemic sex-related hormone levels. Thus, enormous studies have been conducted to reduce or alleviate menopause-associated complications in elderly individuals. Here, the protective properties of melatonin were examined in menopausal rats by monitoring the fibrotic changes and sex-related hormone levels.
Materials and Methods: Twenty-month-old menopausal rats received 20 mg/mL melatonin intraperitoneally twice a week for one month. Twenty-four hours after last injection, group of rats received a single dose of hMG (20 IU) hormone. After 48 hours, rats were euthanized and sex-related hormones, TGF-β family genes, and pathological changes were studied.
Results: Data indicated a significant decrease and increase of Follicle-Stimulating hormone (FSH) ($P<0.01$) and hstradiol (E2) ($P<0.05$) respectively after melatonin therapy compared to control menopausal rats, whereas in the hMG group, increase of FSH level ($P<0.01$) was observed. Of note, no changes were observed in terms of luteinizing hormone (LH) levels. Histopathological evaluation revealed the lack of significant changes in ovarian follicular number between both groups ($P>0.05$). The deposition of collagen fibers was not significantly altered in melatonin-treated rats although the expression of smad2, smad6, smad4 and tgf-β were altered in the presence of melatonin and hMG, however, the differences were not statistically significant ($P>0.05$).
Conclusion: Data suggested melatonin can restore the sex-related hormone profile of menopausal rats without prominent effects on ovarian follicular function, development, and fibrotic changes. Melatonin application can alleviate menopause-related complications in females possibly by regulation of sex-related hormones.
Keywords: Menopause, Rats, Melatonin, Sex-Related Hormones, Fibrosis

P-58: Effect of Crocin on The Regeneration of Beta Cells in Diabetic Model Zebrafish

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Background: Diabetes is caused by destruction of beta cells due to autoimmune reactions, resulting in insulin deficiency. A method of treating diabetes involves regenerating beta cells. Given the prolonged use of medicinal plants, including crocin, in the traditional treatment of diabetes in Iran, it seems that various products derived from these plants can effectively contribute to the restoration of beta cells. Zebrafish has a high similarity to the human body. This study aimed to evaluate the effect of crocin on the regeneration of beta cells in a diabetic model zebrafish.
Materials and Methods: Crocin was evaluated in Tg (ins:CFP-NTR) transgenic zebrafish larvae in doses of 500, 125, 31.25, 7.81, and 1.95 µg/ml. The control groups included NT and NC (untreated and treated transgenic larvae with metronidazole, respectively), as well as NECA (a molecule that promotes the beta cell proliferation). The obtained data from larvae that regenerated beta cells were analyzed using Prism and ImageJ programs. The effect of optimal dose (7.81 µg/ml) on PDX1 and insulin levels was determined using the polymerase chain reaction (PCR) method.
Results: Gene expression analysis of insulin and PDX1 genes revealed that crocin at the concentration of 7.81 µg/ml had a significant effect on beta cell regeneration. The gene expression level of PDX1 was elevated in NECA group compared to the NT group, and the insulin gene was significantly increased both in treatment and NECA groups.
Conclusion: Crocin plays a significant role in regulating insulin levels and restoring beta cells; hence, it could be a promising candidate for diabetes treatment.
Keywords: Beta Cells, Crocin, Diabetes, Restoration, Zebrafish

P-59: Bovine Embryo Sexing Using Spent Embryo Culture Medium Fatty Acid Profiles; A Preliminary Practice in A Dairy Farm IVF Lab

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Background: There is a difference in metabolism between male and female embryos before implantation and gonad development and it is due to the X and Y chromosomes and their gene expression. As the industrial world is moving towards the artificial selection, sexed-embryos for economic reasons, scientists are proposing to use non-invasive methods and finding biomarker(s) to achieve the goal. While these differences can vary from consuming glucose to depletion and accumulation of specific amino acids in the SECM, our objective was to predict bovine embryos' sex using fatty acid profiling.
Materials and Methods: Bovine oocytes were aspirated, matured and fertilized in standard condition and cultured for 3 days, until cleavage stage. Then 19 hatched zygotes were transferred to single drops of culture media and incubated for 7 days. After blastocyst formation, embryos and their SECM were collected separately and frozen. Embryos were used for Polymerase chain reaction (PCR) and SECMs were used for detection of FA profiles.
Results: In the chromatogram of the culture medium the percentage of dihomo-gamma-linolenic acid (C20:3n6) were highest in male SECM (0.3, 0.60 ± 0.28 and 14.21 ± 1.99 % for basal culture media sample, female SECM and male SECM, respectively; mean ± standard division) Deschuras index for C16 fatty acids increased in the culture medium of female embryos (0.10 vs. 0.18%); this index for C18 fatty acid was almost unchanged in male and female cultures.

Conclusion: C20:3n6 fatty acid in SECM seems to be a suitable biomarker for non-invasive sexing in male embryos of bovine embryos.
Keywords: Bovine, IVF Culture Medium, Lipidomics, Non-Invasive Methods, Sex Selection

P-60: Betaine Effects on Apoptosis and Oxidative Stress in Methotrexate-Induced Testicular Damage in Mice

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Background: Methotrexate (MTX) is widely administered to manage various cancers. However, MTX induces spermatogenic defects. This study investigated the protective effects of Betaine (BET) against MTX-induced testicular damage.
Materials and Methods: Forty-eight male mice were randomly divided into six groups: control, BET (300 mg/kg), MTX (20 mg/kg), and MTX (20 mg/kg) + BET (100, 200, and 300 mg/kg) groups. Testosterone levels, histological changes, sperm quality, apoptosis, and oxidative stress biomarkers were assessed to evaluate the protective effects of BET.
Results: MTX disrupted germinal epithelium, reduced sperm quality, and decreased serum testosterone levels, as well as induced apoptosis and oxidative stress in the testicular tissue. BET dose-dependently restored the testosterone levels and Catalase (CAT) and superoxide dismutase (SOD). Furthermore, BET reduced lipid peroxidation, as indicated by decreased malondialdehyde (MDA) levels. BET preserved normal spermatogenesis, improved sperm quality, and reduced histological changes by MTX. Moreover, BET reduced apoptosis by decreasing the Bax/Bcl-2 ratio in the testicular tissue of the MTX-intoxicated mice.
Conclusion: The results indicate that BET mitigates testicular harm triggered by MTX by inhibiting apoptosis and decreasing oxidative stress levels.
Keywords: Methotrexate, Betaine, Spermatogenesis, Apoptosis, Oxidative Stress

P-61: Xylitol Improved Germ Cell Proliferation, Metabolomics Amount, and GSH-Related Reduction Imbalance in High-Fat Diet-Received Mice

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Background: The current research has investigated the protective effect of Xylitol against high-fat-diet-induced negative impact on histological characteristics, and metabolomics as well as glutathione levels.
Materials and Methods: For this purpose, immature male mice were randomly assigned to 6 groups: the control group (received normal diet), high-fat diet-induced obesity resistant (HFD-OR), high-fat diet-induced obese (HFD-O), xylitol-received (Xylitol), HFD-O+ xylitol, and HFD-induced obese which received a HFD for the first 8 weeks and xylitol for the second 8 weeks (HFDW-O+ Xylitol). The HFD was administrated for 16 weeks. The last group received a HFD for 8 weeks and continued by receiving Xylitol. The Repopulation (RI), Tubular differentiation (TDI), and spermiogenesis indices (SPI), mean distributions of leydig and sertoli cells were analyzed. To evaluate potential alterations in germ cell's Intracytoplasmic carbohydrate storage (ICS), the periodic acid-schiff (PAS) staining method was employed. The testicular lactate, lactate dehydrogenase (LDH), glucose level, GSH and GSSG content alongside with relative GSH and GSSG ratio were analyzed.
Results: A significant ($P<0.05$) reduction in the mean percentages of tubules with positive RI was revealed in all experimental groups compared to control group. No significant differences were demonstrated between the experimental groups to each other. The HFDW-O+Xylitol mice showed significant ($P<0.05$) increase in TDI and SPI percentages when compared to HFD-O mice. All the HFD-received groups exhibited significant ($P<0.05$) decrease in mean distributions of Leydig cells/one mm2 compared to control group. Moreover, no significant change was revealed in Sertoli cells number/seminiferous tubule between all experimental groups compared to the control mice. In PAS staining, the results exhibited a significant ($P<0.05$) decrease in ICS in HFD-O mice compared to control group. Remarkable ($P<0.05$) increase in HFD-O+Xylitol and HFDW-O+Xylitol groups was revealed when compared to HFD-O group. The amount of Lactate and Glucose were remarkably decreased in HFD-O group compared to control group. This situation was ameliorated in HFD-O+Xylitol and HFDW-O+Xylitol groups when compared to HFD-O group. Although the testicular LDH level was decreased in all experimental groups, it was upregulated in HFD-O+Xylitol and HFDW-O+Xylitol groups in comparison to HFD-O group. The GSH level was significantly ($P<0.05$) decreased in all experimental groups compared to control group. No remarkable changes were exhibited in GSH level between HFD-O and HFD-O+Xylitol in comparison to control group but the HFDW-O+Xylitol mice revealed a significant decrease in GSH level compared to control and HFD-O groups. All the experimental groups except HFDW-O+Xylitol showed significant ($P<0.05$) increase in relative GSH to GSSG ratio when compared to control group. Moreover, HFDW-O+Xylitol exhibited a remarkable ($P<0.05$) decrease in GSH to GSSG ratio compared to HFD-O group.
Conclusion: These findings showed that with no relation to obesity, chronic HFD consumption is able to disrupt germ cells proliferation, differentiation, and maturation through negatively affecting metabolomics, GSH, and GSH/GSSG relative ratio. Moreover, Xylitol, by rebalancing these parameters is able to ameliorate the HFD-induced impairments.
Keywords: Xylitol, High-Fat Diet, TDI, SPI, Metabolomics

P-62: Assessing The Influence of One-Month Fasting on Loss Weight in A Rat Model of Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common hormonal and endocrine disorder that causes infertility in women of reproductive age. Obesity leads to increases androgen production. Excess androgens cause abdominal obesity, which perpetuates the PCOS hormonal imbalance cycle. Obese patients with PCOS have more metabolic risk factors . weight loss has beneficial effects on metabolic, endocrine outcomes in PCOS. This study was conducted with the aim of investigating the effect of one-month of fasting on loss weight in a rat model of PCOS.
Materials and Methods: 15 female Sprague-Dawley rats were randomly divided into three groups: The control group received a normal diet for 16 weeks. the PCOS group received a high-fat diet for 16 weeks and letrozole for 28 days. The fasting group had all the conditions of the PCOS group ,but they fasted for 12 hours a day for 30 days from the end of the 12th week. The weight of all mice was measured every week and analyzed at the end of the 16th week.
Results: The results obtained from the comparison of the weight of each group showed that the trend of weighing in the PCOS group was increasing compared to the control group, but it was not statistically significant (P=1, 95% CI=-16.14 , 28.02) .Also, the weight of the fasting group decreased compared to the PCOS group, and this change was not significant (P=1, 95% CI=-23.36, 20.8).
Conclusion: These findings suggest that fasting may hold promise as an intervention for managing some aspects of PCOS.
Keywords: PCOS, Obesity, Fasting

P-63: Effect of Polyacrylonitrile Particles on Development of Mouse Embryos

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Background: polyacrylonitrile particles are one of the most widely used precursors in the manufacture of carbon nanotubes. Absorption of plastic particle to pregnant mother and passing through the placenta is harmful for embryos.
Materials and Methods: The research was conducted on 40 Balb/C type mice. After examining the vaginal plaque, the pregnant mouses were divided into 4 groups. Each one were subjected to gavage with polyacrylonitrile particles on the 7th ،8th and 9th days of pregnancy and no gavage was performed for the control group. On the 15th day of pregnancy the uterine horns were opened and the embryos were removed. All the embryos were examined for the presence of abnormalities.
Results: Despite the lack of difference in height and weight of experimental groups compared to the control group, there were significant abnormalities including: closure of the membrane between the fingers (polydactyly), eye abnormalities and closure of the eye cavity, hearing abnormalities due to the closure and non-formation of the ear cavity, head protrusion (exencephaly).
Conclusion: The polyacrylonitrile particle affect on mouse embryos and interfered on many of developmental mechanisms such as differentiation and apoptosis.
Keywords: Polyacrylonitrile Particles, Fetal Growth, Pregnant Mice, Abnormality

P-64: Evaluation of Sheep Ovarian Extracellular Matrix After Decellularization

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Background: Using the extracellular matrix (ECM) and preserving its components in tissue engineering is ever used to regenerate damaged and dysfunctional organs. The best scaffold in tissue engineering is the decellularized ECM of the target tissue which allows researchers to obtain natural, and cell-free ECMs and use the tissue as a cellular model by organizing it in 3D and 2D. This study examines the preservation of the structure of the sheep ovary after decellularization, to create a suitable biological scaffold.
Materials and Methods: Here, we used NaOH together with the DNase1 enzyme to decellularize sheep ovarian tissue. After decellularization, the amount of cell nucleus was analyzed qualitatively by DAPI staining and quantitatively by DNA content, collagen, and glycosaminoglycan content by Masson's trichrome and Allison blue staining, respectively.
Results: Evaluation of ovarian tissue showed that after decellularization, the cells were completely removed and the structure of the ovary remained intact. The average percentage of cells in the decellularized ovary is less than 5% compared to the healthy ovary and has decreased significantly ($P<0.05$).
Conclusion: In general, these results show that the structure of the extracellular matrix such as collagen and glycosaminoglycan in the decellularized ovary remains intact compared to the healthy ovary and is not damaged after decellularization.
Keywords: Sheep Ovary, Extracellular Matrix, Collagen, Glycosaminoglycan

P-65: Fe3O4/Honey Nanocomposite Improves IVF and 2-cell Formation Rates in Mouse Germinal Vesicles Vitrification

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Background: Considering that the oocyte vitrification use for fertility preservation in women causes damages to oocyte and reduces the success rate of fertilization and embryo cleavage. So, the current study intends to reduce this cryodamages by using the Fe3O4/Honey Nanocomposite (FHNC) in the mouse Germinal Vesicles (GV) vitrification medium.
Materials and Methods: The adult female NMRI mice were

applied to collect GV oocytes. The non-vitrified GVs, vitrified GVs and vitrified GVs with FHNC were considered as nVit, Vit and Vit+NC groups, respectively. The warming procedure, Maturation (IVM) and fertilization (IVF) were carried out 7 days post vitrification. Eight hours after IVF, the 2 pronuclear zygotes (2PN) were counted and moved to SAGE medium for develop to Blastocyst stage. In addition, Hoechst staining was used in 2-cell stage embryos to prove the real cell division.
Results: Based on our data, the IVF rate in Vit+NC group (88% ± 0.05) has significantly increased as compared to the Vit group (50% ± 0.05) Also, the 2-cell rate of embryos significantly increased in Vit+NC (75% ± 0.05) comparing to the Vit group (60% ± 0.05).
Conclusion: It seems that Fe3O4/honey nanocomposite can improve the vitrification outcomes: IVF rate and 2-cell embryo formation with a real cleavage.
Keywords: IVF, Fe3O4/Honey Nanocomposite, 2-Cell Embryos, Vitrification, Real Cell Division

P-66: Telomere Length Analysis in Oligozoospermic Men: A Comparative Study of Leukocytes and Sperm Cells

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Background: Telomere length serves as a crucial biological marker for genomic stability, safeguarding DNA integrity and aiding in proper chromosome alignment during replication. The correlation between telomere shortening and sperm quality deterioration highlights its significance in male infertility assessment. Hence, our study aims to evaluate leucocyte and sperm telomere length (LTL&STL), alongside sperm parameters, DNA damage, and protamine deficiency in men with oligozoospermia, contrasting findings with those from fertile men.
Materials and Methods: Blood and semen samples were obtained from 10 oligozoospermic men (sperm count <15 million/mL) and 10 fertile men at the Isfahan Fertility and Infertility Center, with written informed consent. Protamine deficiency (chromomycin A3 test), DNA fragmentation (TUNEL assay), and telomere length (quantitative real-time PCR) were assessed. Statistical analysis was conducted using SPSS 11.5, checking for normality and variances. Group comparisons were made using t-tests, with data presented as mean ± SD, and Pearson correlation used to examine relationships, with significance set at $P<0.05$.
Results: Our findings revealed a marked decrease in sperm parameters (concentration, motility, morphology), as well as in LTL & STL, alongside a notable increase in sperm DNA damage and protamine deficiency in oligozoospermic men compared to fertile individuals ($P<0.05$).
Conclusion: These findings suggest that low sperm concentration in men may indicate potential issues with meiotic and/or mitotic division during spermatogenesis. This condition is not only linked to appropriate chromatin packaging but also to telomere length, which plays a critical role in mitosis and meiosis. Telomeres aid in chromosomal alignment, pairing, synapsis, and crossing over, essential processes during spermatogenesis.
Keywords: Chromatin, Leukocyte, Oligozoospermia, Telomere Length

P-67: A Systematic Review of Alpha-Lipoic Acid's Role in Sperm Function in Rodent Models of Male Infertility

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Objective: Infertility affects 10-15% of couples worldwide, with male factors contributing to half of the cases. Semen's oxidative stress plays a significant role, contributing to 30-80% of cases of male infertility. Studies suggest antioxidants may improve sperm quality and male fertility by protecting cells from oxidative damage. This systematic review explores the protective role of alpha-lipoic acid (ALA), a multifaceted antioxidant, in rodent models for male infertility.
Material and Methods: We searched four databases for papers discussing the impact of ALA treatment on male infertility in animal models. Up to December 2022, we identified 11,787 articles related to the protective effects of ALA. After evaluating the studies for relevance and assessing the risk of bias (CRD42022341370), we narrowed the list to 23 studies that explored the effects of ALA on sperm function in rodents.
Results: Of these 23 studies, 15 suggested that ALA could improve sperm parameters, while six indicated that ALA treatment significantly reduced sperm DNA damage. Seventeen papers highlighted ALA's antioxidant properties, and four noted its anti-inflammatory effects. Additionally, 13 studies suggested that ALA could modulate androgenesis, while 18 showed that ALA could restore normal testicular architecture. Lastly, two studies demonstrated that ALA was also effective in restoring reproductive performance.
Conclusion: This systematic review reveals that ALA has protective effects in rodent models of male infertility, helping restore spermatogenesis and steroidogenesis, and maintaining redox and immune balance. Both low (<50 mg/kg) and high doses (≥50 mg/kg) showed benefits, but a high risk of bias and limited study quality prevented definitive recommendations. More rigorous, placebo-controlled clinical trials are needed to identify optimal dosage and duration.
Keyword: Alpha-Lipoic Acid, Male Infertility, Rodent Models, Systematic Review
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P-68: Assessing The Impact of Advanced Glycation End Products (Ages) on Sperm Health in C57bl/6 Mice

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Objective: Advanced glycation end products (AGEs) are prevalent in metabolic disorders like diabetes, obesity, and infertility-related conditions, where they exert adverse effects on cellular and tissue health. To better understand how AGEs affect both sperm structure and function, our research used mouse models exposed to tailored diets that promote AGE accumulation.
Material and Methods: In this experiment, we divided two groups of 5-week-old C57BL/6 mice: one fed a control diet and the other an AGE-enriched diet. After 13 weeks, we assessed various parameters, such as fasting blood glucose (FBS) and sperm structure and function. Additionally, we examined testicular superoxide dismutase levels, malondialdehyde content, total antioxidant capacity, Johnson score, and the presence of RAGE and carboxymethyl lysine (CML) proteins.
Results: After 13 weeks, we observed significant differences between AGE and control groups. AGE group showed an increase in FBS levels compared with the control group ($P<0.005$). With regard to sperm parameters, AGE group showed lower mean values and a higher percentage of sperm abnormalities, including nuclear histone retention, chromatin deficiencies, DNA fragmentation, increased membrane lipid peroxidation, compared to the control group ($P<0.005$). In addition, AGE group showed a significant reduction in total testicular antioxidant capacity and a lower Johnson score compared to the control group ($P<0.005$). Mean levels of testicular superoxide dismutase did not differ significantly between the two groups ($P>0.005$). However, the AGE group had the highest mean level of testicular malondialdehyde content, as well as higher accumulation of RAGE and CML proteins compared to the control group ($P<0.005$).
Conclusion: AGEs have negative effects on male reproductive health, causing metabolic problems, sperm abnormalities and oxidative stress, highlighting the role these compounds can play in male infertility, particularly in the case of metabolic disorders.
Keyword: Advanced Glycosylation End products, Carboxymethyl lysine, Receptor of advanced glycation end products, Sperm parameters, Sperm function

P-69: Assessment of Streptozotocin-Induced Diabetes Effects on the One-Carbon Cycle and Sperm Functionality in Mice

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Objective: Men with diabetes face an elevated risk of infertility, often characterized by indicators of oxidative damage and reduced methylation in sperm, indicative of a deficiency in the one-carbon cycle (1CC). To delve deeper into this phenomenon, our study sought to explore the impact of diabetes on the one-carbon cycle using mouse models of streptozotocin-induced diabetes, encompassing both type 1 and type 2 diabetes.
Material and Methods: In this experimental study, we divided 50 male mice, aged eight weeks, into four groups: sham, control, type 1 diabetes mellitus (DM1), and DM2. The DM1 group underwent an eight-week regimen of a normal diet (ND), followed by five consecutive days of intraperitoneal Streptozotocin (STZ) injections at a dosage of 50 mg/kg body weight. Conversely, the DM2 group was subjected to an eight-week high-fat diet (HFD), succeeded by a single intraperitoneal injection of STZ at a higher dosage of 100 mg/kg. After twelve weeks, all mice were euthanized for parameter assessment. Notably, the sham group received citrate buffer injections as the solvent for STZ.
Results: Both types of diabetic animals exhibited severe impairment in spermatogenesis, characterized by heightened DNA damage ($P=0.000$), reduced chromatin methylation (percent: $P=0.019$; intensity: $P=0.001$), and compromised maturation ($P=0.000$). Additionally, disruptions in the one-carbon cycle (1CC) were evident, marked by elevated homocysteine levels ($P=0.000$) and diminished availability of carbon units [methionine ($P=0.000$), serine ($P=0.088$), folate ($P=0.016$), B12 ($P=0.025$)] required for methylation processes.
Conclusion: We've noted a distinct impairment of the one-carbon cycle (1CC) in diabetic individuals' testes, likely due to insufficient intracellular glucose and reduced carbon unit supply. Addressing these issues through interventions enhancing glucose uptake into sperm cells and providing extra methyl donors could potentially improve fertility in diabetic patients, pending further clinical validation.
Keyword: Sperm function, Chromatin, Diabetes, Glucose, Methylations
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P-70: High Dna Stainability (Hds): A Reliable Indicator of Sperm Nuclear Integrity?

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Objective: The sperm chromatin structure assay (SCSA®) identifies both the DNA fragmentation index (DFI) and high DNA stainability (HDS), which reflects sperm nuclear compaction. However, the significance and utility of HDS remain unclear. To address this, spermatozoa from 397 infertile men underwent SCSA®, TUNEL, and CMA3 tests, with 100 men additionally undergoing aniline blue (AB) and toluidine blue (TB) staining. This study aims to determine the relevance and reliability of HDS.
Material and Methods: Semen samples from 397 infertile men underwent analysis using the SCSA®, TUNEL, and CMA3 tests. Additionally, a smaller subset ($N = 100$) underwent aniline blue (AB) and toluidine blue (TB) staining, in addition to the SCSA®, TUNEL, and CMA3 tests. All male patients ($n = 397$, mean age = 36.78 years) participating in the study provided signed consent forms. We used SPSS software (version 22; Chicago, IL, USA) for analysis. Descriptive statistics presented means ± SD, while Pearson correlation and ANOVA tests determined relationships and differences ($p <0.05$).
Results: HDS seems to lack reliability as an indicator of nuclear immaturity, given its weak correlation with CMA3, AB, and TB stains. The low association between HDS and sperm DNA fragmentation (TUNEL and SCSA®), as well as DNA condensation (CMA3, AB, and TB) tests, suggests a potential

decoupling of these parameters. In contrast to DFI and TUNEL, HDS has not demonstrated correlation with typical clinical scenarios of male infertility such as asthenozoospermia, teratozoospermia, or astheno-teratozoospermia.
Conclusion: HDS shows weak correlations with tests assessing sperm nucleus maturity. This study represents the first comparison of SCSA®, TUNEL, AB, TB, and CMA3 assays on identical samples, revealing their strengths, weaknesses, and the need for careful interpretation.
Keyword: High DNA stainability, Sperm nuclear integrity, Sperm DNA fragmentation, Sperm DNA condensation

P-71: Association of Varicocele with Reduced Sperm Telomere Length and Genomic Integrity

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Objective: Varicocele, characterized by enlarged scrotal veins, is a leading cause of male infertility. This study explores the correlation between oxidative stress and varicocele-related infertility, particularly its impact on sperm function and telomere length. We assess sperm telomere length as a potential marker of genome integrity in infertile men with grade II or III varicocele, compared to fertile men.
Material and Methods: Blood and semen samples were obtained from 18 infertile men with grade II or III varicocele and 20 fertile men at the Isfahan Fertility and Infertility Center, with written informed consent. Protamine deficiency (chromomycin A3 test), DNA fragmentation (TUNEL assay), and telomere length (quantitative real-time PCR) were assessed. Statistical analysis was conducted using SPSS 11.5, checking for normality and variances. Group comparisons were made using t-tests, with data presented as mean ± SD, and Pearson correlation used to examine relationships, with significance set at $p < .05$.
Results: The mean of sperm parameters quality, sperm and leukocyte telomere length were significantly lower in infertile men with varicocele compared to fertile men ($P<0.05$). Conversely, sperm DNA fragmentation, protamine deficiency, and lipid peroxidation were significantly higher in the varicocele group ($P<0.05$).
Conclusion: The shortened telomere length observed in both sperm and leukocytes is likely linked to heightened oxidative stress associated with varicocele, contributing to increased DNA fragmentation in sperm. Therefore, evaluating leukocyte telomere length may serve as an indicator of antioxidant capacity, influencing sperm function.
Keyword: Sperm parameter, Chromatin, Leukocyte,, Varicocele, Telomere length

P-72: Telomere Length Analysis in Oligozoospermic Men: A Comparative Study of Leukocytes And Sperm Cells

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Objective: Telomere length serves as a crucial biological marker for genomic stability, safeguarding DNA integrity and aiding in proper chromosome alignment during replication. The correlation between telomere shortening and sperm quality deterioration highlights its significance in male infertility assessment. Hence, our study aims to evaluate leucocyte and sperm telomere length (LTL&STL), alongside sperm parameters, DNA damage, and protamine deficiency in men with oligozoospermia, contrasting findings with those from fertile men.
Material and Methods: Blood and semen samples were obtained from 10 oligozoospermic men (sperm count <15 million/mL) and 10 fertile men at the Isfahan Fertility and Infertility Center, with written informed consent. Protamine deficiency (chromomycin A3 test), DNA fragmentation (TUNEL assay), and telomere length (quantitative real-time PCR) were assessed. Statistical analysis was conducted using SPSS 11.5, checking for normality and variances. Group comparisons were made using t-tests, with data presented as mean ± SD, and Pearson correlation used to examine relationships, with significance set at $p < .05$.
Results: Our findings revealed a marked decrease in sperm parameters (concentration, motility, morphology), as well as in LTL & STL, alongside a notable increase in sperm DNA damage and protamine deficiency in oligozoospermic men compared to fertile individuals ($P<0.05$).
Conclusion: These findings suggest that low sperm concentration in men may indicate potential issues with meiotic and/or mitotic division during spermatogenesis. This condition is not only linked to appropriate chromatin packaging but also to telomere length, which plays a critical role in mitosis and meiosis. Telomeres aid in chromosomal alignment, pairing, synapsis, and crossing over, essential processes during spermatogenesis.
Keyword: Chromatin, Leukocyte, Oligozoospermia, Telomere length

P-73: Investigating the Association Between Sperm Telomere Length and Sperm Quality in Infertile Men

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Objective: Telomeres, which are noncoding and repetitive DNA sequences, serve a crucial role in maintaining chromatin integrity. While telomere length is age-dependent in somatic cells, it tends to increase in sperm cells with age. Thus, our objective is to evaluate sperm parameters, chromatin status as well as telomere length in sperm, and leukocytes cells (referred to as LTL and STL) in both infertile and fertile men.
Material and Methods: 38 infertile and 19 fertile men aged between 20 and 50 years were considered for this study. Protamine deficiency (chromomycin A3 test), DNA fragmentation

(TUNEL assay), lipid peroxidation (Bodipy probe) and telomere length (quantitative real-time PCR) were assessed. We analyzed data with SPSS 11.5, checking normality and variances. Group comparisons used t-tests, data were presented as mean ± SD, and Pearson correlation examined relationships, with significance at P<0.05.
Results: A notable decrease in sperm concentration and motility, alongside a marked increase in sperm abnormal morphology, DNA fragmentation, lipid peroxidation, and protamine deficiency, was evident in infertile men compared to fertile counterparts (P<0.05). Additionally, infertile men exhibited significantly shorter mean LTL and STL compared to fertile individuals (P<0.05). Moreover, we identified significant correlations between telomere length and sperm concentration, DNA fragmentation, and lipid peroxidation (P<0.05).
Conclusion: Elevated oxidative stress in spermatozoa of infertile men may lead to chromatin packaging abnormalities, DNA damage, and shortened sperm telomeres, potentially contributing to fertility issues in these individuals.
Keyword: Telomere Length, DNA Fragmentation, Protamine Deficiency, Sperm Parameters, lipid peroxidation

P-74: Celiac Disease and Male Reproductive Health: Investigating Sperm Parameters and Chromatin Integrity

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Background: Celiac disease, a prevalent chronic inflammatory disorder of the small intestine, arises from a permanent intolerance to gluten/gliadin. Research has revealed oxidative stress as a key mechanism implicated in gliadin toxicity, with a documented correlation between oxidative damage and the disease. Likewise, elevated oxidative stress has been frequently observed in infertile men, contributing to compromised sperm function. Hence, our objective was to evaluate sperm parameters and chromatin status in individuals affected by Celiac disease.
Material and Methods: In this study, we collected semen samples from 10 men diagnosed with Celiac disease and 11 fertile men without Celiac disease. Following the guidelines outlined in the World Health Organization (WHO) 2010 protocol, we conducted basic semen analyses. We then evaluated various parameters including the percentage of sperm exhibiting persistent histones, protamine deficiency, DNA fragmentation, as well as levels of malondialdehyde (MDA) and intracellular reactive oxygen species (ROS). These assessments were performed using aniline blue, chromomycin A3, sperm chromatin structure assay, thiobarbituric acid reactive substances (TBARS) assay, and diacetyldichlorofluorescein staining, respectively.
Results: Sperm parameters were similar between men with Celiac disease and fertile counterparts, but those with Celiac disease exhibited significantly higher sperm chromatin maturation issues and DNA damage, along with lower sperm viability (P<0.05). However, there were no significant differences in sperm lipid peroxidation or intracellular ROS levels between the two groups (P>0.05).
Conclusion: Celiac disease exerts a notable influence on the process of sperm chromatin maturation and the occurrence of DNA fragmentation. These findings underscore the substantial impact of celiac disease on male reproductive health, emphasizing the intricate relationship between this condition and fertility-related parameters in men.
Keywords: Celiac; Chromatin; Oxidative stress; Sperm parameters

Embryology

P-75: Taurine's Protective Role Against Acrylamide-Induced Ovarian Stress and Apoptosis in Mice

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Background: Acrylamide (Acr) is a chemical with a variety of applications in industries and for producing laboratory materials. Nonetheless, Acr dietary exposure in humans may cause reproductive toxicity as it can pass through cellular membranes leading to decreased fertility, implantation abnormalities, and lower postnatal survival. Taurine (Tau) is a sulfur-containing amino acid with cell membrane stabilization and antioxidant and scavenging properties. Hence the current study aimed to assess the effect of Tau against Acr-induced stress and apoptosis in mice ovarian tissue.
Materials and Methods: 40 adult healthy mice, 6-8 weeks old, divided randomly into 4 groups including Con (received normal saline orally), Acr (received 50 mg/Kg/day Acr orally) Acr+Tau75 (received Acr and 75 mg/Kg/day Tau), and Acr+Tau150 (Received Acr and 150 mg/Kg/day Tau). The treatment continued for 35 days and then the levels of stress markers and apoptosis were measured using immunofluorescence and tunnel assays.
Results: The findings revealed that both doses of Tau significantly improved the protein and gene expression levels of enzymes involved in response to stress including Glutathione Peroxidase, Super Oxide Dismutase, and Catalase P<0.05). Moreover, the tunnel assay revealed the ameliorative properties of Tau against Acr-induced apoptosis in the ovaries. Similarly, Tau significantly decreased the gene expression levels of Caspase3 and Bax while increasing the Bcl2l1 gene expression (P<0.01).
Conclusion: The current findings suggest the promising properties of Tau in the amelioration of Acr-induced stress and apoptosis in ovarian tissue. Thereby, Tau could be considered a significant contributor to the protection against Acr-induced ovarian toxicity, nevertheless, further studies are encouraged.

Keywords: Taurine, Acrylamide, Ovary, Apoptosis, Antioxidants

P-76: Evaluation of The Effect of Melatonin Antioxidant on Human Arrested Embryos

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Background: Fertilization (IVF) is often challenging due to embryonic developmental arrest. Disruptions in the cell cycle are frequently observed in these embryos. The improvement of culture media is one way to address this issue. This study is focused on examining the impact of melatonin, which is an antioxidant, on the development of type II arrested human embryos .
Materials and Methods: This study utilized 72-hour 4-5 cell human embryos from the embryology department of the Royan Research Institute. After ascertaining the ideal concentration, the embryos were cultivated in control and melatonin groups and incubated for 48-72 hours. Morphological assessments, gene expression, and protein expression were carried out. The data analysis involved the Tukey test, one-way ANOVA, and Chi-square. The significance threshold was set at $P<0.05$.
Results: Results show that 0.002mM is the optimal concentration for the melatonin. A significant decrease in the arrest rate ($P<0.0001$) was followed by an increase in the development rate ($P<0.0001$) and develop up to the pre-morula stage ($P<0.0001$) compared to the control group (non-treated arrested embryos). Compared to the control group (normal blastocysts), there was no significant difference in the expression of the OCT4, NANOG, CCNA2, and CDKN1A genes in the melatonin group. However, the SOX2 level expression was significantly higher than control ($P<0.0001$). Confirmation showed that the NANOG protein expression in the melatonin group matched those of the control group (normal blastocysts).
Conclusion: According to the findings, melatonin antioxidant, results in the formation of embryos with a standard cell cycle and morphology. It has been suggested by studies that the melatonin antioxidant triggers this effect by activating the Phosphoinositide 3-kinases pathway through PTEN inhibition.
Keywords: Embryonic Development, Embryonic Arrest, Melatonin, Antioxidant

P-77: Efficacy of Zeta Potential Sperm Selection Method on Sperm Parameters

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Background: Approximately 50% of couples facing fertility challenges are affected by low-quality sperm. The zeta-potential method allows the recovery of spermatozoa with high motility, normal morphology and minimal damage to the DNA, using a fast, safe, and economical procedure.
Materials and Methods: The study aimed to investigate the effect of zeta potential sperm selection in obtaining spermatozoa with better motility and morphology. In the present study was performed on 20 infertile men with high DNA Fragmentation Index (DFI) (above 25%) and Fertilization (IVF) failure, the semen sample was processed with zeta potential for the motility of spermatozoa analysis by using Computer-Assisted Sperm Analysis Software (CASA) after liquefaction of the semen. The morphology of spermatozoa was evaluated by using Papanicolaou staining.
Results: The paired samples t test was used for statistical analysis. Before the Zeta method, the average percentage of progressive motility of spermatozoa was $9.75 \pm 6.38\%$ and after the Zeta method, the average percentage of progressive movement of spermatozoa increased to 17.4 ± 9.06 ($P<0.05$). The difference in the mean percentage of slow motility after sperm preparation using the zeta method compared to before was not statistically significant ($P> 0.05$). The average percentage of normal morphology before Zeta was $1.05\% \pm 0.82\%$ and after Zeta was $1.60\% \pm 1.04\%$, with a significant increase.
Conclusion: Zeta potential procedures improve the quality of the selected spermatozoa for Intracytoplasmic Sperm Injection (ICSI).
Keywords: Sperm Selection, Zeta Potential, Motility

P-78: Investigation of Mouse Ovarian Encapsulation with Aloe Vera Against Vitrification

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Background: One of the methods of fertility preservation is ovarian tissue cryopreservation. The purpose of this study was to conserve most of the follicular reserves against the destructive effects of cryoprotectant solutions and liquid nitrogen.
Materials and Methods: In this empirical study, the ovaries of Naval Medical Research Institute (NMRI) female mice (8 weeks old) were randomly divided into four groups: Fresh (not vitrified), Vitrification (not encapsulated vitrified), Aloe vera 1 (encapsulated in Aloe vera pieces before placing in vitrification solutions), Aloe vera 2 (encapsulated in aloe vera pieces before placing in liquid nitrogen). After vitrification and warming, histological structure, gene expression *(BAX, BCL2, and P53*) and oxidative stress levels (NO test and MDA test) were examined in each group.
Results: Histological evaluation showed that the average number of primordial follicles in aloe vera2 group and aloevera1 group decreased compared with the vitrification group, although aloevera2 has better performance compared to aloe vera 1, but none of these results were not statistically significant.

Expression of apoptosis-related genes showed that the ratio of BAX/BCL2, P53 and the levels of tissue nitric oxide (NO) and malondialdehyde (MDA) in aloe vera groups 1 and 2 showed a decrease compared with the vitrification group but there was no statistically significant difference.
Conclusion: This study showed that encapsulation of the ovary in aloe vera1 and aloevera2 could not improve the adverse effects of cryopreservation.
Keywords: Vitrification, Cryobiology, Mouse, Aloe Vera, Ovary

P-79: Papaverine Enhances Sperm Quality in Asthenozoospermic Men During Freeze-Thawing

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Background: Cryopreservation of sperm has increasingly become an essential technique that allows sperm to maintain biological function and genetic diversity. However, it is known that the freezing of sperm negatively influences the sperm parameters by producing Reactive Oxygen Species (ROS). The effect of Papaverine (PPV) supplementation on oxidative stress parameters during cryopreservation of semen samples of asthenozoospermic men was assessed.
Materials and Methods: Semen samples of 30 asthenozoospermic men were divided into: Control (fresh), Freeze (treated with cryo-protectant alone), and Freeze + PPV (treated with cryo-protectant + 100 µM PPV solution). Sperm motility was evaluated with light microscope, sperm viability with eosin-nigrosin staining and sperm morphology using the Diff Quick kit. Data were analyzed using Repeated Measure analysis and Bonferroni post-hoc test.
Results: Sperm motility, normal morphology and viability significantly decreased in the Freeze group compared to the Control group ($P<0.001$). Whereas, in the Freeze+ PPV group a significant increase was observed in these parameters compared to the Freeze group ($P<0.001$).
Conclusion: Our results indicated that PPV ameliorates the adverse effects of cryopreservation on sperm quality in the asthenozoospermic men.
Keywords: Asthenozoospermia, Papaverine, Freeze-Thawing, Sperm Parameters

P-80: Correlation Between Age and Sperm DNA Fragmentation: Insights from SCSA® and Flow Cytometry-Assisted TUNEL Assay in A Vast Patient Population

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Background: Sperm DNA integrity is key for reproductive success, yet tests assessing it aren't standard in fertility evaluations. The variety of tests used in clinical trials leads to confusion due to the lack of standardization, compounded by small sample sizes in many studies.
Materials and Methods: For this study, we employed a substantial collection of semen samples representing a diverse range of ages (10000 samples). These samples underwent simultaneous assessment for Sperm DNA Fragmentation (SDF) using two commonly utilized assays: the Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) assay and the Sperm Chromatin Structure Assay (SCSA®). Additionally, we had access to standard seminal parameters such as sperm motility, morphology, and count for these samples, enabling us to explore correlations between age, SDF, and traditional seminal parameters.
Results: Our findings reveal that both SCSA® and TUNEL assessments of sperm DNA fragmentation (SDF) yield consistent results. However, TUNEL consistently reports lower levels of SDF compared to SCSA®. Regardless of the method used, SDF levels increase steadily with age, while the high DNA stainability (HDS) parameter assessed via SCSA® remains stable. Interestingly, conventional sperm parameters do not appear to vary significantly with age in the analyzed cohort. Only sperm volume and motility show significant declines in the oldest age group (50-59 years).
Conclusion: Within the extensive cohort examined, SDF proves to be age-dependent, rising steadily with advancing age. While the SCSA® and flow cytometry-assisted TUNEL assessments of SDF correlate well, TUNEL demonstrates lower sensitivity compared to SCSA®. This variance in sensitivity must be considered when evaluating the actual level of sperm DNA fragmentation in a sample. Classical sperm parameters such as motility, morphology, and sperm count exhibit minimal changes with age, rendering them insufficient for assessing an individual's fertility potential.
Keywords: Sperm Parameters, Sperm DNA Damage, TUNEL, SCSA

P-81: The Impact of More than 10 Years Embryo Storage Time Following Vitrification on Live Birth Rate and Perinatal Outcomes

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Background: The technique of embryo cryopreservation has been increasingly applied in assisted reproductive technologies (ART) laboratory. However, there has been a concern about the safety and efficacy of long-term freezing of embryos. therefore, the aim of this retrospective study was to evaluate the impact of the duration of cryopreservation on pregnancy rate, live birth rate and perinatal outcomes.
Materials and Methods: The study included 62 women who freezed their embryos between September 2009 and February 2013 in Novin Infertility Treatment Center. They had more than 1 vitrification straws. They transferred the thawed cleavaged embryos from their first straw, less than 3 months after vitrification and delivered healthy children. These women transferred another embryo straw after at least 10 years later. For this purpose, we checked the pregnancy rate, live birth rate and prenatal outcome from their second transfer.
Results: From 62 women, 53 women got pregnant again. From

53 pregnancy, 6 chemical pregnancy, 4 miscarriage and 1 legal abortion (Down syndrome) were happened.50 healthy children were born (8 twins). there were No statistically significant difference between the first transfer (embryos with less than 3 months storage time) and the second transfer (embryos with more than 10 years storage time) in pregnancy rate, live birth rate and prenatal outcome. Miscarriage rate increased when the storage time exceeded 10 years.
Conclusion: The duration of vitrification did not significantly affect the pregnancy rate, live birth rate and prenatal outcomes within 10 years period. However, the miscarriage rate increased significantly when the duration of vitrification exceeded 10 years. In addition, postponement of transfer increased the risks of pregnancy at an advanced age.
Keywords: Frozen-Thawed Embryo Transfer, Prolonged Storage Time, Live Birth Rate, Perinatal Outcome

P-82: Do Iron-Honey Nanocomposite Affect Mouse Oocyte Maturation ?

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Background: Maturation (IVM) is an assisted reproductive technology in which immature oocytes are retrieved from the ovaries and matured under laboratory conditions. One of the main issues in the cultivation environment is the presence of reactive oxygen species. Therefore, to improve the IVM process, the utilization of iron-honey nanocomposite (IHN) as an antioxidant can enhance oocyte maturation and yield healthier mature oocytes.
Materials and Methods: The germinal vesicle (GV) oocytes were collected from 6-8-week-old naval medical research institute (NMRI) mice and divided into two groups: control (without IHN) and the experimental (with IHN) group. In the control group, oocyte resumption of meiosis occurred in an IVM medium containing α-MEM, follicle-stimulating hormone (FSH), human chorionic gonadotropin (HCG), and fetal bovine serum (FBS). For the experimental groups, the IHN was added to the IVM medium as an additional supplement. After 16-18 hours, the maturation rate of MII oocytes and the nuclear maturation of MII oocytes (using Hoechst staining) were evaluated. If the p value is greater than 0.05, differences will not be considered statistically significant.
Results: There were no differences observed in the maturation rate of the oocytes between the control and experimental groups. By binding the Hoechst dye to the DNA of the nucleus of the polar body of the oocyte, the maturation of the oocyte was confirmed.
Conclusion: The utilization of honey-based nanoparticles did not have any adverse effect on mouse oocyte maturation .
Keywords: Oocyte, Maturation, Iron-Honey Nanocomposite, Mouse, Antioxidant

P-83: Impact of Curcumin on The Parameters of Human Semen and The Expression of ADD1, PRM1 and PRM2 Genes during The Freeze-Thaw Process

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Background: Sperm cryopreservation is commonly used for male fertility preservation but can reduce sperm quality. Studies suggest antioxidants in freezing media can protect sperm from structural and molecular damage during cryopreservation. This study assesses the impact of curcumin pre-treatment on human sperm parameters after freezing and thawing at different concentrations.
Materials and Methods: Semen samples were obtained from twenty-five normozoospermic males. Subsequently, each of the collected samples was separated into five identical portions: a fresh group and frozen-thawed groups supplemented with varying concentrations of curcumin (0, 20, 50, and 100 μM). The assessment involved pre-cryopreservation and post-thaw analyses to determine the sperm's fertilization potential. This was achieved by measuring the levels of Protamine 1 (PRM1) and Protamine 2 (PRM2) as indicators of male fertility, along with Adducin 1 Alpha (ADD1) as a marker associated with the success of pregnancy.
Results: The mRNA levels of PRM1 were significantly lower in the control group compared to the fresh group ($P<0.001$), while the PRM1 levels after thawing were significantly higher in the curcumin-treated groups. There was also a significant difference in PRM2 mRNA levels between the control and fresh groups ($P<0.001$). Sperm cells frozen with freezing medium plus 50 μM and 100 μM curcumin had significantly higher PRM2 mRNA levels than those frozen without curcumin ($P<0.001$). However, the PRM2 mRNA levels after thawing in the curcumin-treated groups were not significantly different from the control group. Additionally, supplementation with 20 μM, 50 μM, and 100 μM curcumin significantly elevated ADD1 mRNA levels compared to the control group.
Conclusion: The utilization of curcumin as a supplementary component in cryoprotective solutions contributes to reducing the incidence of damage to vital genetic material. Therefore, it can be used as a protective agent to enhance the success rates of assisted reproductive techniques.
Keywords: Cryopreservation, Human Spermatozoa, Curcumin, qPCR

P-84: Protective Effects of Mesenchymal Stem-Cell Derived Exosome During Human Sperm Cryopreservation

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Background: The protection of spermatozoa against the damaging effects of the freezing procedure is of great importance to infertility. Recent approaches showed the beneficial effects of using liposomes and extracellular vehicles (EVs) including exosomes of different origins to ameliorate the damaging effects of cryopreservation on spermatozoa. The present study used mesenchymal stem-cell derived exosome to prepare the human sperm freezing medium during the freezing-thawing.
Materials and Methods: Semen samples of 50 Asthenoteratozoospermia men (random) were collected. Then the sperm parameters were analyzed according based on World Health Organization (WHO, 2010) criteria (2021) and following it each sample divided into 4 groups (E1-E4). E1 (control group). E2: sperm cryopreservation with 25 µg exosome + freezing medium. E3: sperm cryopreservation with 50 µg freezing medium. E4: in this group the cryopreservation sperm with 100 µg + freezing medium. after frozen-thawed sperm were assessed for motility, morphology, viability, lipid peroxidation, total antioxidant capacity (TAC), mitochondrial membrane potential (MMP), DNA integrity.
Results: The post-thawing results indicated that the Mesenchymal stem-cell derived exosome had improved sperm motility ($P<0.05$), morphology ($P<0.05$) and viability ($P<0.05$) compared with untreated samples. The levels of malondialdehyde (MDA) decreased significantly ($P<0.05$), with a consequent decrease in DNA damage ($P<0.05$). The TAC ($P<0.05$) and MMP levels ($P<0.05$) were also significantly improved.
Conclusion: Mesenchymal stem-cell derived exosome could protect spermatozoa from cryopreservation damage.
Keywords: Mesenchymal Stem-Cell Derived Exosome, Sperm, Cryopreservation

P-85: Intracytoplasmic Sperm Injection Outcomes and Pregnancy Rate Oof Cult-Active Medium (Oocyte Activation) in Patients Undergoing Frozen Sperm

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Background: Fertilization failure is the major problems that may be faced in 30–55% of the patients during an Intracytoplasmic sperm injection cycle (ICSI). The aim of the present study was to determine whether Cult-Active medium can improve the fertilization rate, and ICSI outcome in patients with Frozen Sperm (FS) that underwent ICSI.
Materials and Methods: A total of 50 patients who had diminished with FS were included in the study. 30 Patients with were randomized to make artificial oocyte activation with (GM508 Cult-Active- Gynemed-Germany) for 15 minutes just after ICSI (experimental group).20 patients from routine ICSI without Cult-Active medium (Control group). Around 16 to 18 hours after ICSI, fertilization was assessed. The percentage of cleavage and embryo quality were calculated 72 hours after ICSI. Implantation, chemical, and clinical pregnancy, miscarriage rate and, live birth, were determined.
Results: Fertilization rate was significantly lower in the control group compared to experimental group ($P<0.01$). In addition, cleavage ($P<0.001$) and embryo quality (grade I, II) (1.5 ± 0.1vs. 2.4 ± 0.2, $P<0.01$) and (1.7 ± 0.2vs. 2.9 ± 0.4, $P<0.01$) were substantially different between two groups (control and experimental). The pregnancy outcomes showed significant difference in the rates of biochemical pregnancy, clinical pregnancy, implantation, and live births between the control and experimental groups ($P<0.05$).
Conclusion: The findings showed that (GM508 Cult-Active) treatment may fertilization and cleavage rates, which in turn, effect on the implantation, pregnancy and live births rate for patients with frozen–thawed sperm.
Keywords: Cult Active Medium, ICSI, Fertilization, Frozen Sperm

P-86: Fe3O4/Honey Nanocomposite Synthesized Based on The Green Method Increases The Survival Rate and Maturity After Cryopreservation of Immature Mouse Oocytes

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Background: Green synthesized nanocomposites are a new applicable material in cryobiology due to their low-cost production, biocompatibility, and nontoxic properties. In this study, honey based green synthesized Fe3O4 nanocomposites (FHNC) were used to improve the survival rate and maturation condition of immature mouse oocytes during vitrification.
Materials and Methods: Adult female Naval Medical Research Institute (NMRI) mice were used to collect Germinal Vesicle immature oocytes (GV). GVs were divided into three groups: non-vitrified (nVit), vitrified (Vit), and vitrified with nanocomposite (Vit+NC). Immature oocytes were washed in the equilibrium medium for 5 minutes and then in the vitrification medium for 1 minute. Finally, after being transferred to the liquid nitrogen on the Cryotop. 7 days later, the vitrified oocytes were placed in the warming medium containing different concentrations of sucrose. After 45 minutes, they entered the oocyte maturation culture medium and were kept in the incubator for 16-18 hours. Finally, the viability and Maturation (IVM) rates were examined using of the Hoechst staining.
Results: Based on the obtained results, the survival rate of immature oocytes after warming in the Vit+NC group ($71\% \pm 0.05$) meaningfully enhanced comparing to the Vit group ($57\% \pm 0.05$). In addition, the rate of IVM in the Vit+NC group ($75\% \pm 0\text{-}05$) increased significantly as compared to the Vit group ($59\% \pm 0.05$) and was positively close to the nVit group ($88\% \pm 0.05$).
Conclusion: Fe3O4/Honey nanocomposites could improve the vitrification outcomes of mouse immature oocytes.
Keywords: Cryopreservation, Immature Oocytes, Nanocomposite, Green Synthesis, Fe3O4/Honey Nanocomposite

P-87: The microRNA Expression Levels in Serum, Follicular Fluid, and Cumulus Cells Can be Used as Biomarkers for Predicting Oocyte Maturation in Assisted Reproductive Technology

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Background: MicroRNAs play a vital role in regulating the function of the human reproductive system. They can affect the development of oocytes and embryos. The current study focuses on the expression of miRNAs in the serum, Follicular Fluid (FF), and Cumulus Cells (CCs) and their connection to oocyte maturation in women undergoing Intracytoplasmic Sperm Injection (ICSI).
Materials and Methods: The study group consisted of 200 women who went through the ICSI cycle. Patients who had female factor infertility (infertile women) and those who had male factor infertility (fertile women) were divided into case and control groups, respectively. The miRNA level was determined by using Real-Time PCR. All individuals were evaluated on the number and maturity of their oocytes. The serum, FF, and CCs samples were collected and compared for miRNAs expression levels in pairs. The relationship between miRNAs expression level and oocyte maturation was analyzed in each sample, both in the case and control groups.
Results: The expression of two miRNAs, miR-155 and miR-21, was observed in serum, FF, and CC samples. There was a significant reduction in the expression levels of miR-155 and mir-21 in the case group compared to the control group in all three samples (t test, $P<0.05$). Oocyte maturity was positively correlated with the expression level of miR-155 in the serum and the expression of miR-21 in the FF and CCs (Pearson correlation, $P<0.05$).
Conclusion: The expression level of miR-21 and mir-155 is considerably reduced in women with female factor infertility. Human oocyte maturity was crucially related to the level expression of miR-155 in serum and the level of mir-21 in FF and CCs samples. Thus, miR-155 and miR-21 could be involved in the pathology of infertility and can be a non-invasive predictor of oocyte maturation.
Keywords: Infertility, miR-155, miR-21, Oocyte Maturation

P-88: Evaluation of The Effect of CHIR99021 Small Molecule on Human Arrested Embryos *In Vitro*

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Background: A frequent challenge in Fertilization (IVF), is embryonic developmental arrest during the pre-implantation stage. These embryos often experience disruptions in the cell cycle. One approach to address this issue involves improvement of culture media. This study aims to assess the effect of CHIR99021 small molecule on the development of type II arrested human embryos.
Materials and Methods: In this study, 72-hour 4-5 cell human embryos from the Royan Research Institute's embryology department were employed. After determining the optimal concentration, the embryos were cultured in control and CHIR99021 groups at 37°C for 48-72 hours. Morphological evaluations, gene and protein expression were conducted. The Tukey test, one-way ANOVA, and Chi-square were used to analyze data. A significance threshold of $P<0.05$ was adopted.
Results: In the CHIR99021 group, the optimal concentration was 1µM. There was a significant decrease in the arrest rate ($P<0.0001$), increase in development rate ($P<0.0001$), and develop up to the pre-morula stage ($P<0.0001$) compared to the control group (non-treated arrested embryos). Also, five embryos achieved blastocyst formation. The expression of the *OCT4*, *NANOG*, *CCNA2*, and *CDKN1A* gene in the CHIR99021 group, exhibited a non-significant difference when compared to the control group (normal blastocysts). But, *SOX2* gene expression was significantly increased compared to the control ($P=0.01$). The expression of NANOG protein in CHIR99021 group was confirmed to be similar to that of the control blastocysts (normal blastocysts).
Conclusion: The findings suggest that CHIR99021 small molecule leads to the development of embryos with activated cell cycle and normal morphology. Studies suggest that the CHIR99021 small molecule presumably through GSK3 inhibition, triggers this effect through Phosphoinositide 3-kinases pathway activation.
Keywords: Embryonic Arrest, Cell Cycle, CHIR99021, Small Molecule

P-89: The Effect of Timing on The Parameters of Human Spermatozoa After Freezing-Thawing

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Background: Sperm freezing is used as a routine procedure in assisted reproduction clinics. One controversial issue in sperm cryopreservation is determining the optimal timing for sperm evaluation post-thaw. Our study aimed to assess the impact of various time intervals on sperm quality parameters following the thawing process.
Materials and Methods: In this preliminary study, 5 human normal sperm samples were included. All samples were evaluated for sperm motility, viability, morphology, and DNA integrity. Cells were examined in terms of progressive (fast or slow), non-progressive, and immotile sperm cells. Eosin-nigrosine test was used to check cell viability. Diff-quick was used to assess sperm normal morphology. Sperm chromatic dispersion test was used to check the amount of DNA fragmentation. Direct swim-up was used for sperm preparation. Spermatozoa were cryopreserved by the rapid freezing method. Glycerol-egg yolk-citrate was used as a cryoprotectant agent according to WHO protocol. After thawing, three different time points of 0, 5 and 10 minutes were used for evaluation of sperm parameters. all sperm parameters were evaluated after each time point.
Results: We observed a marked decline in total sperm motility at 10 minutes post-thaw compared to pre-freeze levels. However, no significant differences emerged in total motility among the various time points or in comparison to pre-freezing conditions. Progressive motility experienced a notable reduc-

tion at 5 and 10 minutes post-thaw relative to pre-freezing figures. Sperm morphology remained consistent across all groups. While sperm viability and DNA integrity exhibited a downward trend with increased duration post-thaw, these changes did not reach statistical significance.
Conclusion: Immediate evaluation of sperm parameters post-thaw appears to yield higher motility rates. To corroborate these preliminary findings, further research with a more extensive sample size is warranted
Keywords: Sperm, Cryopreservation, Thawing

P-90: Effect of Extracellular Vesicles Derived From Human Theca Progenitor Cells On Human granulosa cells

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Theca cells play an important role in maintaining the structure and integrity of the follicle and estradiol production. To investigate the paracrine effect of theca cells on the growth and proliferation of granulosa cells, the impact of extracellular vesicles of theca progenitor cells resulting from the differentiation of human theca stem cells, on human granulosa cells was evaluated. In this study human theca stem cells differentiated into theca progenitor cells during an 11-day culture period. Theca progenitor-EV was isolated after centrifugation of the collected conditioned medium of the differentiated cells. The morphology, size, and specific markers of theca progenitor-EV were measured. Human granulosa cells were then treated with theca progenitor-EV for 24 hours and survival rate, estradiol hormone expression, and expression of CYP19A1, Cyclin D1, PCNA, and P53 genes were investigated. The results showed that theca progenitor-EV had the minimal criteria for defining extracellular vesicles. These also included two proteins, BMP4 and TGF-β, as specific markers for theca.
Their addition to the culture medium of granulosa cells increased the expression levels of genes related to apoptosis (P53), proliferation (Cyclin D1, PCNA), and estradiol synthesis (CYP19A1) (P<0.05). Also, theca progenitor-EV significantly increased estradiol secretion in granulosa cells (P<0.05). The results of this study demonstrate that theca progenitor-EV can positively affect granulosa cells even in the absence of theca cells directly. Therefore, isolated EVs may improve the media used for the in vitro development of human follicles by affecting the growth and function of granulosa cells.
Keywords: Theca cell , granulosa cell , Extracellular Vesicles , Theca Progenitor cells

P-91: Resveratrol Ameliorates Lipid Peroxidation of Bull Sperm Following Freeze-Thawing Process

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Background: Sperm freezing is widely used assisted reproductive technologies in cattle industry. However, it exerts irreversible detrimental effects on sperm motility, viability as well as fertilizing ability of spermatozoa. Extensive efforts have been undertaken to decrease adverse effects of cryopreservation by supplementation of freezing medium with antioxidants and cryoprotectants. In this study we evaluate addition of different antioxidants such as resveratrol, taxifolin and fetuin in cryopreservation extender on quality and antioxidant potential of bull spermatozoa.
Materials and Methods: A total of fifteen ejaculates from 3 mature bull were collected by artificial insemination. Semen samples were divided into five equal group including control, resveratrol (0.2 mM), taxifolin (50 µM), fetuin (5 mg/ml) and combination of three antioxidant. After equilibration, samples were frozen in liquid nitrogen. Sperm total and progressive motility, membrane integrity, mitochondrial activity, DNA integrity, level of glutathione peroxidase and malondialdehid were evaluated after thawing.
Results: Supplementation of fetuin showed the highest motility and progressive motility compared with other groups. There were no significant differences in viability, membrane integrity, mitochondrial activity and level of glutathione peroxidase between the groups. Level of malondialdehid is significantly decreased by supplementation of resveratrol in freezing medium.
Conclusion: Our result showed that, supplementation of bull sperm freezing medium with resveratrol by decreasing lipid peroxidation could improve post thawed sperm quality.
Keywords: Bull Sperm, Resveratrol, Taxifolin, Fetuin, Cryopreservation

P-92: Lycopene Mitigates The Adverse Effects of Cryopreservation on Sperm Parameters in Asthenozoospermic Men During Freezing-Thawing

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Background: Asthenozoospermia is one of the most prevalent causes of male infertility, significantly affecting all types of sperm disorders. Although cryopreservation is an indispensable part of assisted reproductive centers, it decreases sperm quality. Adding an antioxidant to the cryopreservation medium could effectively mitigate these adverse effects. Lycopene, a red carotenoid and powerful antioxidant, has many positive effects on sperm parameters. This investigation studied the impact of lycopene supplementation on sperm parameters during the cryopreservation of semen samples from asthenozoospermic men.
Materials and Methods: Semen samples were collected from

30 asthenozoospermic men. Each sample was then divided into three groups: control (fresh), freeze (treated with cryoprotectant alone), and Freeze + Lycopene (treated with cryoprotectant and 5 μmol/L lycopene). The samples in the freezing groups were cryopreserved using a human sperm freezing medium and a rapid freezing method. For each sample, sperm motility was assessed according to World Health Organization (WHO) criteria using light microscopy. Viability was evaluated using eosin-nigrosin staining, and sperm morphology was examined using the Diff-Quick kit. Data were statistically analyzed using the Repeated Measures Analysis method

Results: Sperm motility, viability, and normal morphology significantly decreased in the Freeze group compared to the Control group ($P<0.05$). However, in the Freeze + Lycopene group, a significant increase in these parameters was observed compared to the Freeze group ($P<0.05$).

Conclusion: Based on our results we concluded that lycopene reduces the adverse effects of cryopreservation on sperm quality in asthenozoospermic men.

Keywords: Asthenozoospermia, Cryopreservation, Lycopene, Sperm Parameters

P-93: Canthaxanthin Improves Human Sperm Parameters During Cryopreservation

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Background: Sperm freezing can increase oxidative stress, making sperm more susceptible to reactive oxygen species (ROS) and chilling injuries. This study aimed to investigate the impact of canthaxanthin, a carotenoid with antioxidant properties, on human sperm parameters following the freeze-thaw process.

Materials and Methods: Several normozoospermic semen samples were collected and processed using the swim-up method. The supernatant containing motile sperm was divided into eight groups and treated with various concentrations of canthaxanthin (0, 10, 20, 40, 50, 60, 70, 100 μM) for 15 minutes. The samples were then frozen and thawed after 4 weeks, and the effectiveness of each treatment was assessed by measuring sperm motility, vitality, and morphology.

Results: Incubation of sperm with 50, 60, 70, and 100 μM canthaxanthin, in comparison to the control group (0 μM), significantly improved progressive motility (9.85 ± 2.05, 9.29 ± 3.41, 11.43 ± 3.08, and 10.35 ± 2.19) vs. (6.71 ± 1.15) and total motility (59.35 ± 6.18, 62.18 ± 8.32, 60.84 ± 4.25, and 55.91 ± 6.47) vs. (46.12 ± 5.15) after thawing. Additionally, the addition of 50, 60, and 70 μM canthaxanthin to the media resulted in a significant increase in sperm vitality compared to the control group (62.11 ± 5.06, 60.74 ± 4.93, and 57.21 ± 3.25) vs. (47.81 ± 6.52) after the freeze-thaw process. However, different concentrations of canthaxanthin had no significant effects on sperm morphology compared to the control group.

Conclusion: Canthaxanthin, as an antioxidant, can significantly reduce the harmful effects of cryopreservation on sperm parameters. The addition of canthaxanthin to sperm samples during the freeze-thaw process can potentially improve post-thaw sperm quality, particularly in terms of motility and vitality.

Keywords: Canthaxanthin, Sperm Cryopreservation, Antioxidant, Sperm Parameter

P-94: Effect of Different Dose of Ghrelin Hormone agonist (GHRP-6) on Early Maturation of Immature Human Oocyte in Compare with Single-Step Medium Culture le

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Background: In an Maturation (IVM) cycle, immature Oocytes resume meiosis in laboratory and then later subjected to fertilization. So, the culture medium plays a vital role in this process. The role of Ghrelin hormone is discovered in reproduction lately. This study aims to evaluate the effect of six different doses of Ghrelin hormone agonist (GHRP-6) on Oocyte maturation and compare it with single-step culture as the control group.

Materials and Methods: Oocytes collected after 38-40 hours HCG (human chorionic gonadotropin) priming, from 210 women under 35 (non-female factor), referred to Mehr fertility center, Rasht, Iran. Germinal vesicle oocyte (GV), which had delayed cell cycle and were not suitable for intra cytoplasmic sperm injection (ICSI), donated to this study. Each experimental groups and control group had 30 GVs. Apparition of the first polar body evaluated during 24 hours and 48 hours. Fisher's exact test statistical method was used to determine the significance level.

Results: After 24h, 70.0% of GVs in 75ng/ml group was in Metaphase II stage (MII) which is a significant different from other dosses and control group ($P<0.05$). And this number reached to 80.0% in second day which is not significant but still higher than other groups and control group. Additionally, at higher doses, GHRP-6 had negative effect on maturation and Oocyte viability.

Conclusion: It can be concluded that, GHRP-6 has positive effect on early maturation of human GVs in culture media at lower doses.

Keywords: Immature Oocyte, IVM, Ghrelin Hormone, GHRP-6

P-95: Fertilization Rate and Blastocyst Rate of Matured Oocyte Treated by Ghrelin agonist (GHRP-6) In Media Culture

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Background: Increasing the success rate of invitro maturation (IVM) for human Oocytes has a major clinical significance.

Therefore, optimizing media culture could be essential. Effect of Ghrelin hormone reported in different stages of fetus development recently. This study is a comparison of fertilization rate and blastocyst rate in Oocytes which matured in three different doses of GHRP-6 in media culture and single-step culture as control group.
Materials and Methods: 120 germinal vesicle oocyte (GV) were collected from women under35 (non-female factor) referring to Mehr medical center, Rasht, Iran, after 38-40 hours of human chorionic gonadotropin (HCG) priming. GV stage Oocytes, which were not suitable for fertilization, donated to this study. 30 Oocyte cultured in each experimental and control group. Oocyte maturation examined after 24 and 48h of culture. Each matured Oocyte was injected with related husband sperm. Fertilization rate and blastocyst rate were examined after 24 and 6 days post injection. Fisher's exact test statistical method was used to determine the significance level.
Results: The highest rate of maturation in day one was observed in 75 ng/ml which was 24 metaphase II Oocyte. Apart from significant difference between 75 ng/ml and other groups in early maturation ($P<0.05$), there was no significant difference between experimental groups and control group in fertilization rate and pronuclei formation ($P>0.05$). Meanwhile, there was blastocyst development just in control group ($P<0.05$).
Conclusion: It could be concluded, despite the positive effect of GHRP-6 on maturation rate of GVs,the resulting MII Oocytes don't have enough quality to reach blastocyst stage.
Keywords: IVM, GV, Ghrelin Hormone, ICSI

P-96: Investigating The Antioxidant Effects of Aqueous Extract of Black Mulberry on Reproductive Toxicity Induced by Cadmium Chloride in Male Rabbits

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Background: Cadmium chloride is a heavy metal that induces oxidative stress and causes adverse effects on the reproductive system. Antioxidants can be considered a useful strategy to reduce damages induced by oxidative stress. This study aimed to investigate the protective effects of the aqueous extract of black mulberry, containing phytochemical composition, on the destructive effects of cadmium chloride on reproduction in male rabbits.
Materials and Methods: In this study, 15 adult male rabbits were divided into 3 groups: 1. control group (rabbits which received distilled water); 2. cadmium chloride group (rabbits which received cadmium chloride (5 mg/BW)); 3. mulberry + cadmium group [rabbits which received mulberry (300 mg/kg BW), and after 3 hours, received cadmium (5 mg/kg BW)]. After 1 month, reproductive hormones [Testosterone (T), Follicle-Stimulating Hormone (FSH), and Luteinizing Hormone (LH)], antioxidant enzymes (Glutathione: GSH and Catalase: CAT), and lipid peroxidation index (Malondialdehyde: MDA level) in the blood samples and epididymal sperm number were tested in these three groups.
Results: In the cadmium group, GSH and CAT, FSH, LH, T levels, and sperm count significantly decreased ($P\leq0.001$), and MDA level significantly increased ($P\leq0.05$) compared with the control group. Whereas, in the mulberry + cadmium group, the level of reproductive hormones and antioxidant enzymes and sperm count significantly increased ($P\leq0.05$), while the MDA levels significantly decreased compared with the cadmium group.
Conclusion: Cadmium chloride induces adverse effects through oxidative stress and the black mulberry with its potent antioxidant properties ameliorates negative effects induced by cadmium chloride and improves fertility parameters in male rabbits.
Keywords: Black Mulberry, Cadmium Chloride, Oxidative Stress Indicators, Reproductive Hormones

P-97: Coumarin Reduced Apoptosis and Oxidative Stress in A Cyclophosphamide-Induced Premature Ovarian Failure Mouse <odel

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Background: Premature ovarian failure (POF) is defined as the loss of normal ovarian function and the absence of folliculogenesis before the age of 40. Cyclophosphamide (CTX) is a chemotherapy alkylating agent that causes a lot of side effects including POF. The present study aimed to investigate the effects of coumarin (COU), as an antioxidant, on apoptosis and oxidative stress in CTX-induced POF mouse model.
Materials and Methods: NMRI mice were randomly divided into three groups: control group (40 mg/kg/day oral gavage of normal saline for 14 days), POF group (600 mg/kg/day CTX for 6 days), and COU+POF group (40 mg/kg/day oral gavage of COU for 14 days + 600 mg/kg/day CTX for 6 days). Three weeks after establishing the POF mouse models, ovaries were collected and assessment of the oxidative stress status and measurement of the relative expression of apoptosis genes were performed.
Results: Our results showed that in the POF group, the expression of gene Bax significantly increased compared with the POF+COU group. A significant decrease in Bcl-2 gene expression was observed in the POF group compared to control and POF+COU groups. No significant changes in the expression of Caspase3 and Caspase9 genes were observed between the groups. In the POF group, there was a significant increase in Caspase8 gene expression compared to the control and POF+COU groups. The ratio of Bax to Bcl-2 gene expression in the POF group increased significantly compared to the control and POF+COU groups. Biochemical analysis of ovarian tissue for stress oxidative markers showed a significant increase in MDA level and a significant decrease in CAT, SOD, and GPx levels in the POF group compared with the control and POF+COU groups, but combined therapy with COU prevented the increase of MDA level and reduction of CAT and SOD levels in COU + POF group and was close to the control group. Also, the oxidative stress index (OSI) increased significantly in the POF group compared to the control group and POF+COU groups.
Conclusion: Our results showed that coumarin as an antioxidant, can reduce apoptosis and oxidative stress induced-CTX.
Keywords: coumarin, premature ovarian failure, oxidative stress, mice, apoptosis.

P-98: Investigating The Expression Level of Receptor for Advanced Glycation End-Products and Soluble Receptor

for Advanced Glycation End-Products Genes In Granulosa Cells of Patients With Polycystic Ovary Syndrome

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Background: One of the most common diseases of the endocrine system that affects women of reproductive age is polycystic ovary syndrome (PCOS). Advanced glycation end products (AGEs) are known to associate with the pathogenesis of several chronic diseases via interaction with their corresponding receptor (RAGE). The soluble forms of RAGE (sRAGE) are considered as anti-inflammatory agents by inhibiting the consequent adverse effects of AGE. Our aim was to investigate the expression levels of RAGE and sRAGE in women with or without PCOS who underwent controlled ovarian stimulation for Fertilization (IVF).
Materials and Methods: A total of 20 eligible women [10non-PCOS (control) and 10 patients with PCOS (case)] were included the study. The granulosa cells of these people were isolated by the gradient method, and their extracted RNA was synthesized into cDNA, and finally gene expression was measured by real-time PCR. The data were reported as mean ± standard deviation and the significance level ideas 0.05 (independent t-test was performed).
Results: RAGE gene expression in PCOS patients (0.96 ± 1.44) was lower than the expression of this gene in the control group (4.44 ± 5.66) and these results were not statistically significant (P= 0.076). sRAGE gene expression in PCOS patients (378.11 ± 1177.78) was lower than the expression of this gene in the control group (9 ± 15.09), and these results were not statistically significant (P= 0.335).
Conclusion: The present study, for the first time, examined the expression of the sRAGE gene in PCOS patients at the same time and showed that there is no statistically significant difference in the expression of the RAGE and RAGE genes in both the PCOS and non-PCOS groups that it might be because of the low number of samples.
Keywords: PCOS, AGEs, RAGE, sRAGE

P-99: DAPI Fluorescent Staining Technique Is More Efficient to Detect The Cell Penetration into The Artificial Ovary

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Background: Nowadays the number of people surviving cancer has increased. These people had experience of being exposed to chemo or radiotherapy and consequently, their gametes are damaged. One of these techniques is artificial ovary (AO) which tries to provide a microenvironment like a native niche to induce any type of seeded germ cells, restore hormonal function and support the production of fertile oocytes. Aim of the current study is to find a more efficient technique to approve the settle down of injected cells.
Materials and Methods: To achieve this goal, ovarian samples were obtained from patients applying for gender reassignment (transsexuals) with their informed consent. Based on the Royan Institute human ovarian bank's protocol, the ovarian strips were prepared and saved at -196°C. Different materials are utilized in AO construction, including the ovarian natural decellularized scaffold. Some of the cryopreserved strips were decellularized and used as scaffolds. To mimic the natural ovarian environment, a mixture of ovarian cells was needed. The other ovarian strips were considered for taking out the ovarian cells and cultured in a six-well plate. Then at confluency of 80%, the first passage was performed. Cells were transferred into a T25 flask and after the second passage, cells from three flasks were dissolved in around 200 µl of warm culture medium containing 15% FBS, and AO was made by injecting the cells with insulin syringe 30 Gauge. The four seeded scaffolds were cultured 38 h and fixed in Bouin and paraformaldehyde; then evaluated with Hematoxylin and Eosin (H&E) and DAPI staining techniques.
Results: Although, H&E technique showed the cellular penetration into the scaffolds, DAPI staining technique approved that the cells had successfully penetrated deeply into the artificial ovaries.
Conclusion: The fluorescent methods like DAPI staining is more practical to detect the cell penetration more than simple H&E histological technique.
Keywords: Artificial Ovary, DAPI Staining, Cell Injection, Fluorescent Staining

P-100: Assessing The Influence of One-Month Fasting on Improve Ovulation in A Rat Model of Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common hormonal and endocrine disorder that can lead to infertility in women of childbearing age. Being overweight or obese is closely linked with PCOS, often resulting in hyperandrogenism and chronic anovulation. This study aimed to explore the effects of a one-month fasting regimen on Improve ovulation in a rat model simulating PCOS.
Materials and Methods: Fifteen female Sprague-Dawley rats

were divided into three groups: Control, PCOS, and Fasting. The Control group was fed a regular normal diet for 120 days, while the PCOS group received a high-fat diet for 120 days and 28 days of letrozole treatment. The Fasting group followed the same diet and treatment as the PCOS group but also underwent 12-hour fasting daily for 30 days. Ovarian samples were collected and examined using a light microscope for histological and morphometric analysis.
Results: The results of Histological examination of ovarian tissue from the PCOS group revealed numerous cystic follicles, a hallmark of polycystic ovaries. In contrast, the Fasting group displayed a variety of follicles, including secondary, tertiary, and Graafian follicles, as well as a significant number of corpora lutea. Furthermore, cystic and atretic follicles returned to a normal state in the Fasting group, indicating improved follicle growth, ovulation, and egg release.
Conclusion: These findings suggest that fasting may be a potential intervention for certain aspects of PCOS management, Including improving ovulation.
Keywords: PCOS, Ovulation, Fasting

P-101: Evaluating The Effects of Hydroxytyrosol on Level of Reactive Oxygen Species and Sperm Parameters in Human Asthenoteratozoospermia during Incubation

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Background: Asthenoteratozoospermia (AT) is a leading cause of male infertility. Semen samples in men with AT produce higher levels of reactive oxygen species (ROS) during manipulation and are more susceptible to oxidative stress, which can further compromise sperm quality. One promising approach to counteracting this oxidative damage is the use of antioxidants. This study investigates the effects of Hydroxytyrosol (HT) on sperm parameters and ROS level- in AT samples during incubation.
Materials and Methods: Thirty-five AT semen samples were divided into five groups receiving doses of 0, 25, 50, 75, and 100 micrograms per milliliter (µg/ml) of HT. The study proceeded in two phases. In the first phase, 15 AT samples underwent incubation for 30, 45, and 60 minutes. Sperm motility and viability parameters were assessed to determine the optimal conditions. In the second phase, 20 samples were treated with the selected doses and incubation time from the first phase, and mitochondrial membrane potential, intracellular ROS levels, DNA damage, and lipid peroxidation of the plasma membrane were evaluated.
Results: In the first phase, no significant improvement in sperm motility was observed. However, the viability rate at a concentration of 25 µg/ml of HT during 30 minutes significantly increased compared to the control group ($P<0.05$). In the second phase, DNA damage in sperm significantly decreased after 30 minutes of incubation with both 25 and 50 µg/ml of HT. However, no improvement was noted in other parameters such as lipid peroxidation of the membrane, ROS level, and mitochondrial membrane potential.
Conclusion: Incubating human AT samples for 30 minutes with a concentration of 25 µg/ml of HT improved sperm viability. Additionally, doses of 25 and 50 µg/ml of HT lead to a reduction in DNA damage level. These findings suggest the potential utility of HT as an antioxidant in ART procedures for men with AT.
Keywords: Asthenoteratozoospermia, Hydroxytyrosol, Incubation Time, Oxidative Stress, DNA Damage

P-102: The Effects of Adding Cryoprotectant Agent at Different Times on Spermatozoa Parameters

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Background: Sperm freezing is a technique used to preserve sperm in men undergoing chemotherapy, fertility preservation, and other medical situations. During the cryopreservation process, cryoprotectant agents (CPAs) are used to protect sperm cells from damage caused by freezing and thawing, such as the formation of ice crystals within the cells' cytoplasm. Protocols recommend gradually adding CPAs to spermatozoa to minimize osmotic shock; however, there is no consensus on the optimal duration for CPA addition. Our study aims to evaluate the impact of varying CPA loading times on sperm parameters post-thawing.
Materials and Methods: In this preliminary study, five normozoospermic human samples were obtained following direct swim-up. We investigated three different durations for CPA addition: 0 minutes (immediate CPA addition), 3 minutes, and 6 minutes. Sperm parameters assessed included progressive motility, total motility, viability, morphology, and DNA fragmentation, both before freezing and after thawing in the experimental groups.Sperm viability was evaluated using eosin-nigrosin staining, morphology was assessed using Diff-Quik staining, and DNA fragmentation was measured with the sperm chromatin dispersion test. Rapid freezing was employed, utilizing glycerol-egg yolk-citrate as the cryoprotectant agent, following the World Health Organization (WHO) protocol.
Results: There was a significant decrease in total motility in the 6-minute group compared to pre-freezing, while other groups showed no significant difference in total motility. Progressive motility significantly decreased after thawing in all experimental groups, with no significant differences between the groups. Sperm viability was significantly lower in the 0-minute and 3-minute groups compared to pre-freezing levels, although the 3-minute group did not show a significant difference from pre-freezing. Normal sperm morphology did not differ among the groups. Sperm DNA fragmentation remained unchanged in the 3-minute group compared to pre-freezing but significantly increased in the 0-minute and 6-minute groups.
Conclusion: Our data indicated that adding CPA to spermatozoa over a 3-minute period before freezing yielded superior results. However, further studies with larger sample sizes are needed to confirm these findings.
Keywords: Cryoprotectant Agents, Sperm Freezing, Thawing, DNA Integrity

P-103: Walnut Leaf Extract: A Natural Antioxidant to Improve Sperm Parameters and Testicular Dysfunction in Rats Under Heat Stress

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Background: Walnut leaf extract as a natural antioxidant has protective effect on testicular tissues under heat stress condition. The aim of this study was to investigate the effect of walnut leaf extract on testicular tissue and sperm quality in rats under heat stress
Materials and Methods: For this purpose, 60 male albino rats were divided into five groups: T1- healthy control group. T2: Heated-control group. T3 treatment with 40 mg/kg extract. T4 treatment with 60 mg/kg extract. T5- treatment with 150 mg/kg of extract administered orally to walnut leaf extract for 55 days after inducing heat stress for 45 days. The rats were then dissected and their testis tissues were evaluated for pathological changes, sperm parameters, and gene expression of Heat Shock Protein-70 (HSP70), Heat Shock Factor-1 (HSF1), Nuclear Factor Erythroid 2–Related Factor 2 (NRF2), and Silent Information Regulator 1 (SITR1) by Real-Time Polymerase Chain Reaction (RT-PCR).
Results: The T2 group had complete tissue destruction and the lowest sperm motility. Rats in the group T3 exhibited slightly improved motility. Also, the mRNA expression of HSP70 and HSF1 in the 60 and 150 mg/kg groups were significantly higher than those in the heat stress group. The T4 and T5 treatment groups had higher expression of SITR1 and NRF2 than the T2 group.
Conclusion: In conclusion, walnut leaf extract has antioxidant activity that controls testis dysfunction and sperm quality under heat stress conditions.
Keywords: Walnut, Spermatogenesis, RT-PCR, Heat Stress, Antioxidant

P-104: Membrane Lipid Replacement Strategy in Bull Sperm Protection During Cryopreservation

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Background: Sperm cryopreservation is an effective technology of artificial insemination for commercial breeders. However, the efflux of phospholipids from the sperm membrane impairs its function which leads to reduce the fertility potential of thawed sperm. Membrane Lipid Replacement with lipid nanomicelles could restore cellular membrane and increase sperm cryo-survival. This study investigated the effects nano-micelles made from Glycerophospholipid mixtures (GPL) on the cryosurvival of thawed bull sperm.
Materials and Methods: Semen samples were collected from six bulls, twice a week, then mixed and were diluted with the Tris-egg yolk extender containing different concentrations of GPL nano-micelles according to the following groups: control (GPL-0), 0.5% (GPL-0.5), 1% (GPL-1), and 1.5% (GPL-1.5) , then diluted semen was cooled at 5°C during 2 h and stored in liquid nitrogen. The optimum concentration of GPL was determined by evaluation of the quality parameters including motility, viability, plasma membrane integrity, apoptotic-like changes, lipid peroxidation, and mitochondrial activity of thawed sperm.
Results: Exposure of sperm to GPL-0.5 significantly increased total, progressive motility, Average Path Velocity (VAP), Velocity of Straight Line (VSL), and Velocity of the Curved Line (VCL), compared to the frozen control group ($P<0.05$). The percentage of viability and membrane integrity were significantly higher in the GPL-0.5 and GPL-1 compared to the other groups ($P<0.05$). Moreover, the lowest rate of lipid peroxidation and apoptosis was significantly in GPL-0.5 and GPL-1 groups in comparison to the frozen control group. Mitochondrial activity of thawed sperm was not affected by GPL ($P >0.05$).
Conclusion: Our results showed that membrane lipid replacement with GPL micelles (0.5 %, and 1%) could substitute damaged lipids in membrane and protect bull sperm against cryo-injury.
Keywords: Cryopreservation, Bull Semen, Glycerophospholipid, Nanomicelle

P-105: Fe3O4-Honey Nanocomposite Doesn't Have any adverse Effect on Mouse Germinal Vesicle Maturation

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Background: Maturation (IVM) of mammals is one of the assisted reproductive techniques (ART) for adult females. The factors like incorrect PH levels, light exposure and heat in laboratory setup can negatively impact oocyte culture conditions leading to reduction in mitochondrial activity. Utilizing nanoparticles, particularly nanocomposites (NC), represents a promising new approach to enhance the quality of matured oocytes in environments. Honey, known for its antioxidant and antibacterial properties, is used as a coating for Fe_3O_4 magnetite nanoparticles.
Materials and Methods: Immature oocytes from 6-8 weeks old NMRI female mice were placed in an maturation solution without any supplement (control group) and with nanocomposite (IVM+NC). After 16-18 hours, the maturation rate was assessed. Subsequently, matured oocytes were vitrified using vitrification techniques. After 7 days, the warmed oocytes were examined for nuclear maturation rate using Hoechst fluorescent staining, and their survival rate was evaluated using trypan blue staining. JC-1 assay was carried out for mitochondrial activity assessment in the MII oocytes after vitrification/warming.
Results: The IVM rates in the Control and IVM+NC groups both reached ~80% in a non-significant manner. Furthermore, the post warming survival rate was about 60% in both groups. After evaluating the red to green fluorescent colors, it was observed that the mitochondrial activity in the nanocomposite group (IVM+NC) was not different from the control one.
Conclusion: There were no antagonistic impacts in maturation,

mitochondrial activity and post vitrification survival rates after using of Fe3O4/Honey nanocomposite in maturation of mouse immature oocytes.
Keywords: Nanocomposite, Maturation, Fe_3O_4, Vitrification, Mitochondrial Activity

Epidemiology and Health

P-106: The Moral Status of Parents' Decision on Gene Editing

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Background: Gene editing, as an emerging technology, has been making a vast progress so much so that it has motivated some parents to spend a good deal of money for having a genetically healthy child. In addition, a group of parents seek to enhance physical or mental capacity of their children or even prolong their children's life span. Are such decisions ethical? On the other hand, if they withhold utilizing the technology for treatment or enhancement of their children, is this decision unethical? Have they violated their moral responsibility? Not only does the parents' decision to utilize the gene editing facilities (i.e. that of the germline gene editing) leave impact on their children, it will undoubtedly leave an impact on next generations; a reality that overshadows the afore-said moral questions.
Materials and Methods: This research is a theoretical-analytical study. In this work, we will first study the opinions and views. Secondly, we would make attempt to analyze and evaluate those opinions and views with the aim of reaching a conclusion on the status of moral decision in this regard.
Results: Various positions have been taken towards the moral status of parents' decision on utilizing gene editing for treatment or enhancement. They may be classified into two general outlooks: individual and societal. The individual position emphasizes on autonomy of parents, on one hand, and on the principle of beneficence, on the other. This position does not necessarily lead to a defense of a decision to utilize the technology. The second outlook is more concerned with social discrimination and inequalities, and with the possibility of eugenic policies that remind us of the bitter experience of the twentieth century totalitarian systems in Europe.
Conclusion: Any moral evaluation of parents' decision to utilize the gene editing technology, therapeutic or otherwise, should pay attention to both individual and social aspects of such decisions. A theoretical attempt to take into account the two aspects would give rise to a reliable moral appraisal in this field.
Keywords: Gene Editing, Treatment, Enhancement, Beneficence, Autonomy

Female Infertility

P-107: Enhanced VEGF, VEGFR1 And VEGFR2 After Intentional Endometrial Injury in Unexplained Repeated Implantation Failure Patients, An RCT Study

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Background: Although advances in assisted reproductive techniques (ART) have greatly improved the overall outcomes in patients with infertility, the failure of implantation is the main cause of unexplained repeated implantation failure (uRIF) patients. The cellular, vascular, and immunological changes required for endometrial preparations occur within the window of implantation (WOI). To establish an optimal endometrium for implantation, sufficient uterine vascularity is required at the time of implantation. Therefore, endometrial scratching (ES) preceding the IVF treatment cycle, may be an approach to induce endometrial angiogenesis factors such as VEGF, VEGFR1, and VEGFR2. This randomized controlled trial (RCT) study evaluated the expression of VEGF, VEGFR1, and VEGFR2, as key factors for angiogenesis, in the endometrium samples of uRIF patients after intentional endometrial injury in the ES group compared to the non-ES group.
Materials and Methods: The RCT study was registered on the Iranian Registry of Clinical Trials (IRCT20210316050723N1), June 2021-April 2022. Twenty uRIF women were randomly enrolled in 2 groups of ES (n = 10) (twice endometrial sampling in follicular and luteal phases) and non-ES (n = 10) (only luteal phase sampling) in this study. Gene expression analysis on the endometrial samples was performed using QPCR.
Results: The results showed that *VEGF*, *VEGFR1*, and *VEGFR2* genes significantly increased in the ES group compared with the non-ES group ($P<0.05$).
Conclusion: The increased expression of VEGF and its receptors, *VEGFR1* and *VEGFR2*, during the implantation time may be the mechanism responsible for improving implantation in uRIF patients through enhanced angiogenesis.
Keywords: Endometrial Injury, Repeated Implantation Failure, VEGF, VEGFR1, VEGFR2.

P-108: The Effect of Calligonum Comosum (Escanbil) Extract on Pregnancy and Live Birth Rate in Mice Model of Endometriosis

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Background: Endometriosis is a chronic disease in which en-

dometrium tissue grows outside the uterus and causes severe pelvic pain and pregnancy problems. Calligonum comosum is a medicinal plant that grows in desert areas of Iran and is used in traditional medicine for menstrual cramps.
Materials and Methods: This study aimed to investigate the effect of Calligonum comosum total extract (CCTE) on pregnancy and live birth rate in mice models of endometriosis. In this study, 24 female NMRI mice were modeled by autologous and grafting uterine tissue to the abdominal wall. The mice models were randomly allocated into two groups: the first group received 50 mg/kg of CCTE and the second group received normal saline. After 4 weeks of treatment and after mating, pregnancy rate, live birth rate, number and size of endometriosis lesions, histology of lesions, uterus and ovaries, and growth indices of infants were investigated.
Results: The findings showed that the effect of CCTE on the pregnancy rate was more than 50 percent compared to the control group. The live birth rate in the CCTE group was more than 50 the control group. The number and size of the lesions in the treated group were significantly ($P<0.05$) lower than the control group. Histology of ovaries also showed that the quality and number of oocytes in the treated group were better than the control group. CCTE had no negative effect on growth indices of infants The growth indices of infants in the treatment group were better than the control group and the difference was significant ($P<0.05$).
Conclusion: These findings suggest that CCTE can be a promising treatment for treating and improving fertility in women with endometriosis. However, more research is needed to confirm these findings in humans. One of the mechanisms of CCTE on fertility in women with endometriosis is reducing pelvic inflammation, which is one of the main factors in creating fertility problems.
Keywords: Endometriosis, Pregnancy, Laboratory Mice, Scanbil, Growth Indices

P-109: Investigating The Effect of The Use of Sex Hormones on Ovarian Hyper stimulation Syndrome in Predicting Pregnancy Outcome of Fertilization in Women with Polycystic Ovarian Syndrome

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Background: Ovarian hyper stimulation syndrome (OHSS) as a known complication in women with polycystic ovarian syndrome (PCOS) may occur following inducible fertility treatments such as fertilization (IVF) and can affect the sequels of these treatments. This study aimed to assess the effects of OHSS on pregnancy outcomes through IVF in women with PCOS. Also, we assessed the value of baseline sexual hormones to predict the pregnancy's success.
Materials and Methods: This retrospective case-control study was conducted on 180 consecutive women suffering from PCOS who were candidates for IVF at Fatemieh hospital in Hamadan, Iran, from May-July 2022. The women were assigned to the case group (with OHSS, n=129) and the control group (without OHSS, n=51). The case group consisted of people in whom the stimulation of controlled ovulation led to OHSS, while in the control group, the pointed condition was not observed Frozen embryos were transferred in both groups. Embryos were cultured after ICSI for 3-5 days. Embryo quality was assessed before the transfer approximately 72 hours (8-cell stage) after insemination with a maximum of 3 embryos. Corpus luteal support was provided on the day of oocyte retrieval, with progesterone injections (i.m, 50-100 mg/day), until the pregnancy test Frozen embryo transfer was performed by first administering in the middle of the luteal phase (day 21 of the cycle) GnRH agonist until the beginning of the period or menses. Measuring the sexual hormones was performed using the ELISA technique.
Results: Participants with OHSS had significantly lower BMI, had a higher number of oocytes, and suffered more from hirsutism. Concerning hormonal status, the mean serum level of AMH was significantly higher in the group with OHSS. At the same time, we found no difference in the levels of prolactin, TSH, FSH, or LH between the 2 groups. The mean of endometrial thickness (9.17 ± 0.85 vs. 9.21 ± 0.87, $P= 0.785$) and the number of transferred embryos (2.98 ± 1.31 vs. 3.04 ± 1.47, $P= 0.807$) did not differ between the two groups with and without OHSS. Although the rate of chemical pregnancy and clinical pregnancy were both significantly higher in the OHSS group than the control group ($P<0.001$), in the multivariable logistic regression model, OHSS could not predict the likelihood of clinical or chemical pregnancy following IVF. None of the baseline sexual hormones could predict the successful chemical or clinical pregnancy in PCOS women following IVF.
Conclusion: It can finally be concluded that no significant difference is expected in IVF-related outcomes, including clinical or chemical pregnancy, between the PCOS groups with and without OHSS. In other words, the occurrence of OHSS in such women may not be a main determinant for IVF poorer outcomes. Contrary to popular belief, laboratory markers, especially sex steroids, may not predict the outcome of IVF in these women.
Keywords: Ovarian Hyperstimulation Syndrome, Fertilization, Polycystic Ovary Syndrome.

P-110: Effectiveness of Endometrial Scratching in Follicular And Luteal Phases in Pregnancy Rate of Frozen Embryo Transfer Candidate Women

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Background: Endometrial scratching (ES) has been reported as a strategy to improve the outcome of IVF. Considering that the follicular phase and luteal phase have their own specific characteristics and hormonal secretions, we investigated whether scratching in different phases affects a woman's chances of becoming pregnant following frozen embryo transfer.
Materials and Methods: A total of 300 frozen embryo transfer candidate women with normal cavities and good embryo quality were randomly divided into two groups: group A: with ES in the follicular phase; group B: with ES in the luteal phase. In both groups, endometrial scratching was performed before IVF. The rate of pregnancy and baseline characteristics, such as age,

education, and embryo quality, were compared between the two groups.
Results: Our results showed no significant differences in baseline characteristics between the groups. Furthermore, no significant differences were observed between the women who underwent ES in the follicular phase and those in the luteal phase for the outcome of IVF and chemical or clinical pregnancies.
Conclusion: ES in different phases of the cycle preceding frozen embryo transfer did not affect the outcome of pregnancy.
Keywords: Endometrial Injury, Follicular Phase, Luteal Phase, Pregnancy Rate.

P-111: The Effect Of Human Papilloma Virus on Female Infertility: A Systematic Review

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Background: Human papillomavirus (HPV) is one of the most common viral STDs in the United States. Infection with HPV, especially with 16 and 18 types, is one of the main risk factors for cervical neoplasia and the second most common cause of cancer in women around the world. Pap smear is the best diagnostic method but it doesn't work in the latent phase. There are more than 100 varieties of HPV. Some types of HPV infection cause warts, and some can cause different types of cancer. 20–60% of female infertility cases are caused by sexually transmitted infections, which can eventually lead to pelvic inflammation and tubal obstruction. This study aimed to explore the reproductive concerns of women infected with HPV.
Materials and Methods: We conducted an extensive search across electronic databases, including PubMed, MEDLINE, Embase, Google Scholar, and ResearchGate, and explored the available English-language literature. The Mesh terms were" Human papillomavirus (HPV)" OR" Genital Warts" OR "; "Female Infertility". The articles included in this review adhere to the following criteria: they encompass studies solely focused on progress in comprehending and novel treatment approaches, and they are studies conducted in the English language within the last decades. We have used Ryan's AI in this review to screen articles, where it was done with the help of colleagues to visually separate articles using their reading as well as keyword readers.
Results: Some types of genital HPV can cause cancer of the lower part of the uterus that connects to the vagina (cervix). Other types of cancers, including cancers of the anus, penis, vagina, vulva and back of the throat (oropharyngeal), have been linked to HPV infection.
Conclusion: There is no general agreement on the best way to treat patients with genital HPV infection. The Gardasil vaccine is effective against types 16, 18, 6, and 7 (people aged 9-26 get it).
Keywords: Human Papillomavirus, Genital Warts, Female Infertility

P-112: Correlation of Thyroid Stimulating Hormone and Gonadotropins in Women with Secondary Infertility

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Background: Secondary infertility refers to a condition in which a woman has experienced at least one clinical pregnancy but cannot experience it again. In this study, we evaluate the correlation of thyroid stimulating hormone (TSH) and gonadotropins (LH and FSH) in women with secondary infertility.
Materials and Methods: Ninety-four women with history of secondary infertility were participate in this study. Women with age more than 40, PCOS, and endometriosis were excluded from the study. Blood samples were collected from the participants and analyzed to determine the concentration of TSH and gonadotropins. Statistical analysis was performed via SPSS software and p value less than 0.05 was considered statistically significant.
Results: There was a negative significant correlation between TSH and LH levels (p= 0.19; correlation coefficient= -2.4); although the correlation between TSH and FSH levels was not statistically significant. (p= 0.78; correlation coefficient= 0.02).
Conclusion: Our study highlighted the correlation of TSH and LH levels in women with secondary infertility that should be considered in planning treatment of such patients.
Keywords: Secondary Infertility, LH, FSH, TSH

P-113: The Effect of Ovulation Stimulation Drugs on Peripheral Blood NK Cells in Women With Endometriosis during IVF/ICSI Cycles: Preliminary Data

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Background: Endometriosis is an estrogen dependent disease in women of reproductive age. Although the exact etiology of this disease remains unclear, genetic factors, hormonal changes, and the immune system alterations have been identified in its pathogenesis. Natural killer (NK) cells are one of innate immune cells characterized as CD3- CD56+ cells which their changes was reported in endometriosis. Considering the importance of NK cells in the pathogenesis of endometriosis, the aim of this study is to evaluate the effect of ovulation stimulation drugs on peripheral blood NK (pNK) cells in women with endometriosis during fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles.
Materials and Methods: In this cohort study, 40 infertile women with endometriosis who underwent ovulation stimulation with long gonadotropin-releasing hormone (GnRH) agonist or GnRH antagonist protocols during IVF/ICSI cycles at

Royan Institute will be included. The blood samples will be collected at least on two time points: 1) before the start of the ovulation stimulation cycle on day 2-5 of the menstrual cycle and 2) ovum pickup day. If women come to Royan clinic on gonadotropin triggering day, another blood sample will be collected. Each blood samples were analyzed with specific antibodies against CD56 (NK cell surface marker), CD16 (another NK surface marker), CD3 (T cell marker), CD107a (NK cell activity marker) by flow cytometry.
Results: Till now, 7 women were enrolled (mean of body mass index (BMI): 27.35 Kg/m2 and mean age: 31.71 years old). Comparison on pNK cells frequency and activity was done between ovulation stimulation starting day and day of Ovum pick up. No significant differences were observed in percentage and activity of pNK cells between these two time points.
Conclusion: Any definite conclusion could not be draw because of incomplete sample size.
Keywords: Endometriosis, Natural Killer Cells, Long GnRH Agonist, GnRH Antagonist, IVF/ICSI

P-114: Comprehensive Study on Various Types of Herbal Medicines Effective on Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects women's health and fertility. It affects 1 in 10 women globally and has various complications. and involves multiple pathways and is associated with the development of particular malignancies. This article aims to explore the impact of natural compounds and medicinal plants in treating PCOS.
Materials and Methods: We searched for articles and abstracts on PCOS in PubMed, Scopus, EMBASE, Web of Knowledge, and MeSH from 2015 to 2024. Out of 5000 articles, 150 met our inclusion criteria.
Results: PCOS is a common endocrine disorder affecting approximately 3.4% of women worldwide. The condition is characterized by the formation of cysts in the ovaries, which leads to hormonal imbalances and various symptoms such as irregular periods, hirsutism, obesity, acne, and infertility. PCOS is also associated with insulin resistance and diabetes mellitus. Combining conventional medical treatments, lifestyle and dietary adjustments, and natural remedies based on evidence could potentially provide better management for women with PCOS. This comprehensive treatment strategy could lead to improved metabolic and reproductive outcomes. However, further research is needed to establish standardized guidelines for using natural treatments for PCOS.
Conclusion: PCOS, is a hormonal disorder that affects women and can cause various health problems, including infertility. The typical treatment for PCOS involves using hormonal contraceptives to regulate menstrual cycles, anti-androgens to address hyperandrogenism, and insulin sensitizers to improve metabolic parameters. Long-term pharmacotherapy may produce potential side effects, making natural alternatives more appealing. In this review, we aimed to investigate the effectiveness of herbal remedies in treating polycystic ovaries, including specific supplements such as Fennel, Chaste berry, and Nigella Sativa, as well as other plants with insulin-sensitizing, anti-inflammatory, and endocrine-modulating properties.
Keywords: Polycystic Ovary Syndrome, Herbal Medicines, Inflammatory, PCOS Symptoms

P-115: Implications of Immune Checkpoint Molecules in The Pathophysiology of Preeclampsia: Potential Biomarkers And Therapeutic Targets

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Background: Immune checkpoint molecules play a crucial role in mediating immune tolerance during pregnancy. This study aimed to investigate the potential implications of these molecules in the pathophysiology of preeclampsia (PE) by analyzing the levels of both transmembrane and soluble forms in PE patients.
Materials and Methods: The expression levels of transmembrane CTLA-4, PD-1, PDL-1, and Tim-3 on peripheral blood mononuclear cells (PBMCs) were assessed using PCR and Western blotting. Additionally, the soluble forms of these molecules in serum were measured using ELISA. The correlation between transmembrane immune checkpoints and their soluble counterparts was also examined.
Results: PE patients exhibited reduced expression levels of CTLA-4, PD-1, and Tim-3 on PBMCs, while PDL-1 expression was increased compared to the healthy control group. Furthermore, the serum levels of soluble CTLA-4 (sCTLA-4) and soluble PDL-1 (sPDL-1) were decreased, whereas soluble PD-1 (sPD-1) and soluble Tim-3 (sTim-3) levels were elevated. The results also demonstrated a positive correlation between the expression of CTLA-4 on PBMCs and sCTLA-4 levels. Conversely, the expression of PD-1, PDL-1, and Tim-3 negatively correlated with their soluble forms.
Conclusion: Abnormalities in the expression levels of transmembrane CTLA-4, PD-1, and Tim-3 molecules on PBMCs, as well as the corresponding soluble levels in serum, may contribute to the pathogenesis of PE. These findings suggest that these molecules could potentially serve as biomarkers for distinguishing PE and aid in the development of effective treatments for the condition.
Keywords: Immune Checkpoint Molecules, Preeclampsia, Transmembrane Expression, Soluble forms, Biomarkers

P-116: Clinical Pregnancy Rate Comparison between Two Protocols for Endometrial Preparation in Freeze Embryo Transfer Cycles

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Background: Various factors including drugs used for endometrial preparation can influence the outcome of frozen embryo transfer cycle. The study objectives were to compare clinical pregnancy rate in two endometrial preparation protocols for FET cycles (suppression with or without Gn-Rh agonist before transfer cycle.
Materials and Methods: This retrospective cohort study involved 186 women undergoing FET at IVF center in Emam-Khomeini University Hospital in Sari between January and March 2024. The endometrial preparation protocols used were artificial hormone therapy (HRT) alone and HRT with suppression with GnRh agonist.
Results: There was no significant differences in ages, BMI, duration of infertility, number of embryo transfer. The rate of clinical pregnancy was higher in artificial hormone replacement therapy (HRT) group (14 from95 women) than HRT with GnRh down regulation group(18 from 91women)but there was no significant differences (14/7 vs. 19.8%) (P<0.05).
Conclusion: Freeze embryo transfer cycles outcome were similar between the two protocols used for endometrial preparation.
Keywords: Freeze Embryo Transfer, Endometrial Preparation, Hormone Replacement Therapy, Pituitary Down Regulation

P-117: Comparison of Ovarian Stimulation Response in Patients with Breast Cancer and Oocyte Donors Undergoing GNRH Antagonist Controlled Ovarian Stimulation

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Background: Increased attention has been paid to fertility Preservation and reproductive problems in cancer survivors. In this study, an attempt was made to compare ovarian stimulation response in women with breast cancer and oocyte donors undergoing GnRH antagonist controlled ovarian stimulation.
Materials and Methods: This retrospective study was conducted between 2014 and 2022 on 144 women with breast cancer (n=78) and oocyte donors (n=66) who underwent IVF/ICSI treatment for the first time. In this study, women with diagnosis of breast cancer who indicated for chemotherapy and had a desire to preserve fertility and women with age <35 years included in the study. The patients with poor ovarian response, polycystic ovary syndrome (PCOS), endometriosis, severe male factor infertility and those used oral contraceptives during the last three months were excluded from the study. The primary outcome measure was retrieved mature oocytes. SPSS software was used for data analysis. In all tests, a significance level of less than 0.05 was considered.
Results: The mean age was significantly higher and mean BMI was significantly lower in the breast cancer group than in the oocyte donor group. There were also significant differences between groups in terms of hormonal profiles (LH, FSH, AMH), vitamin D, gonadotropin starting dose, total dose of gonadotropin used, rate of MII oocytes and number of embryos obtained by IVF/ICSI method. Based on the results, there was a significant correlation between MII and AMH in breast cancer group (r= 626; P<0.001).
Conclusion: Women with breast cancer disease should expect a lower rate of MII oocytes retrieved after COH for fertility preservation compared with donor oocytes women. Multicentric studies are needed to evaluate the true effect of breast cancer on ovarian response.
Keywords: Breast Cancer, Fertility Preservation, Ovarian Response

P-118: Translation and Cross-Cultural Adaptation of The Persian Version of The WERF Ephect Endometriosis Patient Questionnaire: A Cross-Sectional Study

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Background: The purpose of the World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Bio banking Harmonization Project (Ephect) is to provide the possibility of valid and robust epidemiological, multicenter research to investigate the causes of endometriosis, new diagnostic methods, and better treatments through the formation of international consensus. To use a valid and reliable tool in other countries, it is necessary to translate that tool. Therefore, the purpose of the current study is cultural compatibility validity and reliability of the Persian version of the standard Endometriosis Patient Questionnaire (EPQ) for use in epidemiological and clinical studies of endometriosis in Iran.
Materials and Methods: In this cross-sectional study, 37 women the age of 18-45 years who underwent diagnostic sonography or laparoscopy to assess for endometriosis or with symptoms of dysmenorrhea, dyspareunia, pelvic pain unrelated to menstruation, pain during defecation more than 6 months, were evaluated between December 2020 and December 2023. The research process was divided into two stages: translation and cross-cultural adaption, and cross-cultural validation. The cross-cultural translation and adaptation of the Persian version of WERF EPHect were performed adhering to the recommended guidelines The content validity of this questionnaire was measured by six experts in content, methodology, and face va-

lidity by seven participants, in a qualitative manner. For checking the reliability of the final Persian questionnaire, 30 endometriosis patients completed it on two occasions with an interval of 2 weeks, and the repeatability was examined separately for each question using the Kappa statistics.
Results: 30 patients with endometriosis completed the translated questionnaire and the completion time was about 50-60 minutes. The final changes were made to the questionnaire by Iranian culture and the opinions of professors and study participants. In general, the results indicate the validity and reliability of the Persian version.
Conclusion: The present study showed that the Persian version of WERF-EPHect is a valid and reliable questionnaire and is culturally valid. Further validation studies in other languages will allow language and cultural differences to no longer be a barrier to collaborative research and a large prospective international cohort on endometriosis.
Keywords: Endometriosis, Questionnaire, WERF Ephect, Translation, Cross Cultural Adaptation.

P-119: Comparison of Ovarian Stimulation Cycle Outcome in Medroxyprogesterone Acetate and Gonadotropin Protocol with Antagonist and Gonadotropin Protocol in Polycystic Ovarian Syndrome Patients Undergoing IVF-ICSI: A Clinical Randomized Controlled Trial

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Background: The aim of the study was to analyze cycle characteristics and embryological outcome using gonadotropins in combination with medroxyprogesterone acetate (MPA) in comparison with antagonist protocol for PCOS patients who are undergoing IVF/intracytoplasmic sperm injection (ICSI) treatments.
Materials and Methods: This randomized controlled trial study was conducted between 2018 and 2022, on 165 PCOS women undergoing IVF/ICSI. Patients with age > 39, BMI > 30, poor ovarian response in previous cycles, stimulation in the last 3 months, contraindications for ovarian stimulation, sever male factor infertility didn't include the study. Women were randomly assigned to one of the two groups according to the ovarian stimulation protocols: gonadotropin (rFSH) and MPA protocol (n=80) or antagonist protocol (n=85). The primary outcome measure was fertilization rate and secondary outcome measures were mean number of retrieved oocytes and MII oocytes, OHSS risk and premature LH surge. For data analysis, SPSS software was used. In all tests, the significance level was considered less than 0.05.
Results: Basic characteristics such as infertility duration, age, body mass index and cause of infertility were comparable in both groups. The FSH dosage and ovarian stimulation duration were comparable between groups. No significant differences were found in the mean number of oocytes retrieved, high-quality embryos and fertilization rate between groups. No incidence of premature LH surge was seen in both groups. The serum LH level on triggering day was significantly higher in study group than control group (3.34 ± 0.5 vs 1.78 ± 0.21, P=0.007). The incidence of OHSS was low between the 2 groups, with no significant difference. The clinical pregnancy rate and miscarriage rate was comparable between two groups.
Conclusion: This study showed that the application of Progestin-primed ovarian stimulation (PPOS) protocol with Med Roxy progesterone Acetate in PCOS patients could achieve comparable oocyte retrieval, embryological and pregnancy outcomes to GnRH-ant protocol.
Keywords: Polycystic Ovary Syndrome, Medroxyprogesterone Acetate, Assisted Reproductive Technique, Intracytoplasmic Sperm Injection.

P-120: Investigating The Role of Endometrial Scratching on Pregnancy Success Rates in Patients With a History of Unsuccessful Embryo Transfer

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Background: Endometrial scratching is a procedure that the lining of the uterus (the endometrium) is 'scratched' using a small sterile plastic tube. This procedure has been studied to improve implantation rates and decrease the incidence of implantation failure. The evidence describing the impact of endometrial injury is controversial; therefore, we investigate the role of endometrial injury on pregnancy success rate in patients with a history of unsuccessful embryo transfer in Emam-Khomeini hospital, sari.
Materials and Methods: In this historical cohort study, patients with a history of unsuccessful embryo transfer (at least 2 time) were included. In hysteroscopy scratching group, it was performed on the 21th day of the cycle, then on the third day of next cycle they underwent endometrial preparation with hormone therapy. In other group, this procedure was not done but the preparation of the endometrium was similar. Two weeks after embryo transfer, B-HCG levels were measured, and at 5 weeks of gestation, transvaginal ultrasound was done to confirm the pregnancy sac.
Results: 132 participants were included in this study. The participants were women aged 20-40 years, and all these women had grade A and B embryos that were frozen at the 8-12 cell stage. 69 patients were in the control group and 63 patients were in the intervention group. Demographic characteristics of patients including age, BMI, and duration of infertility were not significantly different between groups (P>0.05). Clinical pregnancy rate was 26% (18 cases of 69 patients) in the control group and 46% (29 cases of 63 patients) in the intervention group (P=0.017).
Conclusion: The results show that endometrial injury increase the clinical pregnancy rate in patients with a history of unsuccessful embryo transfer.
Keywords: Repeated Implantation Failure, Endometrial Scratching

P-121: Association between Oocyte Quality and Serum Vitamin D Levels in Women Undergoing Fertilization Treatment

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Background: Vitamin D3 has been considered as a crucial factor influencing female reproductive health. It plays a vital role in follicular development, oocyte maturation, and subsequent embryo quality, making it an essential factor in assisted reproductive technologies such as fertilization (IVF). Vitamin D3 levels have been positively correlated with ovarian reserve markers and anti-Müllerian hormone (AMH) concentrations, indicating its potential impact on fertility potential. This study aimed to investigate the relationship between oocyte quality and vitamin D3 levels in women undergoing IVF treatment.
Materials and Methods: Serum vitamin D3 concentrations were quantified before initiating IVF cycles. A total of 115 oocytes were collected from 35 women, with a mean age of 32 ± 7 years. The quality of the retrieved oocytes was subsequently evaluated by an embryologist. Following the removal of cumulus cells, individual mature oocytes were imaged to evaluate morphometric characteristics. Serum vitamin D levels and oocyte quality were compared between the groups. The patients were classified as having sufficient (≥ 30 ng/mL) or insufficient (<30 ng/mL) serum vitamin D3. SPSS software (version 27.0) was used for statistical analysis.
Results: The results indicated no significant difference between serum vitamin D levels and the mean ooplasm diameter and area. However, patients with sufficient serum vitamin D levels (≥ 30 ng/mL) exhibited better oocyte quality. They had a circular oocyte shape, standard ooplasm coloration, and a normal polar body shape (less fragmentation of the polar body), and thin and uniform zona pellucida were observed in this group.
Conclusion: Low serum vitamin D levels are associated with decreased oocyte quality, and since oocyte quality affects the success rates of IVF, it can be concluded that vitamin D may influence the outcomes of IVF procedures. However, the effects of serum vitamin D levels on reproductive outcomes should be examined in future comprehensive cohort studies.
Keywords: Vitamin D, IVF, Quality, Oocytes.

P-122: Enhancing Fertility Prospects Through Frozen Embryo Transfer in Endometrioma-Afflicted Patients

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Background: Endometrium, the quintessential clinical presentation of endometriosis, is indicative of the disease's severity. fertilization (IVF) coupled with embryo transfer constitutes a critical therapeutic modality for the amelioration of infertility concomitant with endometriosis. Nevertheless, the determination of the optimal embryo transfer technique (fresh versus frozen) and its consequent impact on the rates of successful pregnancies remains an area of active debate, underscored by a paucity of investigative studies. This study aimed to elucidate the differential outcomes in fertility and neonatal health consequent to fresh versus frozen embryo transfers in women diagnosed with endometrioma experiencing infertility.
Materials and Methods: Employing a retrospective cohort design, this investigation analyzed data extracted from the medical records of 200 women diagnosed with endometrioma-associated infertility who underwent treatment at the Royan Institute from 2016 to 2021.
Results: Comparative analysis of fertility outcomes between the two groups, which were demographically comparable and exhibited no significant variance in endometrioma dimensions, revealed a notable disparity solely in the live birth rate. The incidence of live births was 36.4% within the groups undergoing frozen embryo transfer, as opposed to 22.8% within the fresh embryo transfer group (P=0.04). Neonatal outcomes demonstrated no significant differences between the groups.
Conclusion: The findings of this study advocate for the utilization of frozen embryo transfer as a strategy that may augment the likelihood of live births in women diagnosed with endometrioma.
Keywords: Endometrium, Embryo Transfer, Fertility Outcomes, Pregnancy Outcome, Neonatal Outcomes.

P-123: Investigating The Hydro alcoholic Extract of Cloves on Increasing Fertility by IUI Method in The Ovaries of Adult Rats

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Background: Paying attention to the importance of reproduction in the continuation of the life of animals, it is important to find a way to eliminate the factors that cause infertility. One of the causes of infertility among women is ovarian torsion, which, if not treated in time by cutting off the blood supply to the ovary, causes it will disappear. The use of medicinal plants has been the basis of traditional medicine, and these plants are the structural basis of many chemical medicines today. Cloves have always been considered among various medicinal plants and spices for their antioxidant properties and can probably be a suitable option for improving fertility or treating infertility caused by ovarian torsion. On the other hand, many laboratory and clinical treatments for infertility are being done. IUI is one of them. This method is low cost and less invasive than IVF method. In the present study, the protective effect of hydroalcoholic extract of cloves on the ovary under torsion in rats and its effect on the treatment of infertility resulting from ischemia/reperfusion after performing the IUI method were investigated.
Materials and Methods: For this purpose, 20 rate were randomly divided into 4 groups. After IUI, the number of pregnant rate was counted using this method. The first group as a control group, the second group of rate that underwent torsion and did not receive treatment with hydroalcoholic extract of cloves, the third group of rate that were torsioned and treated with 30 mg of cloves, the fourth group of rate that were torsioned and treated with 60 mg extract of cloves were treated.
Results: The data showed that the use of this plant is effective in increasing the ovarian follicles of female rats and increases the number of offspring through follicles.
Conclusion: It seems that the hydroalcoholic extract of cloves has a role in the treatment of infertility, so it is recommended for additional animal and human studies.
Keywords: Ovarian Torsion, Rat, Ischemia/Reperfusion, Clove Extract, IUI

P-124: Evaluation of Hereditary Thrombophilia Variants of FII And MTHFR with Recurrent Implantation Failure Referred to Royan Institute in Women

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Background: Assisted reproductive methods (ART) are used as the therapeutic choices in infertile couples in which implantation of the growing embryo is one of the essentials steps. Recurrent implantation failure (RIF) as the inability of the embryo to attach the endometrium after repeated transfer of high quality embryos is a multifactorial phenomenon of which, hereditary thrombophilia has been controversially suggested to involved in etiology.
Materials and Methods: In this study, we examine the frequency of FII(G20210A), MTHFR A1298C, MTHFR C677T factors in two study groups; controls consist of 100 women younger than 40 years who have experienced clinical pregnancy following their first or second ART cycle (ART+) and 100 women clinically diagnosed as RIF with at least three failed ICSI or IVF treatment cycles including at least 4 good-quality embryos. Genotyping of the variants were done using PCR-RFLP and confirmed by Sanger sequencing. Statistical analysis was done by Chi-square test.
Results: Our data showed no significant difference in frequencies of FII(G20210A) and MTHFR C677T variants between the RIF and ART+ groups for both genotype as well as allele frequency. For MTHFR A1298C variant, frequency was significantly higher in RIF group compared to ART+ for both mutant genotype as well as mutant allele. In addition, cumulative frequencies of mentioned variants (presence of at least one mutated allele) showed a relative increase in RIF patients compared to control groups, although data was not statistically significant. Although several studies have reported significant associations between hereditary thrombophilia and RIF, the data attained through this research could not fully support this, except for MTHFR A1298C variant. Controversy observed in the results of various studies can be due to variations in ethnic back grounds of each population, lack of agreement on a common definition for RIF, the small sample sizes and different control groups.
Conclusion: MTHFR A1298C is possibly correlated with implantation failure in RIF patients and can be proposed as a potential risk factor. For other two studied variants, we suggest for more extensive studies and inclusion of normal fertile control group.
Keywords: Hereditary Thrombophilia, Recurrent Implantation Failure

P-125: Evaluation of Hereditary Thrombophilia Variants of FII (G20210A) And MTHFR (A1298C), MTHFR (C677T) with Recurrent Implantation Failure Referred to Royan Institute in Women

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Background: Assisted reproductive methods (ART) are used as the therapeutic choices in infertile couples in which implantation of the growing embryo is one of the essentials steps. Recurrent implantation failure (RIF) as the inability of the embryo to attach the endometrium after repeated transfer of high quali-

ty embryos is a multifactorial phenomenon of which, hereditary thrombophilia has been controversially suggested to involved in etiology.
Materials and Methods: In this study, we examine the frequency of FII(G20210A), MTHFR A1298C, MTHFR C677T factors in two study groups; controls consist of 100 women younger than 40 years who have experienced clinical pregnancy following their first or second ART cycle (ART+) and 100 women clinically diagnosed as RIF with at least three failed ICSI or IVF treatment cycles including at least 4 good-quality embryos. Genotyping of the variants were done using PCR-RFLP and confirmed by Sanger sequencing. Statistical analysis was done by Chi-square test.
Results: Our data showed no significant difference in frequencies of FII(G20210A) and MTHFR C677T variants between the RIF and ART+ groups for both genotype as well as allele frequency. For MTHFR A1298C variant, frequency was significantly higher in RIF group compared to ART+ for both mutant genotype as well as mutant allele. In addition, cumulative frequencies of mentioned variants (presence of at least one mutated allele) showed a relative increase in RIF patients compared to control groups, although data was not statistically significant.
Conclusion: MTHFR A1298C is possibly correlated with implantation failure in RIF patients and can be proposed as a potential risk factor. For other two studied variants, we suggest for more extensive studies and inclusion of normal fertile control group.
Keywords: Hereditary Thrombophilia, Recurrent Implantation Failure, Assisted Reproductive Technology

P-126: Association between Disease Activity, Adenosine Monophosphate-Activated Protein Kinase And Mammalian Target of Rapamycin Genes Expression in Rheumatoid Arthritis Patients During Pregnancy

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects patients' lives, including their reproductive health. This study aims to evaluate the association between disease activity, gene expression of AMPK and regulatory protein of mTOR (Raptor) in RA patients during pregnancy compared to non-pregnant RA patients and healthy controls.
Materials and Methods: A total of 45 participants were included, divided into three groups: RA patients during pregnancy, non-pregnant RA patients, and healthy controls. Using Real-time PCR, we assessed the gene expression levels of AMPK and Raptor in all participants.
Results: RA patients during pregnancy exhibited a significant decrease in DAS-28 compared to non-pregnant RA patients (P=0.001). Moreover, the expression of the Raptor gene was significantly lower in RA patients during pregnancy compared to non-pregnant RA patients and the healthy control group (P=0.031 and P=0.012, respectively). Conversely, pregnant women with RA displayed a higher level of AMPK expression compared to non-pregnant RA patients (P=0.041). Notably, there were no significant differences in birth weight between the two patient groups (P=0.114).
Conclusion: Our study highlights the association between disease activity, gene expression of AMPK and Raptor in RA patients during pregnancy. Pregnancy appears to contribute to a significant decrease in disease activity, as indicated by lower DAS-28 scores. Furthermore, the altered expression of AMPK and Raptor genes in pregnant RA patients suggests their potential role in ameliorating the inflammatory condition of patients during pregnancy.
Keywords: Rheumatoid Arthritis, Pregnancy, Birth Weight, AMPK, m-TOR

P-127: Malondialdehyde as A Biomarker for The Diagnosis of Infertility in Women: A Systematic Review

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Background: Reproductive failure is defined as the failure to conceive a recognized pregnancy after 12 months of regular unprotected intercourse. About half of infertility cases are female factors such as polycystic ovary syndrome (PCOS). Research has shown that high levels of malondialdehyde (MDA) are associated with conditions such as PCOS. MDA is a highly reactive compound that can react with various biomolecules. It is derived from lipid peroxidation and it is a biomarker to measure oxidative stress (OS). Evaluation of the impact of OS on women's fertility represents a significant gap in our knowledge about treatment infertility, so in this systematic review we aimed to find out the impact of OS on pregnancy success by monitoring MDA levels in women.
Materials and Methods: A literature search was conducted in PubMed, ProQuest, and Web of Science for relevant studies. This paper provided a thorough examination of the different studies that published from January 2015 to May 2024. Search terms included "Infertility ", "Malondialdehyde ", "Female factors ", and "Oxidative stress". However, out of 198 studies involving the effect of malondialdehyde level on female fertility, only 113 were original article. Reviews were excluded.
Results: Our research showed that pregnancy rates were decreased in higher malondialdehyde levels. Also pervious study showed that the mean MDA levels per age and BMI were 47% increase in women with PCOS. Results showed a significant decrease in the number of primordial, secondary, and antral follicles associated with reduced mRNA levels of genes essential for follicle maturation and ovulation that they are affected by the increase in MDA level and OS.
Conclusion: The role of OS in female infertility and subfertility is an area deserving of continued research. MDA can be used as a marker of OS and a potential marker in predicting assisted reproductive techniques outcome. Since high levels of free radicals and a lower antioxidant status have been reported to induce infertility, employing treatment strategies that involve the use of antioxidant compounds to retard the free radical induced oxidative damage and prevent infertility is necessary.
Keywords: Infertility, Malondialdehyde, Female Factors, Oxidative Stress

P-128: No Title Altered Expression of Interleukin-6, Heparin-Binding Epidermal Growth Factor, And Glycodelin a in Endometrium of Women with Hydrosalpinx following Sal-

pingectomy: A Case-Control Study

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Background: Hydrosalpinx, as one of the known diseases of the fallopian tubes, reduces the embryo implantation rate and success in *in vitro* Fertilization (IVF). It can exhibit its destructive effects through Inflammatory conditions in the uterine endometrium which may lead to alterations in the expression of cytokines and transcription factors involved in the ER and embryo implantation, such as Interleukin-6 (IL-6), Heparin-binding epidermal growth factor (HB-EGF) and Glycodelin A (GdA). These molecules have a crucial role in ER and embryo implantation. Hence, we evaluate the mRNA expression of the mentioned genes in the endometrium of women with hydrosalpinx following salpingectomy
Materials and Methods: This case-control study was performed at Royan Institute. A total of 30 volunteers were recruited for this study: Fifteen patients with hydrosalpinx and fifteen fertile women as the control group. All subjects underwent uterine endometrial sampling by Pipple on days 19 to 24 of the menstrual cycle. The Real-time polymerase chain reaction (PCR) technique was used to quantitatively analyze gene expression.
Results: mRNA expression of IL-6 showed a significant increase in patients with hydrosalpinx before salpingectomy compared to the fertile group, and after salpingectomy, it was significantly decreased. *HB-EGF* and *GdA* gene expression was significantly reduced before salpingectomy, and after the removal of hydrosalpinx was significantly elevated.
Conclusion: *IL-6*, *HB-EGF*, and *GdA* gene expression in the hydrosalpinx group are lower than in the fertile group, and salpingectomy can be beneficial for the recovery of endometrium from the destructive effects of hydrosalpinx and improves the expression pattern of the key molecules involved in ER and embryo implantation.
Keywords: Interleukin-6, HB-EGF, Glycodelin A, Hydro Salpinx, Endometrial Receptivity

P-129: Altered Expression of Interleukin-6, Heparin-Binding Epidermal Growth Factor, and Glycodelin A in Endometrium of Women With Hydrosalpinx following Salpingectomy: A Case-Control Study

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Background: Hydro salpinx, as one of the known diseases of the fallopian tubes, reduces the embryo implantation rate and success in Invitro Fertilization (IVF). It can exhibit its destructive effects through Inflammatory conditions in the uterine endometrium which may lead to alterations in the expression of cytokines and transcription factors involved in the ER and embryo implantation, such as Interleukin-6 (IL-6), Heparin-binding epidermal growth factor (HB-EGF) and Glycodelin A (GdA). These molecules have a crucial role in ER and embryo implantation. Hence, we evaluate the mRNA expression of the mentioned genes in the endometrium of women with hydrosalpinx following salpingectomy.
Materials and Methods: This case-control study was performed at Royan Institute. A total of 30 volunteers were recruited for this study: Fifteen patients with hydrosalpinx and fifteen fertile women as the control group. All subjects underwent uterine endometrial sampling by Pipple on days 19 to 24 of the menstrual cycle. The Real-time polymerase chain reaction (PCR) technique was used to quantitatively analyze gene expression.
Results: mRNA expression of IL-6 showed a significant increase in patients with hydrosalpinx before salpingectomy compared to the fertile group, and after salpingectomy, it was significantly decreased. *HB-EGF* and *GdA* gene expression was significantly reduced before salpingectomy, and after the removal of hydrosalpinx was significantly elevated.
Conclusion: Due to inflammatory conditions in the hydrosalpinx group, *IL-6* gene expression is elevated, but *HB-EGF* and *GdA* gene expression are lower than in the fertile group, and salpingectomy can be beneficial for the recovery of endometrium from the destructive effects of hydrosalpinx and improves the expression pattern of the key molecules involved in ER and embryo implantation.
Keywords: Interleukin-6, HB-EGF, Interleukin-6, Hydrosalpinx, Endometrial Receptivity

P-130: Comparison of The Effects of Metformin and Empagliflozin on Antimullerin Hormone Levels In Polycystic Ovary Syndrome Patients Undergoing Intracytoplasmic Sperm Injection

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Background: Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder, typically characterized by anovulation, infertility, Hyperandrogenism, Insulin resistance and increased levels of anti-Müllerian hormone (AMH) .Metformin has long been used in the treatment of polycystic ovary syndrome. Recently, it has been reported that empagliflozin can be effective in improving the symptoms of PCOS. Therefore, in this research, we decided to investigate the effects of metformin and Empagliflozin on Antimullerin hormone levels in PCOS patients.
Materials and Methods: Participants were randomly assigned to receive metformin, empagliflozin, and placebo. The treatment was performed 2 months before the start of the ovulation cycle and continued until the day of the puncture.
Results: The serum level of Antimullerin Hormone (AMH) before and after treatment showed a significant decrease in both metformin and empagliflozin groups, but this decrease was significant in the empagliflozin group (P=0.01) and not significant in the metformin group (P=0.08). Also, serum AMH level decreased after 2 months of drug use between metformin and empagliflozin treatment groups with placebo, and this decrease was significant in empagliflozin group. However, the AMH level of follicular fluid (FF) in metformin and empagliflozin group after 2 months of drug use was not significantly different compared to placebo (P=0.58).
Conclusion: Therefore, it can be said that empagliflozin can perform better in reducing serum levels and modulating AMH in PCOS patients who have higher AMH compared to metformin.
Keywords: PCOS, Antimullerin Hormone, Empagliflozin, Metformin, Infertility

P-131: Investigating The Predictive Value of FOI (Follicle-To-Oocyte Index), AMH, FSH, LH Hormone And Baseline Serum Levels of FSH/LH With Fertility Outcomes in Infertile Women Undergoing ICSI Cycles in Kamali Infertility Center in 2024

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Background: The success rate of assisted reproductive techniques (ART) is still low, especially in women with poor ovarian responses. Considering the risks of hormone therapy, it is necessary to use cheap, available and highly predictive indicators to ensure the correct selection of patients and to express the chance of success rate in consultation with patients. For this purpose, many biomarkers have been utilized. AMH and antral follicle count (AFC) are considered to be the most commonly available methods. Other advanced methods have been proposed, such as the ratio of the number of oocytes obtained during puncture to the number of follicles greater than 17 mm in the ovary on the trigger day (FOI) and the FSH /LH ratio. Objective: This study was designed to investigate the Predictive value of FOI, FSH/LH ratio and baseline serum levels of FSH and AMH with fertility outcomes in infertile women undergoing ICSI cycles.
Materials and Methods: This retrospective cohort study was performed on 200 infertile women undergoing ICSI treatment to investigate the relationship between FOI, FSH/LH ratio and baseline serum levels of FSH and AMH as independent variables and average variables (fertility outcomes).
Results: The receiver operating characteristics (ROC) showed statistically significant AUC values for FOI (0.604,p=0.014) and AMH (0.711, P<0.001),while FSH and FSH/LH were not significant. Logistic regression revealed that the strongest predictors of live birth were FOI [RR (95% CI): 3.1(1.5-6.3), P=0.002] and AMH [4.11 (1.98-8.55), P<0.001].
Conclusion: These findings suggest that FOI has a comparable predictor value to AMH, and both are useful biomarkers for predicting the likelihood of live births in this population.
Keywords: Live birth, Antral Follicle, AMH, FOI

P-132: Effects of Long-Tterm and Short-Term Administration of Testosterone and Nandrolone Deconate on Sperm Parameters and Testicular Tissue in rats following exercise

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Background: Synthetic testosterone derivatives, such as nandrolone (N), are commonly used in medicine to maximize anabolic effects while minimizing androgenic effects. Illicit use of anabolic androgenic steroids is prevalent among adolescent and bodybuilders because of their anabolic proprieties and their capacity to increase tolerance to exercise. Exogenous testosterone (T) adversely affects spermatogenesis in fertile men, but discontinuation generally leads to recovery. The aim of this study was to investigate the impact of short and long-term administration of testosterone and nandrolone on testis histopathology and sperm parameters following exercise.
Methods: The study consisted of two components: short-term and long-term administration of T and ND. Thirty-six male NMRI mice were used for each component. Animals were divided into six groups: control, exercise, N (0.3 mg/kg), T (0.15 mg/kg), N+E, and T+E. The treatment duration was 14 days for the short-term component and 36 days for the long-term component. Sperm parameters, testis histology (number of spermatogonia, spermatocyte, Sertoli cell, Leydig cells, round and elongated sperm) and stereological study (index of tubular differentiation, spermatogenesis, Sertoli cell, and meiosis, total volume of testis, seminiferous tubules and interstitial tissue) were analyzed.
Results: There were no differences between the control and exercise groups. Short-term administration of N and long-term treatment with N and T significantly reduced sperm quality. Both short and long term administration of N and T had delete-

rious effects on testicular histology and stereological indices. Exercise was found to improve sperm parameters and testicular histopathology particularly in the long-term group.
Conclusion: Nandrolone demonstrated more detrimental effects on testicular tissue compared to testosterone. However, the harmful effects of anabolic androgens on testicular tissue can be mitigated by exercise. These findings shed light on the potential impact of testosterone and nandrolone administration on male reproductive health and the potential benefits of exercise in counteracting their adverse effects.
Keywords: Testosterone, Nandrolone deconate, testicular tissue, exercise

Genetics

P-133: Evaluation of Gene Expression of GREM1 as An Oocyte Maturation Marker in Follicular Fluid of Women with Endometriosis

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Background: Endometriosis (EM) is a chronic inflammatory and estrogen-dependent disease that is defined as the migration and implantation of endometrial lesions outside the uterus and is associated with pelvic pain and infertility. Poor oocyte quality and the subsequent impairment of embryo quality are considered two critical parameters in EM-related infertility. Oocyte analysis is not usually possible; therefore, indirect assessments of oocyte quality must be used. Follicular Fluid (FF) is a non-invasive method to assess oocyte quality and contains biologically active molecules essential for follicular development. Gremlin 1 (GREM1) is a highly conserved protein and a bone morphogenetic protein antagonist that affects cumulus cells function, oocyte developmental competence, and follicle development. For this reason, this study aims to find a relationship between oocyte quality and GREM1 expression in the FF of women with EM.
Materials and Methods: In this study, we investigated the expression level of GREM1 gene in FF of women with EM (n=10) compared to women without EM as a control group (n=10) using qRT-PCR. The with and without EM groups were not significantly different in age, BMI, and levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and anti-müllerian hormone (AMH).
Results: The result indicated that the expression of GREM1 on the level of mRNA has no significant difference in the EM group compared to those from healthy control women (P=0.1972).
Conclusion: Therefore, it seems that there is a need to confirm this data with more patient samples to conclude whether there is a difference between endometriosis patients compared to healthy control women in terms of gene expression of GREM1.
Keywords: Endometriosis, GREM1, Follicular Fluid, Oocyte Quality

P-134: A Missense Variant in FSHR Associated with Polycystic Ovary Syndrome in An Iranian Family

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Background: Infertility is defined as the inability to conceive after one year of regular unprotected sex. Polycystic ovary syndrome (PCOS) is a common endocrine metabolic disorder that affects 5-15% of women of reproductive age and is one of the most important causes of female infertility. It is a complex and multifactorial disease in which genetic factors play a significant role in the occurrence of this disease. The purpose of this study is to investigate the genetic causes of female infertility in three infertile members of an Iranian family using Whole Exome Sequencing (WES).
Materials and Methods: In this study, a family with 3 infertile sisters, two of whom had PCOS, was selected. WES was performed in the infertile sister with PCOS and Sanger sequencing was achieved in other family members to confirm the candidate genes.
Results: Based on WES data, several genes were candidates and finally only a heterozygous missense mutation in the FSHR gene (NM_000145:exon5:c.C445T:p.Q149X) was confirmed in two affected sisters.
Conclusion: It seems that the found variety changes the amino acid of the produced protein, as a result of which the structure and function of the FSHR protein are changed. Heterozygote mutations in the FSHR gene could be a pathogenic agent for PCOS in an Iranian family.
Keywords: Female Infertility, PCOS, WES, FSHR

P-135: Evaluation of The Genetic Variants in Exon 5 of The *SEPTIN12* Gene in Infertile Men with Acephalic Syndrome

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Background: Acephalic sperm syndrome represents a rare form of teratozoospermia with significant implications for male fertility. It is characterized by sperm displaying headless flagella, heads lacking flagella, or abnormal head-to-flagella connections, all stemming from genetic factors. Among the genes implicated in this syndrome is SEPTIN12, crucial for the final differentiation of male germ cells, particularly expressed in the head, neck, and sperm annulus post-meiosis.
Materials and Methods: A total of 20 infertile men with acephalic sperm syndrome (<30% acephalic spermatozoa in total sperm population) were considered as the case group and 20 men with normal spermogram as the control group. DNA was extracted from the blood samples of selected individuals. After designing primers, PCR reactions were done for each DNA

sample. Then, DNA sequencing was performed for PCR products of exon5. Ultimately sequencing was used to determine genetic changes in the mentioned exons. The results were analyzed by Finch TV and Blast.
Results: The results of sanger sequencing showed that a homozygous mutation, c.474G>A, in exon 5 of the *SEPTIN12* gene was identified in an individual of Iranian descent affected by acephalic sperm syndrome.
Conclusion: By examining the genetic change of exon 5 of the *SEPTIN12* gene, a synonymous nucleotide change was observed in exon 5 of one of the Iranian subjects studied by us, which may play a role in the development of acephalic sperm syndrome.
Keywords: Acephalic Sperm Syndrome, SEPTIN12, Genetic Variations

P-136: Evaluation of Genetics Variations of Exon 2 of The *AURKC* Gene in Patients with Macrocephalic Spermatozoa Referring to Royan Institute

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Background: Macrocephalic is defined as large-headed, multiflagellated spermatozoa in infertile men and results in the father's existence unable to have a biological child. Sperm macrocephalic syndrome is caused by the mutation of the Aurora Kinase C (*AURKC*) gene situated on the long arm of chromosome 19 at 19q13.43. The *AURKC* gene encodes the third member of the Aurora subfamily of serine/threonine protein kinases and is usually expressed in the testis. The AURKC gene plays exigent roles in centrosome function, homologous chromosome segregation, and cytokinesis for meiosis. The purpose of the current study was to evaluate genetic variations of exon 2 of the **AURKC** gene in infertile men with sperm macrocephalic syndrome defect.
Materials and Methods: A total of 15 infertile men with sperm macrocephalic syndrome were considered as the case group and 15 men with normal spermogram as the control group. DNA was extracted from the peripheral blood samples of selected individuals. After designing primers, PCR reactions were done and DNA sequencing was performed for PCR products. The results were analyzed by Finch TV and Nucleotide Blast.
Results: Results of sanger-sequencing revealed no mutations in men with sperm macrocephalic syndrome or the control group.
Conclusion: According to our study, it can be concluded that there is no relationship between the occurrence of sperm macrocephalic syndrome disorder and nucleotide changes in exon 2 of the *AURKC* gene and it is suggested to review other exons and Regulatory regions.
Keywords: Infertile Men, Sperm Macrocephalic Syndrome, AURKC Gene

P-137: DNA Methylation on The Human Mesoderm-Specific Transcript Has Negative Effect on Male Fertility

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Background: There have been lots of investigations to find reasons which cause infertility in male gender. By developments in genetic and epigenetic sciences, focuses on relationship between changes in fundamental elements of genome function and fertility loss is growing. The below content evaluates probable mistakes outcrop in Mesoderm-Specific Transcript (MEST) Differentially Methylated Region (DMR), one of common reasons of this problem.
Materials and Methods: In the course of methylation arrangement of the MEST recognition, semen samples were assembled from forty-five men which divided into three groups: fifteen people with normal sperms considered as the control group, fifteen ones with asthenospermia, and fifteen men suffering from both oligospermia and asthenospermia (oligoasthenoteratospermia) as the cases group. The first test utilized in the procedure was standard semen analysis including sperms count, size, condensation, shape and motility. The next step was chromatin quality and sperm maturity that accomplished by Aniline Blue (AB) dye to color the head of spermatozoa. Then, the DNA of sperms extracted and treated with sodium bisulfite to distinguish unmethylated sequences. Quantitative methylation-specific polymerase chain reaction (qMSP) was used for MEST gene DMRs measurement.
Results: The evaluations indicated lesser size, number and normal spermatozoa morphology meanwhile more histone transition (immature sperms) in infertile individuals than normospermia cases. In oligoasthenoteratospermia cases in comparison with normospermia and asthenospermia showed a momentous higher histone abnormalities and MEST methylation.
Conclusion: Observations and data analysis illustrates the inverse relationship between normal sperm criteria and histone anomality and methylation in MEST genes and betokens the findings to diagnose and predict loss of fertility earlier than before.
Keywords: Male Infertility, MEST Gene, DNA Methylation, Sperm Abnormalities, qMSP

P-138: Unveiling Genetic Variations in Spontaneously Aborted Aneuploid Fetuses of Consanguineous Couples with A History of Recurrent Pregnancy Loss by Whole Exome Sequencing

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Background: Recurrent pregnancy loss (RPL) impacts 5% of couples globally, posing physical and mental health challenges. Chromosomal abnormalities are prevalent causes of miscarriage in consanguineous couples. In this study genes linked to miscarriage and aneuploidy using whole exome sequencing (WES) have been identified.
Materials and Methods: The first-trimester aneuploid products of conception (POC) of five consanguineous couples with a history of RPL were selected. The ACMG guideline was considered for variant classification. All the probable causal variants were confirmed by Sanger sequencing in all POC and

available couples' blood DNA.
Results: In five pedigrees, four pathogenic/likely pathogenic and 12 uncertain variants of genes associated with miscarriage etiologies have been identified, some cause aneuploidy in studied models. Nine of them were previously reported in *PZP, AXL, CGAS, CCNB1, CFTR, COL4A2, SULT1A1, MAD1L1*, and *CD109* genes, and two novel variants were found in NCAPH and LRRFIP1. Additionally, five genes were identified as novel candidates for human miscarriage/aneuploidy based on available evidences. These genes are associated with processes such as implantation, placentation, coagulation, immune response, metabolism, fetal growth, cell cycle, and ovarian functions.
Conclusion: These findings contribute to understanding the genetic factors involved in reproductive health. The genomic findings could help such couples and their relatives for a healthier life, considering crucial role of genes involved in aneuploidy in somatic cells, and increase the likelihood of live birth via Preimplantation Genetic Testing (PGT) in future conceptions.
Keywords: Whole Exome Sequencing, Aneuploidy, Miscarriage, Recurrent Pregnancy Loss

P-139: Deciphering The Genetic Blueprint of Germ-Line Stem Cells: A Pathway to Novel Therapeutic Strategies

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Background: Male germline stem cells (mGSCs) and female germline stem cells (fGSCs) are crucial for transmitting genetic information to future generations. mGSCs, or spermatogonial stem cells, located in the testes, produce spermatozoa via spermatogenesis. Their key regulatory pathway involves GDNF, activating RET, and GFRα1 receptors to promote self-renewal and proliferation. In contrast, fGSCs, or oogonial stem cells, located in the ovaries, participate in oogenesis, regulated by the KIT ligand, its receptor c-KIT, and the PI3K/Akt pathway. Germline stem cells hold significant potential in regenerative medicine and fertility treatments. mGSCs can be used in gene therapy to correct genetic defects before transmission, while fGSCs can generate oocytes for women with diminished ovarian reserves or premature ovarian failure. mGSCs maintain sperm production throughout life, whereas fGSCs' oocyte production declines with age. Understanding these cells' gene transcription profiles and signaling pathways reveals functional differences, paving the way for clinical applications in genetic disease and infertility treatments.
Materials and Methods: We utilized microarray data (GEO accession number: GSE51313) to identify differentially expressed genes in fGSCs and mGSCs. Using the STRING database, we predicted the protein-protein interaction (PPI) network of adjacent proteins of these genes. Cytoscape and the Gephi app filtered the network's relevant nodes based on parameters. Enrichment analysis highlighted significant pathways and genes. Fluidigm real-time PCR and immunostaining validated our bioinformatics analysis.
Results: We found notable upregulation of Jun, FOS, SOX9, CDH5, KLF4, and CXCL12 in fGSCs compared to mGSCs, while KIT, POU5F1, and JAK2 were significantly downregulated in fGSCs. PPI analysis revealed five clusters with specialized functions. Enrichment analysis underscored critical pathways, including Signal Transduction, Extracellular matrix organization, RET signaling, and Regulation of Insulin-Like Growth Factor (IGF) transport. Immunocytochemistry confirmed protein presence in fGSCs, and Fluidigm qPCR validated significant gene expression differences in ZBTB16, KLF4, and POU5f1 highlighting distinct molecular profiles between mGSCs and fGSCs.
Conclusion: In summary, our study elucidates the regulatory mechanisms of mGSCs and fGSCs, highlighting their applications in regenerative medicine and fertility treatments. By analyzing gene expression and protein interaction networks, we identified key genes and pathways, providing a foundation for therapeutic strategies for infertility and genetic disorders.
Keywords: Germline Stem Cells, Regulatory Pathways, PPI Network, Gene Expression Analysis, Regenerative Medicine

P-140: Altered Expression of TLR5, NFKB, IL-6, and *TNFα* Can Reduce Endometrial Receptivity in The Patients with Hydrosalpinx Compared with The Control Group

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Background: Pathological inflammation can disrupt the interaction between the embryo and the endometrium, so changes the expression of a series of important genes and molecules in implantation and can cause pregnancy failure. One of the causes of implantation failure can be hydrosalpinx disease. Hydrosalpinx fluid contains some inflammatory factors, including inflammatory cytokines and prostaglandins, etc. May changing expression inflammatory factor that disrupts the processes involved in endometrial receptivity. The effective cleaning of pathogenic factors requires a finely tuned balance of cytokine production by host immune systems. Innate immune system against infection is the identification of pathogenic agents through pattern receptors called the Toll-like receptor family. TLRs play a key and important role in the innate immune system, which is the first line of defense against pathogens.
Materials and Methods: Endometrial samples were obtained from the case (N=10 hydrosalpinx) and the control groups (N=10 male Factor). the expression of *TLR5, NFKB, IL-6* and *TNFα* mRNA genes was evaluated using Real-time PCR method.
Results: The mRNA expression level of TLR5 was significantly decreased Also, mRNA expression of *NFKB, IL-6* and *TNFα* were significantly increased in the case group compared with the control group. ($P \leq 0.05$).
Conclusion: Reduction expression of TLR5 gene can disrupt the implantation process because NF-κB is one of the important downstream pathways of TLR that because initiates the expression and production of inflammatory cytokines. With the increase of NF-KB, two important inflammatory factors (IL-6/ TNFα) that are involved in the normalization of inflammation increase. And as a result, it causes a decrease in endometrial receptivity and embryo implantation failure.
Keywords: Implantation, IL-6/ TNFα, NF-KB, TLR5, Inflammation

P-141: Investigation of Differentially-Expressed MicroR-

NAs and Related Genes in Cervical Cancer Using An Integrated Bioinformatics Analysis

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Background: Cervical cancer is the fourth leading cause of cancer death in women worldwide, with 70% of cases occurring in developing countries. The disease is causally related to persistent infection by certain oncogenic Human Papillomavirus (HPV) infections, with high-risk types 16 and 18 in most cases. MicroRNAs (miRNAs) control the expression of their target genes through mRNA degradation or translation suppression. Modulating the miRNA activities may offer intriguing new possibilities for cancer therapy as our understanding of the target genes for miRNAs and the cellular behaviors that they affect grows. miRNAs can act as tumor suppressors or oncogenes. The regulation of signaling pathways associated with cell proliferation, epithelial-mesenchymal transition, apoptosis, cell migration, and invasiveness by sexual steroid hormones functioning through their receptors is crucial for the growth of tumors.
Materials and Methods: microarray dataset (GSE30656) was retrieved from the Gene Expression Omnibus (GEO) database and was subsequently integrated employing the "sva" package in the R programming environment. The analysis encompassed the identification of differentially expressed miRNAs using the "limma" package. Subsequently, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted to unravel functional and pathway enrichment in the context of the disease. To finalize the investigation the main miRNAs and the related genes were pinpointed through the application of CytoHubba, an algorithmic tool for identifying hub genes and regulatory RNAs in biological networks.
Results: As a result, a noteworthy differential expression was observed in 4 miRNAs (hsa-miR-370, hsa-miR-203, and hsa-miR-370 were downregulated, while hsa-miR-21 was upregulated). Furthermore, a subset of key related genes to these miRNAs indicates that MAP3K1 and ELL2 can be significant genes in the signaling pathways related to cervical cancer.
Conclusion: These miRNAs and related genes can be investigated as biomarkers for the treatment of cervical cancer, but further validation tests should be done.
Keywords: Cervical Cancer, Bioinformatics, miRNA, Biomarkers

P-142: In Silico Analysis and Validation of Differentially Expressed Imprinting Genes RASGRF1 in Endometrial Tissue of Recurrent Implantation Failure

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Background: Epigenetic alterations, including DNA methylation and gene expression, play a pivotal role in the control of imprinting genes. Notably, some of these genes are active in the endometrium, the uterine lining, where they can impact the endometrium's ability to support embryo implantation. In mice, the paternal allele of RASGRF1 undergoes imprinted methylation at a differentially methylated domain (DMD) located 30 kilobases upstream of the promoter. This DMD, acting as an enhancer blocker, can bind to CTCF in a manner sensitive to methylation. The way this DMD controls imprinted methylation is linked to recurrent pregnancy loss in mice.
Materials and Methods: Three microarray (GSE111974, GSE26787 and GSE106602) and one single cell datasets (GSE183837) related to Recurrent Implantation Failure (RIF) were obtained from the GEO database, integrated in R. Analysis included identifying differentially expressed genes. Gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses revealed functional and pathway enrichment for RIF-associated genes influenced by imprinting. Validation involved collecting 10 RIF and 10 healthy cases, followed by real-time PCR for gene expression analysis. The "sva" package in R integrated three microarray datasets on endometrial tissue of RIF, mitigating variability. Differential expression analysis with the "limma" package compared RIF groups, identifying relevant genes. Gene ontology and KEGG analyses elucidated functional and pathway enrichment for RIF-associated genes influenced by imprinting. Validation, comprising RIF and healthy cases, confirmed findings via real-time PCR. This comprehensive methodology guarantees dependable insights into the molecular mechanisms of RIF.
Results: The findings of this study shed light on the intricate molecular landscape within the endometrial tissue of individuals grappling with RIF. The identified differential expression of genes associated with imprinted gene regulation underscores the potential role of epigenetic mechanisms in the etiology of RIF. To unravel the functional implications of these genetic variations, a comprehensive functional enrichment analysis was conducted, revealing noteworthy insights into the affected biological processes. The G-protein-coupled receptor signaling pathway emerged as a focal point in the altered gene expression profile, suggesting its potential involvement in the impaired implantation process. Additionally, the regulation of interleukin-1 beta production and the binding of phosphatidylinositol bisphosphate were implicated, hinting at the intricate interplay between immune regulation and cellular signaling in the context of RIF. Delving deeper into the molecular intricacies, key differentially expressed genes (DEGs) such as RASGRF1 were pinpointed as central hub genes in the pathogenesis of RIF. The microarray and single cell analysis revealed that RASGRF1 was upregulated. Real-time PCR analysis showed significant RASGRF1 expression in RIF compared to healthy case ($P<0.05$).
Conclusion: Key DEGs like RASGRF1, validated through real-time PCR analysis, could serve as potential biomarkers. Understanding the central hub genes involved in RIF provides a molecular basis for refining assisted reproductive technologies. This knowledge may contribute to the optimization of IVF

protocols and other ART procedures.
Keywords: Endometrial, Recurrent Implantation Failure, Imprinting Gene, Microarray, DNA Methylation

P-143: Two Dominant Novel Variants in PANX1 and DCC Cause Primary Ovarian Insufficiency with Hearing Loss

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Background: Primary ovarian insufficiency (POI) includes a range of infertility in women with ovarian dysfunction to early menopause, which causes a decrease in ovarian reserve. There are limited reports on coincidence of POI and hearing loss. Here we report genomic analysis in a woman with POI and hearing loss and confirmatory genetic testing in her relatives.
Materials and Methods: Whole Exome Sequencing (WES) was applied for the proband, and causal variants were confirmed using Sanger sequencing in proband and her family.
Results: Two dominant novel variants in PANX1 and DCC were identified as causal mutations. PANX1 is a candidate gene in female infertility that has been previously in a woman with POI and hearing loss but the splicing heterozygous variant that we found has not been reported in POI so far. DCC as a known gene in deafness has been reported in hypogonadism as well, but the identified missense variant in our case is novel in POI. Considering her father's hearing loss, her parents and brother were also assessed by Sanger sequencing. It revealed that her father carries a heterozygous mutation in PANX1 and DCC, while her normal brother carries a heterozygous mutation just in PANX1 and her normal mother was normal for both variants.
Conclusion: Two dominant novel variants in PANX1 and DCC were identified as causal mutations. *PANX1* is a candidate gene in female infertility that has been previously in a woman with POI and hearing loss but the splicing heterozygous variant that we found has not been reported in POI so far. DCC as a known gene in deafness has been reported in hypogonadism as well, but the identified missense variant in our case is novel in POI. Considering her father's hearing loss, her parents and brother were also assessed by Sanger sequencing. It revealed that her father carries a heterozygous mutation in PANX1 and DCC, while her normal brother carries a heterozygous mutation just in PANX1 and her normal mother was normal for both variants.
Keywords: Primary Ovarian Insufficiency, PANX1, DCC, Whole Exome Sequencing, Hearing Loss

P-144: Protective Effects of N-Acetylcysteine on Granulosa Cells Derived from Women with Polycystic Ovarian Syndrome

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Background: Polycystic ovary syndrome (PCOS) is recognized as one of the most significant endocrine disorders, impacting 10% of women in their reproductive years. This study aimed to assess the impact of N-acetylcysteine (NAC) on apoptosis rates, mitochondrial membrane potential (MMP), and Reactive Oxygen Species (ROS) production in granulosa cells isolated from PCOS patients.
Materials and Methods: Conducted on PCOS women who volunteered for Fertilization (IVF), participants were divided into two groups: the NAC group (PCOS women receiving NAC) and the placebo group (PCOS women receiving rehydration salts). Following NAC and placebo administration, granulosa cells were isolated from the aspirated follicular fluid. Cell validation included flow cytometry for CD45 (a specific white blood cell marker, used as a negative marker) and immunostaining for Follicle-Stimulating Hormone Receptor (FSHR). To analyze the impact of NAC, expression of apoptotic and anti-apoptotic genes, as well as the rate of apoptosis, MMP, and ROS, were measured in both groups.
Results: Real-Time PCR revealed an up-regulation of anti-apoptotic markers in the NAC-treated group and a down-regulation of apoptotic genes in the placebo group. MMP was enhanced in the NAC group, while the rate of ROS decreased compared to the placebo group.
Conclusion: The findings of this study suggest that NAC may confer protective effects against cellular stress and subsequent apoptosis in granulosa cells, potentially improving fertility competence.
Keywords: Polycystic Ovary Syndrome, N-Acetyl Cysteine, Granulosa Cells, Mitochondrial Membrane Potential, Reactive Oxygen Species

P-145: Association Study of Rs3808350 Polymorphism of GPER30 Estrogen Receptor Gene in Women with Recurrent Implantation Failure Referred to Royan Institute

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Background: GPER30 estrogen receptor possibly plays an important role in the regulation of fertility, particularly in women, through modification of estrogen responses. Previous studies have linked GPER30 polymorphisms to various females' reproductive disorders such as infertility and recurrent pregnancy loss, endometriosis, as well as leiomyoma, breast and ovarian cancers. Here, for the first time, we evaluated the association between rs3808350 polymorphism of GPER30 with Recurrent Implantation Failure (RIF) in infertile women referred to Royan institute.
Materials and Methods: In this study, the frequency of rs3808350 in GPER30 gene was evaluated in 300 Iranians including 100 fertile women as control, 100 RIF cases and finally 100 women with successful ART outcome (clinical pregnancy in their first or second IVF or ICSI cycle) as second control

group. Genotyping of rs3808350 polymorphism was carried out using ARMS-PCR method and the results confirmed by Sanger sequencing.
Results: Genotype frequencies in RIF patients, ART+ group and fertile women were as AA: 50%, 29% and 45%, AG: 44%, 67% and 40%, GG: 6%, 4% and 15%, respectively. The allele frequencies in RIF, ART+ and fertile groups were: A (wild): 72%, 62.5% and 65%, G (Mutant): 28%, 37.5% and 35%, respectively. Based on statistical analysis, the differences in genotype frequency among all three studied groups were statistically significant (p-value=0.00), although for allele frequencies the differences were not statistically significant (p-value>0.05). For a better judgment to prove association between rs3808350 polymorphism and implantation failure we compared the differences between RIF and ART+ groups, where the results were statistically significant both in terms of genotype and allele frequencies (P=0.005, P=0.041).
Conclusion: Our results suggested that rs3808350 in GPER30 gene is associated with significant increase in the risk of recurrent implantation failure. Therefore, GPER30 and rs3808350 polymorphism can be considered as contributors to the process of embryo implantation possibly through modification GPER30 expression and consequent change in estrogen responses within the endometrium. Thereby, rs3808350 can be considered as a potential risk factor for human embryo implantation.
Keywords: Recurrent Implantation Failure (RIF), Polymorphisms, rs3808350, GPER30

P-146: Investigating The Expression of Paternally Imprinted Genes in The Sperm Cells of Infertile Men Referred to Royan Institute

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Background: According to the World Health Organization report, infertility affects approximately 80 million couples worldwide, with 50% of them being attributed to male factors. Since spermatogenesis transfers diverse genetic information to the oocyte, playing a crucial role in post-fertilization events, embryo formation, and growth, any disruption in this process may impact the fertility of men. Recent reports indicate that genetic disorders affecting spermatogenesis may be responsible for the majority of cases of unexplained male infertility. One identified factor contributing to infertility is the expression of various genes, including the paternally imprinted genes with a role in fertility. This study aimed to determine the expression of the Paternally-Expressed gene 3 (PEG3), Paired-Box gene 8 (PAX8) and Retrotransposon Gag-Like 1 gene (RTL1) in the sperm cells of infertile men.
Materials and Methods: In this experimental study, semen samples were collected from 20 infertile men and 20 normozoospermic men with similar age and demographic conditions who were referred to Royan Institute in Tehran. After washing the sperms, expression of genes was investigated using Real-Time Polymerase Chain Reaction (PCR) method.
Results: The expression levels of PEG3 and PAX8 genes in the infertile group were significantly lower compared to the normal group (P = 0.039 and P = 0.024, respectively). Additionally, the study found a decrease in the expression level of the RTL1 gene in the infertile group, but this change was not statistically significant (P = 0.560).
Conclusion: The findings of our study suggest considering the roles of imprinted genes in fertility process which could be a contributing factor to male infertility. Any changes in the expression and epigenetic patterns of these genes may affect the success of fertility and embryonic development.
Keywords: Male Infertility, Epigenetics, Imprinting Genes

P-147: Identification of A Novel Candidate Variant in The *PAX6* Gene Associated with Unexplained Male Infertility in An Iranian Consanguineous Family Using Whole Exome Sequencing

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Background: Infertility is a worldwide problem, affecting an estimated 15% of couples globally. Male factors are responsible for up to 50% of infertility cases, and Unexplained Male Infertility (UMI) accounts for 15-30% of all infertile couples. UMI refers to the inability to conceive, despite normal results in semen analysis, sexual history, and physical examination, with female infertility factors ruled out. Studies have demonstrated that genetic factors play a significant role in unexplained infertility. This study aims to identify novel genes and mutations that may be associated with UMI.
Materials and Methods: This study was conducted on an Iranian consanguineous family comprising three brothers with unexplained male infertility, who were referred to the Royan Institute. Whole exome sequencing (WES) was performed on two of the patients and their respective parents. Subsequently, Sanger sequencing was used to confirm the segregation of the identified variant in these individuals and the rest of the family.
Results: A splice site heterozygous mutation in the PAX6 gene (NM_001258462.3:c.-51-1G>C) was identified as the possible cause of unexplained male infertility for the first time. Furthermore, the identified variant underwent validation by Sanger sequencing and demonstrated segregation with the phenotype.
Conclusion: Our discovery in this study expands the pheno-

typic spectrum that could be associated with the *PAX6* gene, which was previously reported as a causative gene for ocular development and other processes.
Keywords: Male Infertility, Unexplained Infertility, Familial Exome Sequencing, PAX6

P-148: Effects of Resveratrol on The Apoptotic Pathway in Granulosa Cells of Healthy and Polycystic Ovary Through Klotho Modulation

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Background: Polycystic Ovary Syndrome (PCOS) is related to apoptotic signal imbalance. Previous research has shown that resveratrol plays a role in modulating apoptotic pathways in the different cell cultures. The Klotho hormone is attributed to attenuated cellular apoptosis and enhanced longevity. Therefore, we investigated the effects of resveratrol on the apoptotic pathway of human Normal Granulosa Cells (N-GCs) and Granulosa Cells from Polycystic Ovaries (PCO-GCs) via Klotho modulation.
Materials and Methods: Isolated GCs of 10 healthy women and 10 women with PCOS were divided into two parts and treated with media alone (control) and 10 μM resveratrol (treated). After 48 hours, cell viability and apoptosis were detected by Annexin-V/propidium iodide detection kit. To assess Klotho gene expression, total cellular RNA was extracted, reversely transcribed into first-strand cDNA, and qRT-PCR was accomplished using SYBR Green PCR Master Mix. Experiments were performed in triplicate.
Results: The frequency of the apoptotic cells significantly ($P<0.05$) decreased in resveratrol-treated N-GCs, whereas it increased in treated PCO-GCs ($P<0.001$). Basal Klotho gene expression by PCO-GCs was significantly higher than N-GCs. Resveratrol significantly decreased Klotho gene expression in PCO-GCs compared to N-GCs. In the presence of resveratrol, Klotho gene expression in the PCO-GCs was not significantly different from controls.
Conclusion: Resveratrol had beneficial impacts on luteinized GCs through modulation of apoptotic pathways. It can improve PCO-GCs to normal conditions by affecting the main aspects of apoptosis and Klotho gene expression.
Keywords: Granulosa Cells, Polycystic Ovary Syndrome, Resveratrol, Apoptosis, Klotho

P-149: Investigation of RAGE Protein Inhibition by Potential FDA-Approved Drugs for Treatment of Polycystic Ovary Syndrome: An In Silico Study

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Background: Polycystic ovary syndrome (PCOS) is a disease in women of reproductive age characterized by ovulation disorders, ovarian cysts, and irregular menstrual cycles. Advanced Glycation End Products (AGEs) are the end products of a non-enzymatically react that play important role in development of PCOS. Recent studies have found that significantly higher level of AGEs in serum of woman with PCOS. AGEs interact with its receptor (RAGE) and disrupts ovulation. There is currently no specific drug for treating PCOS, but among the FDA-approved drugs, 30 drugs have been suggested through in silico calculations and also 12 drugs commonly used for PCOS by doctors. The aim of this study is investigation the effect of these drugs to inhibit RAGE by using computational methods.
Materials and Methods: Structures of the drugs and RAGE were downloaded from DrugBank and RCSB databases respectively. Software tools such as Auto Dock Vina, Molegro, and Pyrx 0.8 were used to optimize structures and perform docking. Finally, the binding energy between each drug and RAGE was determined.
Results: Four drugs showed potential for inhibiting RAGE. The binding energies and stability of these drug-RAGE complexes were notable. The binding energies of Cyproterone Acetate, Ulipristal, Chlormadinone Acetate and Drospirenone were -7.4, -7.2, -7.0, -7.0 kcal/mol respectively.
Conclusion: The study identified several FDA-approved drugs with potential to inhibit the RAGE protein, which is implicated in the development of PCOS. To validate the evidence of this study, further and *in vivo* studies are required.
Keywords: Computational Methods, RAGE, PCOS

P-150: Family-Based Whole Exome Sequencing in Male Infertility: Unlocking The Potential of Genetic Analysis

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Background: It is widely believed that genetic factors contribute significantly to male infertility. However, the identified genes related to male infertility remain limited. The NGS technologies particularly Whole Exome Sequencing (WES), emerged as a powerful tool for genetic research in male infertility. This study aimed to identify candidate genes associated with male infertility.
Materials and Methods: Semen analyses of three families with infertile siblings were conducted on the affected family individuals. WES was performed on the genomic DNA extracted from the peripheral blood. The obtained fastq files were subjected to identify pathogenic candidate variants according to the

ACMG guideline.
Results: Following semen analyses, the families were diagnosed with azoospermia, asthenozoospermia, and unexplained male infertility, respectively. Bioinformatic analysis identified several pathogenic variants (listed in the table) in the affected individuals. Importantly, these variants were not present in the same genotype in normal individuals. Some of these candidate variants are located in genes known to be associated with male infertility, but others such as *CLCN1* are novel genes, which have the potential to exert an effect on male infertility. The candidate variants are subjected to co-segregation and subsequent studies to provide valuable insights into the validation and functional implications of these variants on male infertility. Asthenozoospermia Family: MT-ND5 (p.Ile257Val) MT-ND3 (p.Ala114Thr) MT-CYB (p.Ile7Thr) Azoospermia Family: CLCN1 (c.1471+1G>A) SERPINA6 (c.344T>A:p.Leu115His) USF3 (c.446_4417insGCA:p.Gln1472_Gln1473insAla) Unexplained Family: CATSPER3 (p.Glu387del) ARHGAP22 (exon1:c.34+1G>C).
Conclusion: This study such as some previous studies indicated that family-based WES is a very suitable method to find the genotype-phenotype correlation of male infertility and uncover novel genes for the expansion of the genetic panel for infertility-related genes.
Keywords: Whole Exome Sequencing, Variant, Asthenozoospermia , Azoospermia , Unexplained Male Infertility

P-151: Whole Exome Sequencing, Defective piRNA Processing, and Non-Obstructive Azoospermia

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Background: Non-obstructive azoospermia (NOA) is a severe form of male infertility characterized by the absence of sperm in the ejaculate due to spermatogenesis failure. Numerous NOA genes have been identified through genetic studies. The main aim of this study is to identify the mutation associated with NOA in the studied family.
Materials and Methods: The pedigree of a consanguineous Iranian family with three NOA individuals was analyzed using family-based Whole Exome Sequencing (WES), segregation analysis, in silico protein modeling, and single-cell RNA sequencing data analysis.
Results: Segregation analysis, in silico protein modeling, and single-cell RNA sequencing data analysis demonstrated a missense variant in the protein poly(A)-specific (*PNLDC1*) gene (NM_173516:exon9:c.710G>A;p.Gly237Asp) inherited in homozygosity. The 3′ ends of pre-piRNAs are trimmed by the PNLDC1 in an exonucleolytic style, resulting in mature piRNAs.
Conclusion: It suggests that PNLDC1 plays a role in meiosis and spermatogenesis by piRNAs processing, leading to NOA and serving as the genetic cause in this idiopathic NOA family. These findings contribute to a more effective diagnosis in clinical settings and improve genetic counseling for idiopathic NOA cases.
Keywords: Whole Exome Sequencing, piRNA, Spermatogenic Failure Diseases, Transposable Elements, CAF1 Domain

P-152: Association between Disease Activity, *AMPK* and *mTOR* Genes Expression in Rheumatoid Arthritis Patients During Pregnancy

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects patients' lives, including their reproductive health. This study aims to evaluate the association between disease activity, gene expression of Adenosine Monophosphate-Activated Protein Kinase (AMPK) and Regulatory Protein of mTOR (Raptor) in RA patients during pregnancy compared to non-pregnant RA patients and healthy controls.
Materials and Methods: A total of 45 participants were included, divided into three groups: RA patients during pregnancy, non-pregnant RA patients, and healthy controls. Using Real-time PCR, we assessed the gene expression levels of AMPK and Raptor in all participants.
Results: RA patients during pregnancy exhibited a significant decrease in DAS-28 compared to non-pregnant RA patients (P=0.001). Moreover, the expression of the Raptor gene was significantly lower in RA patients during pregnancy compared to non-pregnant RA patients and the healthy control group (P=0.031 and P=0.012, respectively). Conversely, pregnant women with RA displayed a higher level of AMPK expression compared to non-pregnant RA patients (P=0.041). Notably, there were no significant differences in birth weight between the two patient groups (P=0.114).
Conclusion: Our study highlights the association between disease activity, gene expression of AMPK and Raptor in RA patients during pregnancy. Pregnancy appears to contribute to a significant decrease in disease activity, as indicated by lower DAS-28 scores. Furthermore, the altered expression of AMPK and Raptor genes in pregnant RA patients suggests their potential role in ameliorating the inflammatory condition of patients during pregnancy.
Keywords: Rheumatoid Arthritis, Pregnancy, Birth Weight, AMPK, mTOR

P-153: Identification Crucial Genes in Pelvic Inflammatory Disease and Infertility Through Protein-Protein Interaction and Gene Regulatory Networks: A Comprehensive Systems Biology Study

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Background: Pelvic inflammatory disease (PID) is an infection of the female reproductive system. PID is usually caused by infection with Chlamydia Trachomatis (CT) and neisseria gonorrhoeae (NG). Women with PID have an increased risk of

becoming infertility. The aim of this study is to determine the molecular mechanisms that influence infertility and embryonic development in PID with CT and NG infections.
Materials and Methods: Microarray data were extracted from the Gene Expression Omnibus (GEO), and the protein-protein interaction network was constructed using Cytoscape software. Network analysis was performed to identify hub-bottlenecks and sub-networks. The functional mechanisms for critical genes were identified using Webgestalt and DAVID server. Finally, new drug candidates were repurposed using the drug-gene interaction database.
Results: RPL13, EEF1G, JAK2, MYC, IL7R, CD74, IMPDH2, and NFAT5 were identified as crucial genes in protein-protein interactions and gene regulatory networks in CT and NG infections of PID. Ribosome, hematopoietic cell lineage, platelet activation, and Chagas disease, JAK-STAT pathway, eukaryotic translation elongation, Rap1 pathway, apoptosis, protein processing in the endoplasmic reticulum, progesterone-mediated oocyte maturation, and Epstein-Barr virus infection were identified as significant signaling pathways involving in CT and NG infections.
Conclusion: Our model suggests novel critical genes, and functional pathways involved in CT and NG infections, establishing a link between these infections and infertility. However, further studies and *in vivo* are needed.
Keywords: Pelvic Inflammatory Disease, Infertility, Bacterial Infections, Protein-Protein Interaction Network, Computational Biology

P-154: Identification of Blood miRNAs in Women with Unexplained Recurrent Spontaneous Abortion Using The Systems Biology Approach

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Background: Recurrent spontaneous abortion refers to the occurrence of two or more consecutive pregnancy losses before the 20th gestational week. Miscarriage is a common disorder, affecting approximately 15–25% of pregnant women. Due to the absence of dependable screening methods for early diagnosis, accurate prediction, and effective treatment strategies, it is crucial to investigate the pathogenesis of URSA and identify novel molecular targets to address these challenges. The objective of the present study was to obtain a comprehensive understanding of the expression patterns of miRNAs in patients with URSA using bioinformatics analysis.
Materials and Methods: The non-coding RNA microarray dataset of URSA (GSE178619) was extracted GEO database. Differentially-Expressed Genes (DEGs) were identified according to (P<0.05) and (log2 fold change >0.5 and <-0.5). The miRNA-Gene, miRNA-TF, TF-miRNA, and TF-Gene relationships were identified. These relationships were input and merge for creating gene regulatory network (GRN) in cytoscape software. Network analysis done for determining crucial genes. Finally, enrichment analysis done for identifying functional pathways.
Results: miR-106b, miR-124, miR-145, miR-155, miR-16, miR-17, miR-20a, miR-223, miR-92a, miR-93, and let-7awere identified as the critical genes in GRN. These miRNAs regulate cell cycle, hormone-mediated signaling pathway, regulation of stem cell, cell proliferation and division, immune response, aging, angiogenesis, inflammation, and cell death.
Conclusion: Some of the critical genes were confirmed based on the experimental data. Further and *in vivo* studies are required for the newly predicted biomarkers.
Keywords: Abortion, Infertility, Gene Regulatory Network, miRNA

P-155: Investigating The Expression of *BARX1*, *VAX1*, *ISL2*, and *OTX2* Genes in The Follicular Fluid of Endometriosis Patients Under Treatment of IVF/ICSI Cycles

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Background: Endometriosis, a hormone-dependent disease, affects 10-15% of women and causes pain and infertility. Hormonal imbalance leads to inflammation, angiogenesis, and fertility problems. Some homeobox genes that regulate adult reproductive function may be involved in various aspects of endometrial growth and function, such as proliferation and differentiation. For example, they play an important role in regulating cyclic regeneration of the endometrium. In addition, *HOXA10* and *HOXA11* regulate endometrial receptivity, and *HOXC* and *HOXD* genes are involved in early endometrial growth and proliferation. Research by the Royan Institute on endometrial growth genes, especially HOX genes, and cofactors *BARX1*, *VAX1*, *ISL2*, and *OTX2* shows significant changes in mRNA expression in ectopic tissues. To evaluate the expression of *BARX1*, *VAX1*, *ISL2*, and *OTX2* genes in the follicular fluids of endometriosis patients undergoing IVF/ICSI treatment cycles.
Materials and Methods: In this study, follicular fluid samples were collected from 10 infertile women with endometriosis (stages 3 and 4) and 10 healthy women (non-endometriosis) who were undergoing assisted reproductive ART treatment, after obtaining informed consent and approval from the Medical Ethics Committee. Then RNA was extracted from follicular fluid. Real-time PCR investigated the expression of *BARX1*, *VAX1*, *ISL2*, and *OTX* genes.
Results: This study showed decreased *VAX1*, *ISL2*, and *OTX2* gene levels in patients compared with those in the control group (P=0/2898, P=0/0898, P=0/3527) but BARX2 levels increased in patients vs controls(P=0/0630), despite altered gene expression in the follicular fluid patients but none of them changing was significant. (Statistical significance level: P<0.05).
Conclusion: The study revealed fluctuations in *VAX1*, *ISL2*,

BARX1, and *OTX2* gene expression within the follicular fluid of patients with endometriosis, suggesting their potential as noninvasive biomarkers for diagnosing endometriosis in the future. Previous research has indicated significant changes in the expression of these genes in blood and tissue. Given the impact of endometriosis on fertility and oocyte quality, we extended our examination to the follicular fluid.
Keywords: Endometriosis, ISL2, OTX2, BARX, VAX1

P-156: Genetic Insights into Familial Primary Ovarian Insufficiency Through Whole Exome Sequencing

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Background: Primary ovarian insufficiency (POI) is related to a heterogeneous genetic condition and characterized by amenorrhea before the age of 40. The purpose of this study was to identify the variants and genes related to POI to better understand genetic etiology of POI and help in the early diagnosis of this disease.
Materials and Methods: Whole Exome Sequencing (WES) were done for eight noniatrogenic Iranian POI women whom at least one of their relatives had POI as well. To classify the pathogenicity of the variants, we used the ACMG guidelines in addition to SIFT, REVEL, Polyphen and CADD bioinformatic predictor tools. The deleterious variants were confirmed by Sanger sequencing in the probands and available relatives.
Results: In the studied probands, we identified 32 variants in 25 genes, of which 12 were Pathogenic (P) or Likely Pathogenic (LP). Five of P/LP variants are considered novel genes and 6 of these 12 variants were located in novel genes for POI, which means based on available evidence they have not been reported in POI so far. MSH4, TBP, LRP5, TP53, ZP1, CYP21A2 were genes with P/LP novel variants in this study. To name a function of some key known genes associated with POI, MSH4, involved in meiotic division regulation; ZP1, playing a role in oocyte maturation; and TP53, is a checkpoint in mitotic division.
Conclusion: In accordance to recent large cohort studies, POI seems to be oligogenic in Iranian patients as well. Diagnosis of the genetic causes of POI provides the possibility of genetic counseling and planning for pregnancy and preservation of ovarian tissue or oocyte preservation in the future.
Keywords: Primary Ovarian Insufficiency, Amenorrhea, Whole Exome Sequencing

P-157: Evaluation of The Expression Of *EN1*, *ARX*, *LBX1*, and *PITX2* Genes in Follicular Fluid of Endometriosis Patients Undergoing IVF/ICSI Cycles

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Background: Endometriosis, a hormone-dependent disease, affects 10-15% of women and causes pain and infertility. Treatments are limited by a poor understanding of its cellular and molecular mechanisms. Hormonal imbalances lead to inflammation, angiogenesis, and fertility issues. Diagnosing diseases like endometriosis is difficult without specific blood biomarkers, leading to costly and invasive tests. Efforts are underway to find non-invasive biomarkers for uterine diseases. Organoid models using utopic and ectopic tissues study HOX gene methylation and inflammation to identify genes involved in endometriosis. The Royan Institute's research on endometrial growth genes, particularly HOX genes and cofactors ARX, OTX2, LBX1, EN1, shows significant mRNA expression changes in ectopic tissues. Objective: to evaluate the expression of genes EN1, ARX, LBX1, and PITX2 in the follicular fluid of endometriosis patients undergoing IVF/ICSI treatment cycles.
Materials and Methods: Follicular fluid from women with endometriosis at stages 3 and 4 of the disease and healthy women (with male factor) (20 individuals in each group) was collected during the Assisted Reproductive Technology (ART) process for therapeutic purposes, following written informed consent and approval from the local ethics committee. Patient eggs were used for therapeutic purposes, and then we extracted RNA from this follicular fluid and quantitatively assessed the expression of these genes in the follicular fluid using the qPCR method.
Results: Our results were somewhat similar to previous studies conducted on peripheral blood and tissue. The genes EN1, ARX, PITX2 showed a pattern that was consistent with previous research on blood and tissue and were marginally significant. However, the gene LBX1 exhibited a dissimilar pattern compared to previous studies, and we observed a significant reduction in this gene.
Conclusion: The study reveals fluctuations in EN1, LBX1, ARX, and PITX2 gene expression within endometriosis patients' follicular fluid, suggesting their potential as non-invasive biomarkers for diagnosing endometriosis in the future. Previous research indicated significant expression changes for these genes in blood and tissue. Given the impact of endometriosis on fertility and egg quality, we extended our examination to follicular fluid.
Keywords: Endometriosis, EN1, LBX1, ARX, PITX2

P-158: Evaluation of The Expression of EN1, ARX, LBX1, and PITX2 Genes in Follicular Fluid of Endometriosis Patients Undergoing IVF/ICSI Cycles

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Background: Endometriosis, a hormone-dependent disease, affects 10-15% of women and causes pain and infertility. Treatments are limited by a poor understanding of their cellular and molecular mechanisms. Hormonal imbalances lead to inflammation, angiogenesis, and fertility issues. Diagnosing diseases like endometriosis is difficult without specific blood biomarkers, leading to costly and invasive tests. Efforts are underway to find non-invasive biomarkers for uterine diseases. Organoid models using utopic and ectopic tissues study HOX gene methylation and inflammation to identify genes involved in endometriosis. The Royan Institute's research on endometrial growth genes, particularly HOX genes, and cofactors ARX, OTX, LBX1, and EN1, shows significant mRNA expression changes in ectopic tissues. Objective: to evaluate the expression of genes EN1, ARX, LBX1, and PITX2 in the follicular fluid of endometriosis patients undergoing IVF/ICSI treatment cycles.
Materials and Methods: Follicular fluid from women with endometriosis at stages 3 and 4 of the disease and healthy women (with male factor) (10 individuals in each group) was collected during the Assisted Reproductive Technology (ART) process for therapeutic purposes, following written informed consent and approval from the local ethics committee. Patient eggs were used for therapeutic purposes, and then we extracted RNA from this follicular fluid and quantitatively assessed the expression of these genes in the follicular fluid using the qPCR method.
Results: Our results were similar to previous studies on peripheral blood and tissue. The genes EN1, ARX, and PITX2 showed a pattern that was consistent with previous research on blood and tissue and were marginally significant (P= 0/4359, P=0.1333, P=0.3266). However, the gene LBX1 exhibited a dissimilar pattern compared to previous studies, and we observed a significant reduction in this gene (P=0/0076).
Conclusion: The study reveals fluctuations in EN1, LBX1, ARX, and PITX2 gene expression within endometriosis patients' follicular fluid, suggesting their potential as non-invasive biomarkers for diagnosing endometriosis in the future. Previous research indicated significant expression changes for these genes in blood and tissue. Given the impact of endometriosis on fertility and oocyte quality, we extended our examination to follicular fluid.
Keywords: Endometriosis, EN1, LBX1, ARX, PITX2

P-159: Study on The Impact of Quercetin on The Regeneration of Beta Cells in A Zebrafish Model of Diabetes Mellitus

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Background: Diabetes is a metabolic disease associated with elevated blood sugar levels or insulin resistance. Controlling diabetes is challenging; however, regenerating beta cells is a treatment option. Considering the application of traditional Iranian medicine in treating diabetes, quercetin could be an effective compound in managing diabetes. The human body is similar to that of zebrafish. The present study evaluated the impact of quercetin on regenerating beta cells in a zebrafish model of diabetic mellitus.
Materials and Methods: Quercetin was assessed in Tg (ins:CFP-NTR) transgenic zebrafish larvae at 500, 125, 31.25, 7.81, and 1.95 µg/ml concentrations, as well as in the control groups, comprising NT and NC (untreated and treated transgenic larvae with metronidazole, respectively) and NECA (a molecule that increases beta cell proliferation). The analysis of data obtained from larvae regenerated beta cells was performed using Prism and ImageJ programs. The impact of optimal dose (1.95 µg/ml) on PDX1 and insulin levels was assessed using the PCR method.
Results: Analyzing the expression of PDX1 and insulin revealed significant impact of quercetin (at dose of 1.95 µg/ml) on the regeneration of beta cells. The gene expression level of PDX1 was significantly increased in the treatment group compared to the NT and NC groups, and insulin gene expression was elevated in the NECA group.
Conclusion: Quercetin, as a potent antioxidant, can neutralize oxidative agents, thereby increasing beta cells and expression levels of PDX1 and insulin genes. Therefore, it has the potential to be used as an effective drug in the treatment of diabetes.
Keywords: Beta Cells, Diabetic Model, Diabetes Mellitus, Restoration, Quercetin

P-160: Unlocking The Potential of Long Non-Coding RNAs in Male Infertility: From Spermatogenesis to Novel Therapeutic Strategies

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Background: Long Non-Coding RNAs (lncRNAs) play critical roles in regulating spermatogenesis and male fertility. Recent research has identified numerous lncRNAs with differential expression patterns throughout spermatogenesis, indicating their involvement in this complex process. Understanding the functions and mechanisms of lncRNAs in the male reproductive system could shed light on infertility causes and facilitate the development of innovative diagnostic and therapeutic strategies.
Materials and Methods: This systematic review adhered to PRISMA guidelines and was registered in PROSPERO (CRD42022356032). A thorough literature search was conducted on PubMed and Web of Science databases using relevant keywords related to lncRNAs and male infertility. Inclusion criteria encompassed studies investigating the correlation between lncRNAs and sperm parameters or male infertility in adult males. After meticulous screening and selection, 20 articles were included for analysis.
Results: Numerous lncRNAs, including Mrhl, Drm, Spga-lncRNAs, NLC1-C, HongrES2, Tsx, LncRNA-tcam1, Tug1, Tesra, AK015322, Gm2044, and LncRNA033862, have been validated for their roles in spermatogenesis. In humans, 15 lncRNAs consistently exhibited differential expression across multiple studies on germ cells. Additionally, a study on male in-

fertility identified 9879 differentially expressed lncRNAs, with specific high expression in immotile sperm. Moreover, various lncRNAs were dysregulated in different subtypes of male infertility.
Conclusion: LncRNAs offer promising avenues for understanding the molecular mechanisms underlying spermatogenesis and male infertility. While their exact roles remain largely unknown, the identified lncRNAs hold potential as biomarkers or therapeutic targets for male infertility. Further research is essential to uncover their specific contributions and translate these findings into clinical applications.
Keywords: lncRNAs, Spermatogenesis, Male Infertility

P-161: Evaluation of Hereditary Thrombophilia-Related Variants of FVL, FXIII, And PAI-1 Genes in Women With Recurrent Implantation Failure Referred to Royan Research Institute

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Background: Recurrent Implantation Failure (RIF) as the inability of the embryo to attach the endometrium following repeated transfer of high-quality embryos, is a multifactorial condition. Thrombophilia is considered as one of the important, yet controversial contributing factors. In this case –control study, we evaluated hereditary thrombophilia-related variants of FVL, FXIII, and PAI-1 genes in patients with RIF at Royan institute.
Materials and Methods: In the RIF group, 100 women younger than 40 years with at least three failed ICSI or IVF treatment cycles were included in which a total of at least 4 good-quality embryos were transferred. The control group comprised 100 women under 40 years old who have achieved clinical pregnancy during their first or second IVF or ICSI treatment cycles. In all participants, thrombophilic-related variants of Factor V leiden, FXIII (Val34leu), and PAI-1 (4G/5G) were genotyped using PCR-RFLP and their frequencies were compared between case and control groups.
Results: The results showed no significant associations between genotype frequency of Factor V Leiden (Wild.t: 94/100 (94%), Heterozygote: 5/100 (5%), Mutant.t: 1:100 (1%) in RIF group and Wild.t: 93/100 (93%), Heterozygote 7/100 (7%), Mutant.t 0/100 (0%) in control Group, p-value: 0.77), Factor XIII (Val34Leu) (Wild.t: 76/100 (76%), Heterozygote: 21/100 (21%), Mutant.t: 3:100 (3%) in RIF group and Wild.t: 75/100 (75%), Heterozygote: 22/100 (22%), Mutant.t 3/100 (3%) in control group, p-value: 0.985), and PAI-1 (4G/5G) (Wild.t: 26/100 (26%), Heterozygote: 52/100 (52%), Mutant.t: 22:100 (22%) in RIF group and Wild.t: 21/100 (21%), Heterozygote: 53/100 (53%), Mutant.t 26/100 (26%) in control group, p-value: 0.646). Moreover, in comparing allele frequencies, no significant differences was observed between RIF and control group for Factor V Leiden (wild allele: 193/200 (96.5%), mutant allele: 7/200 (3/5%) in RIF group and wild allele: 193/200 (96.5%), mutant allele: 7/200 (3.5%) in control group, p-value: 1.000), Factor XIII (Val34Leu) (wild allele: 173/200 (86.5%), mutant allele: 27/200 (13/5%) in RIF group and wild allele: 172/200 (86%), mutant allele: 28/200 (14%) in control group, p-value: 0.885), and PAI-1 (4G/5G) (4G allele: 96/200 (48%), 5G allele: 104/200 (52%) in RIF group and 4G allele: 105/200 (52.5%), 5G allele: 95/200 (47.5%) in control group, p-value: 0.368). In addition, cumulative frequencies of mentioned variants (presence of at least one mutated allele) showed a relative increase in RIF patients compared to control group, although this data was not statistically significant (combined allelic mutations: 198/200 (99%) in RIF group and 188/200 (94%) in control group, No allelic mutations: 2/200 (1%) in RIF group and 12/200 (6%) in control group, p-value: 0.118).
Conclusion: In conclusion, while some studies observed significant associations between hereditary thrombophilia with RIF, the data obtained through this research could not support this. A part of the contraversy observed in the results of various studies can be due to variations in the frequencies of thrombophilic related genetic polymorphisms in different ethnic population with various genetic backgrounds, lack of agreement on a common definition of RIF, the small sample sizes and different control groups. Thereby, we recommend more comprehensive studies to be done in each population in order to achieve conclusive results.
Keywords: Hereditary Thrombophilia, Polymorphisms, Recurrent Implantation Failure

P-162: In Silico Evidence on The Efficacy of Milk Peptides in The Treatment of Recurrent Implantation Failure

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Background: Despite significant advances in Assisted Reproductive Technologies (ARTs), the rate of embryo implantation following Fertilization (IVF) has not increased as expected. Recurrent Implantation Failure (RIF) is defined as the inability to achieve pregnancy after at least three embryo transfers using high-quality embryos. Tumor Necrosis Factor-Alpha (TNF_α) is a pro-inflammatory cytokine primarily produced by activated macrophages. Increased expression and activation of TNF_α could lead to RIF. Therefore, inhibiting TNF_α might be a strategic approach to enhance implantation rates in women with RIF. Scientific evidence suggests that milk proteins may

effectively modulate RIF. Hence, this study aimed to assess the effectiveness of milk peptides in inhibiting TNF_α using computational methods.

Materials and Methods: Main milk proteins were extracted from the Bomiprot database, converted into 15-amino acid peptides, and screened based on charge, solubility, and peptide stability using Pepcalc and Expasy databases. Ultimately, 55% of milk peptides successfully passed these screening stages. The resulting peptides underwent docking evaluation with TNF_α.

Results: Based on the results of the hydrogen bond analysis in Molegro, peptides with common bonds with the main TNF_α inhibitory ligands were extracted from the peptide library. The results of these stages showed that 50% of the peptides derived from milk proteins exhibited stability, desirable solubility, and appropriate binding energy with a ΔG less than -200 KJ/mol.

Conclusion: Our computational results demonstrated for the first time that peptides present in milk may have the potential to inhibit TNF_α and could possibly be effective in increasing implantation rates in women with RIF.

Keywords: Type 2 Diabetes, Milk Peptides, Tumor Necrosis Factor-Alpha, Computational Methods

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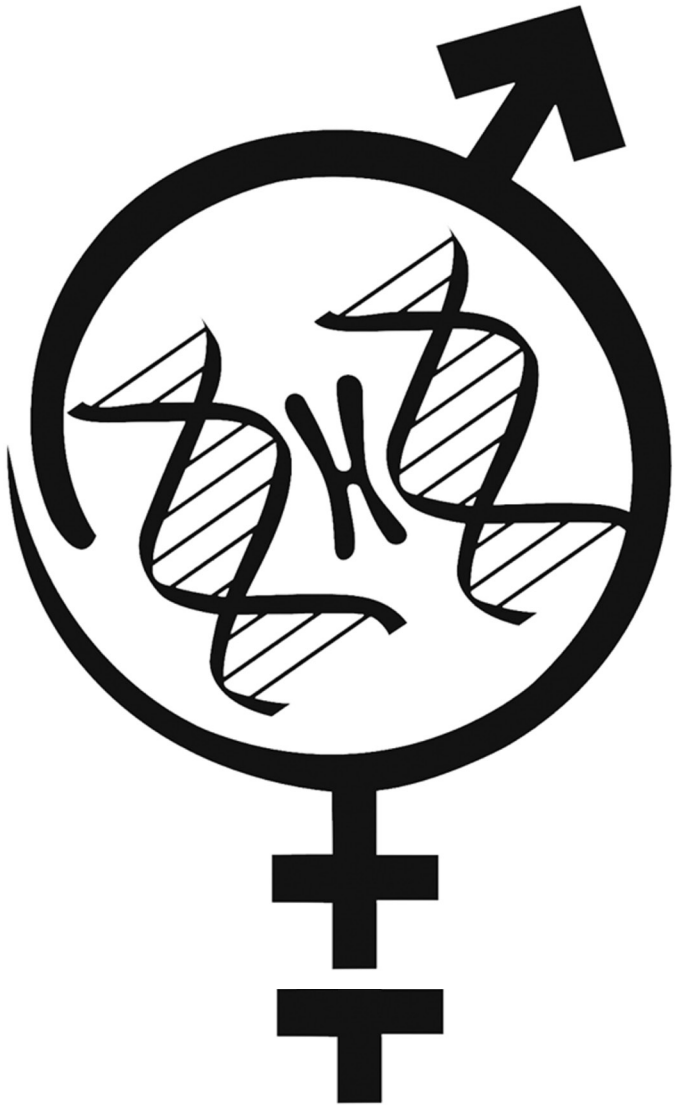
Abstracts of

Royan International Hybrid Twin Congress

19th Seminar on Nursing and Midwifery
28-29 August 2024



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Invited Speaker

Inm-1: Applications of PRP in Infertility

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Inm-2: This Study Aimed to Investigate the Relationship between Follicular Fluid Bisphenol A

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(BPA) concentrations with alterations of ICAM-1 and ICAM-2 genes and proteins expression as well as methylation profiles in the cumulus cells of poor ovarian response (POR) women based on their healthy lifestyle habit. Materials and Methods: Eighty women under the age of 35 were divided into two groups: 1-POR without using plastic containers (n = 40) and 2-POR with using plastic containers (n = 40). The *ICAM-1* and *ICAM-2* genes and protein expressions were examined by the quantitative PCR and western blotting technique. The methylation pattern was investigated by the methylation-specific PCR. Total BPA in follicular fluid was measured with high-performance liquid chromatography technique and the detection limit was 1.14 ng/ml. *ICAM-1* and 2 genes were differentially expressed between the two groups studied. Results: *ICAM-1*, *ICAM-2* genes, and protein expressions in group 1 were upregulated compared to the second group (P < 0.05). While DNA methylation status in group 1 were decreased compared to the other group (P < 0.05). The concentration of BPA in the follicular fluid of group 1 was lower compared to the second group (P < 0.05). The oocyte quality and clinical pregnancy ratio showed significantly higher in group 1 than in the other ones (P < 0.05). The alteration of ICAM-1 and ICAM-2 gene expressions in POR women is probably related to BPA concentration. Conclusion: As a result Lifestyle habits may also affect the methylation pattern and protein levels in the cumulus cells of POR women. Additionally, lifestyle habits may be considered as a marker for ovulation, oocyte maturation, preimplantation and clinical pregnancy process.

Inm-3: The Effect of Endometriosis on Oocyte and Embryo

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Endometriosis (EMS) affects about 10% of reproductively aged women and up to 40–50% of infertile women. Endometriosis according to implant and adhesion scores divides into four stages: minimal (stage I), mild (stage II), moderate (stage III), and severe (stage IV). Inflammatory, fibrotic, and oxidative responses caused by EMS reduced ovarian reserve. Also, follicle quality, changes in normal pelvic physical environment, decreased endometrial receptivity, and immunological dysfunction are the main reasons for EMS impeding female fertility. On the other hand, the development of the human embryo is directly influenced by the nuclear and cytoplasmic maturation of the oocyte. Several studies have examined the quality of embryos derived from the oocytes of women with endometriosis to determine the impact of endometriosis on embryo quality, with conflicting results. A large meta-analysis in women undergoing assisted conception for tubalfactor infertility reported that women with stage I/II endometriosis have reduced fertility and implantation rates compared to women without endometriosis. Other IVF outcomes, including the number of oocytes retrieved and fertilization rate, were affected by the presence of endometriosis at all stages, suggesting that endometriosis affects fertility, oocyte and embryo quality, and endometrial receptivity. In other study, women with endometriomas undergoing IVF have been reported to have a significantly lower number of oocytes and number of MII oocytes retrieved compared to controls with tubal and male-factor infertility, there is no difference in their total number of embryos, number of top-quality embryos, clinical pregnancy rate, implantation rate or live birth rate. Therefore, the presence of endometriosis may reduce oocyte quality and embryo quality. However, this does not appear to translate to a clear clinical impact on IVF outcomes.

Inm-4: The Effect of Environmental and Chemical Contaminants on Male Infertility

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Inm-5: Management of The Freezing Process

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According to the World Health Organization, by 2030, it is estimated that 1.4 million women of reproductive age will be diagnosed with cancer annually. Fortunately, in many cases, cancer is no longer considered an incurable disease. From 2008 to 2014, 85 percent of women under 45 with cancer survived. This increase in survival rates has shifted the focus from an exclusive focus on preserving life to a focus on preserving quality of life after treatment. One aspect of this is maintaining the ability to have a biological family. Providing treatment options

that preserve fertility in cancer patients has become a critical component of survivorship care. This leads to improved quality of life and allows survivors to become mothers even in the seemingly unfavorable circumstances of cancer. However, in recent years, there has been continuous improvement in cancer treatment and diagnosis, which has led to a significant improvement in the survival rate of cancer patients. But treatments that include chemotherapy, radiotherapy, surgery, or combination therapy have numerous side effects that may lead to premature ovarian failure in women or significant loss of male germ cells. However, although there are guidelines regarding fertility preservation in the context of neoplasms, physicians routinely do not consider it and do not discuss these options with their patients. It is important for patients to be informed of the options available for fertility preservation and encouraged to make informed decisions in collaboration with their medical team. Although performing fertility preservation is considered an aspect of comprehensive oncology care. However, there is still no unified guideline for oncologists and infertility specialists to treat cancer patients. In the first step, it is necessary to consider fertility preservation counseling before cancer treatment. Then the fertility preservation options for different patients using different treatments should be available and suitable. In this review, we discuss the knowledge, methods, and options related to fertility preservation and how these new strategies help oncologists, surgeons, pediatricians, and hematologists preserve fertility. We also discuss the unique challenges and considerations, including ethical dilemmas, for providing timely and comprehensive care.

Keywords: Fertility Preservation, Oncofertility, Reproductive Counseling, Quality of Life

Inm-6: Surgical and Non-Surgical Treatments

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Endometriosis is defined as the presence of ectopic endometrial tissue (glands and stroma) outside the confines of the uterine cavity and musculature. The lesions are typically located in the pelvis but can occur at multiple sites including the bowel, diaphragm, and pleural cavity. Endometriosis lesions in the pelvis can be categorized as superficial peritoneal, ovarian, and deeply infiltrating. ectopic endometrial tissue and resultant inflammation can cause dysmenorrhea, dyspareunia, chronic pain, and infertility. Primary laparoscopic surgery is indicated for staging and treating of endometriosis, improving fertility, and reducing pain. Women with asymptomatic endometriosis do not require surgical treatment, even if the endometriosis has not been previously surgically removed. Women with infertility and an asymptomatic endometrioma typically proceed with ART. For women younger than 35 years who desire a trial of natural conception, we advise six months of timed intercourse. For women ≥35 years of age, we typically proceed with ART but also offer clomiphene if ART is not possible. Women with surgically staged moderate (stage III) to severe (stage IV) endometriosis, including those with endometriomas, benefit from ART.

Inm-7: Social Fertility, Oncofertility

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Inm-8: Care and Lifestyle in Preventing the Effect of Environmental and Chemical Contaminants on Infertility

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According to the World Fertility and Family Planning (2020) report from the United Nations (UN), there was a reduction of nearly %20 in the number of live births per female between 1990 and 2019 in less than 30 years (3.2 to 2.5 live births). This indicates a concerning and undeniable trend in the overall fertility rate, which research indicates will continue to drop in the upcoming years. Even though a large portion of the global fertility decline is due to personal choice, an increasing number of couples—particularly in developed nations—are having difficulty conceiving, and damaged neonates are becoming more frequent. It is abundantly evident from studies that chemicals and metals found in food, water, air, and beauty products negatively impact fertility in a variety of ways. Men's sperm counts and functions are steadily declining as a result of these contaminant, and women's anovulation, implantation difficulties, and fetal viability are getting worse. To summarize, there are four ways in which contaminants reduce fertility: 1. Endocrine disruption chemicals that interfere any part of the function of hormones 2. Damage to the female reproductive system 3. Damage to the male reproductive system 4. Impaired fetal viability. This study has been done to investigate environmental and chemical contaminant affecting fertility and their mechanism of action and explain care and lifestyle in reducing the effects of this contaminant. PubMed, Embase, Cochrane, and Scopus databases were searched based on related keywords. According to studies contaminant such as pesticides/ Herbicides (for example dibromochloropropane, Organophosphates, Atrazine), Radiation Exposure, Heat exposure, air pollution, heavy metals (for example Cadmium, Lead, Mercury, Arsenic) plastic materials (for example Phthalates, Bisphenol A) and even noise pollution can have negative effects on fertility. It is almost impossible to remove these pollutants from our living environment, but Adherence to several clinical recommendations leads to the modification of the harmful effects of contamination on fertility.

Keywords: environmental contaminants- chemical contaminants- infertility

Inm-9: The Effect of Environmental and Chemical Contaminants on Female Infertility

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Inm-10: Endometriosis Definition, Causes and Diagnosis

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Endometriosis is an estrogen-dependent benign inflammatory disease characterized by the presence of ectopic endometrial implants. Implants typically occur in the pelvis but have also been seen in the upper abdomen, peripheral and axial skeleton, lungs, diaphragm, and central nervous system. The most common sites of endometriosis, in decreasing order, are the ovaries, anterior/posterior cul-de-sac, broad ligaments and uterosacral ligaments, uterus, fallopian tubes, sigmoid colon and appendix. Because the growth of the implants is dependent on ovarian produced steroids, it is a disease that most severely affects women ages 25–35 years. Patients can present with a wide- range of symptoms ranging from being asymptomatic to infertile. In addition to infertility, it is commonly associated with symptoms such as dyspareunia, dysmenorrhea, bladder/bowel symptoms, and chronic pelvic pain. Endometriosis has been estimated to affect up to 10–15% of reproductive aged women. The association between endometriosis and infertility is well supported throughout the literature, but a definite cause-effect relationship is still controversial. The prevalence of endometriosis increases dramatically to as high as 25–50% in women with infertility and 30–50% of women with endometriosis have infertility. The fecundity rate in normal reproductive age couples without infertility is estimated to be around 15 to 20%, while the fecundity rate in women with untreated endometriosis is estimated to be anywhere from 2 to 10%. Women with mild endometriosis have been shown to have a significantly lower probability of pregnancy over 3 years than women with unexplained fertility (36% vs. 55%, respectively). IVF studies have suggested that women with more advanced endometriosis have poor ovarian reserve, low oocyte and embryo quality, and poor implantation. Despite the well supported association between endometriosis and infertility, the difficulty in proving a causal relationship likely stems from the multiple mechanisms by which endometriosis can impact fertility and the heterogeneity and variations in the phenotype of the disease. This article will discuss endometriosis-associated infertility including a basic background on endometriosis, its presumed pathophysiology in causing infertility, and both current and potential treatments. The definite pathogenesis of endometriosis is still unknown but there are a number of leading theories including retrograde menstruation, altered immunity, coelomic metaplasia, and metastatic spread. Newer research is also proposing stem cell and genetic origins of the disease.

Inm-11: Familiarity with New Protocols of Embryo Transfer

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Since the birth of the first child in 1982, following a frozen embryo transfer, significant advances have been made in cryopreservation techniques. The implementation of new policies that limit the number of embryos transferred in fresh cycles to reduce the risk of ovarian hyperstimulation syndrome (OHSS) has led to an increased desire to transfer frozen embryos. The methods for preparing the endometrium for embryo transfer differ significantly. It is essential to acknowledge that only a suitable endometrium does not guarantee the success of frozen embryo transfer, and various factors, including the quality of the embryo and the number of embryos transferred, also play a crucial role in this outcome.

Five primary methods for preparing the endometrium are:

Natural cycle
Natural cycle with placental gonadotropin hormone injection
Hormonal cycle (artificial)
Hormonal cycle with agonist pre-treatment
Ovulation induction cycle (ovulation triggering)

Each of these cycles has been thoroughly evaluated over the years in terms of cost, ease of implementation, patient acceptance, and most importantly, pregnancy and success rates. A detailed analysis will reveal the benefits and drawbacks of each method, allowing for a comprehensive comparison.

Inm-12: Life Style and Care in Women with Endometriosis

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Endometriosis is a chronic inflammatory disorder characterized by the presence of ectopic endometrial-like glands and stroma, often involving the pelvic organs and frequently leading to anatomical distortion within the pelvis. The prevalence of this disease ranges between six and ten percent, while the incidence is believed to be above 33% for patients with acute pelvic pain. As long as the etiology of endometriosis is not fully understood and the condition has no definitive treatment, women suffering from this chronic disease may greatly benefit from insights into environmental factors or interventions that could prevent, modify, or cure endometriosis. The relationship between childhood and adolescent weights and the development of endometriosis is counterbalanced. Current evidence suggests that endometriosis symptoms may be reduced by physical activity. In a prospective cohort study, breastfeeding was a protective factor for endometriosis-related symptoms. Several studies have identified an association between alcohol consumption and symptoms related to endometriosis, whereas others have not. The high intake of red meat, trans-unsaturated fatty acids, and omega-6 fatty acids derived from the diet are the precursors of the pro-inflammatory prostaglandins PGE2 and PGF2α, which likely increase uterine cramps and cause the painful symptoms of endometriosis. Antioxidant vitamins (D, E, and B-group vitamins), as well as foods rich in calcium and omega-3 fatty acids, may protect against the development of endometriosis. Fasting can help preserve energy level, thereby providing the body time to regenerate and heal. High fat consumption is associated with oxidative stress and inflammation – two key features of endometriosis. Oxidative stress, chronic inflammation, and immunological disorders are features shared between coeliac disease and endometriosis. The literature is scarce regarding the association between these two diseases.

Inm-13: The Need for A Training Software among Iranian InfertileCouples: A Qualitative Study

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Background: Training needs are multidimensional requirements affected by social and cultural background, level of knowledge and personal and health conditions. This study was conducted to explain the needs for a training software among Iranian infertile couples. Materials and Methods: In this qualitative study, we used content analysis to examine the need among ten infertile participants (four men and six women) and six health care professionals (including two gynecologists, two reproductive health specialists and two midwives). The present research was carried out from January 2017 to July 2018 at Rouyesh and Ibn Sina infertility treatment centers in Karaj, Iran. The participants were selected through purposive sampling with maximum variation. Four focus group discussions with the health care professionals and twelve semi- structured, in-depth interviews with the infertile participants were held for data collection. Data were analyzed using conventional content analysis in MAXQDA-10.
Results: Data analysis led to the extraction of a central theme of “a multidimensional training application” and its four main categories, including "pre-treatment training", "diagnostic training", "mid- and post-treatment training" and “continuous psychological training". These main categories also had 20 subcategories.
Conclusion: Based on the results of this study, infertile women and men have multidimensional training needs before and after treatment and during the process of diagnosis; psychological aspects should also be considered. The inter- viewed health care professionals helped to explain these training needs. A training software thus needs to be designed based on the real needs of the infertile community.
Keywords: Infertility, Knowledge, Qualitative Research, Training Programs

Inm-14: Sperm Freezing

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Oral Speaker

Onm-1: The Comparison of General Health Between Fertile and Infertile Women in Hamadan, Iran

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Background: Noticing the significant role of fertility in Iranian families, the incidence of infertility and its social and cultural dimensions, this study was carried out to compare general health status of infertile women with fertile women.
Materials and Methods: This observational case-control study, we compared the GHQ of 147 women as the control group and 147 infertile patients as the case group who were matched in terms of influential variables. Data collection was done through demographic questionnaire and general health questionnaire (GHQ-28) which were completed by both groups. The results were analyzed by logistic regression analysis, t test, and chi-square using STATA 10 software.
Results: Means of GHQ score in fertile and infertile women were 18.32 ± 8.83 and 27.06 ± 9.87, respectively. Here the mean score of the infertile women was significantly higher in comparison with the fertile women (P<0.001). Physical symptoms, anxiety, social interaction, and depression scores of infertile women were significantly higher in comparison with the fertile women (P<0.001). There were no significant differences in the means of age and duration of marriage between the two groups and the distribution of educational level, occupation, and income levels were the same in the two groups.
Conclusion: Average scores of general health and physical complaints, anxiety, impaired social interaction, and depression in infertile women were higher than those in fertile women. This indicates their involvement with some degrees of public health diseases, it is necessary to emphasize the training courses to provide counseling to women and health policy makers should pay special attention to the health status of fertile women by creating various fields.
Keywords: Fertile women, Infertile women, Public health,

Onm-2: Recurrent Implantation Failure and Sexual Function in Infertile Iranian Women: A Comparative Cross-Sectional Study

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Background: Recurrent implantation failure (RIF) which means failing to implant after two or more high-quality embryo transfer cycles, affects 3% to 5% of women worldwide. The aim of this study was to assess the relationship between recurrent implantation failure and sexual function in infertile Iranian women.
Materials and Methods: This was a comparative cross-sectional study on 180 infertile Iranian women (90 infertile women with recurrent implantation failure and 90 infertile women who did not start infertility treatment). A demographic questionnaire and the Female Sexual Function Index were used for data collection. Data were analyzed using Chi-square, independent t-test, and multiple linear regression.
Results: The mean scores of different domains of sexual function (desire, lubrication, arousal, orgasm, pain, and satisfaction) were significantly lower in the group with RIF compared to the group without RIF. The total score of sexual function was significantly lower in the RIF group compared with the group without RIF (23.11±2.24, vs. 25.99±2.35, P<0.001).
Conclusion: The results of this study showed that women with RIF had significantly lower sexual function than that in women without RIF. Therefore, sexual function issues should be treated as an important component of comprehensive care. This study did not measure the impact of economic factors on sexual function, however, the majority of the sample were classified as having weak or moderate economic status and this, along with the high cost of infertility treatments, could potentially have played a role in the participants' experience.
Keywords: Recurrent implantation failure, Sexual function, Infertility

Poster Presentation

Pnm-1: The Effect of Human Papillomavirus on Female Fertility

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Background: This disease is the most sexually transmitted disease in the world. High-risk strains of Human Papillomavirus (HPV) cause about 5% of cancers worldwide, and an estimated 570,000 women and 60,000 men develop HPV-related cancers each year. The purpose of this study is to investigate the effect of this virus on fertility and its consequences on pregnancy.
Materials and Methods: A literature review of studies on the effect of HPV infection on male and female fertility was performed using databases such as PubMed and Google Scholar. Search terms include: "HPV and Fertility"
Results: This virus, which is a very common sexually transmitted disease, can disappear on its own within a year or two, but in repeated infections it is associated with cervical, uterine, varopharyngeal and anogenital cancers. The effect of this virus on women's fertility has not yet been proven, but based on some available articles, it probably affects the rate of abortion, premature birth, implantation success rate, reduced success rate of assisted reproductive methods and reduced live birth, but needs to be investigated. More Among the causes of infertility, endometriosis is more common in women with HPV. Changes in oocyte maturation/fertilization/implantation/abortion/live birth are debated and not yet confirmed, but the rate of quality embryos in infected women is lower than in healthy women for this virus.
Conclusion: This review article suggests that HPV infection likely affects women's fertility, but more research is needed in larger numbers of infertile couples.
Keywords: Fertility, Infertility, HPV

Pnm-2: The Effect of Human Papillomavirus on Male Fertility

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Background: This disease is the most sexually transmitted disease in the world. High-risk strains of Human Papillomavirus (HPV) cause about 5% of cancers worldwide, and an estimated 570,000 women and 60,000 men develop HPV-related cancers each year. The purpose of this study is to investigate the effect of this virus on male fertility.
Materials and Methods: A literature review of studies on the effect of HPV infection on male and female fertility was performed using databases such as PubMed and Google Scholar. Search terms include: "HPV and Fertility"
Results: This infection can disappear by itself within one or two years, but in repeated infections, it is associated with cancers of the anus, penis, tonsils, and throat. The important effects expressed on male fertility are: a) (influence on seminal fluid parameters and possibly reducing sperm motility and penetration, b) (increasing the probability of sperm "DNA fragmentation index" and reducing the success rate of "intrauterine insemination"), c) reducing the speed of sperm infection in blastocyst formation and possibly reduced implantation, d) a higher percentage of oligospermia and spermatozoa have been observed in HPV-positive infertile men, F) also, the sperm can transfer DNA infected with the virus to the egg and thus affect the development of the fetus.
Conclusion: This review article shows that HPV infection has an effect on male fertility, but the study of its effect on the success rate of assisted reproductive methods and adverse pregnancy outcomes is ongoing and requires further investigation.
Keywords: HPV, Male Fertility, Pregnancy Outcomes

Pnm-3: The Effect of Narrative Writing on Stress, Depression, Sexual Satisfaction and Fatigue in Fertilitle Couples Undergoing Assisted Reproductive Technology Treatment: A Randomized Controlled Study

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Background: Infertile couples undergoing fertility treatments may experience stress, depression, sexual dissatisfaction and fatigue and could benefit from psychological intervention. Narrative writing has shown promising results on various psychological outcomes, yet no study has applied the method to infertility couples. The aim of this study was to the effect of narrative writing on stress, depression, sexual satisfaction and fatigue in infertile couples undergoing assisted reproductive technology (ART) treatment.
Materials and Methods: In this randomized controlled study, 80 couples enrolling in their first ART treatment at the Isfahan Fertility and Infertility were offered to participate. A total of 80 couples were randomized to home-based narrative writing (n=40) and control group (n=40). Completed an infertility-related stress, questionnaire; The Beck Depression Inventory, Larson sexual satisfaction questionnaire and Mental Fatigue Scale at treatment enrollment, 3 weeks later (at the time of down regulation), and 6 weeks after the intervention. The intervention took place 2 weeks after treatment start
Results: The experimental group demonstrated significant improvements in stress ($z = 6.528$, $P<0.001$) and sexual satisfaction ($z = 3.148$, $P= .003$) and significant reductions in depression ($z = -4.850$, $P<.0001$) and fatigue ($z = -4.597$, $P<0001$) in six weeks after the intervention.
Conclusion: The preliminary results suggest narrative writing to be a feasible, cost-effective, and efficient method for alleviating psychological disorders in infertile couples, although results should be considered preliminary and further testing with a larger sample is warranted.
Keywords: Narrative Writing, Sexual Satisfaction, Infertile Couples, Assisted Reproductive technology

Pnm-4: The Efficacy of Acupuncture in Thyroid Function, Fertility Improvement Between Female with Hashimoto Thyroiditis: A Systematic Review

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Background: Hashimoto thyroiditis (HT) is highly prevalent among reproductive-aged women and has a substantial negative impact on fertility. Currently, there is no specific treatment for HT. It has been reported that acupuncture can halt or delay the progression of HT and improve fertility in child-bearing period female; thus this present study was conducted to determine efficacy of acupuncture in thyroid function, fertility improvement between female with HT.
Materials and Methods: This systematic review was performed in Medline, EMBASE, Cochrane library, Science direct and Springer databases to find relevant articles. Search terms include: acupuncture, effectiveness, fertility, function, HT between clinical trial, semi-experimental, cohort studies, case-control studies assessing the results in effect of acupuncture in thyroid function, fertility improvement between female with HT were included. Out of 32 papers identified through initial search, 26 relevant studies were selected from which, 19 paper s were included in this systematic review.
Results: All women received acupuncture at points RN23, ST9, RN17, RN4, RN6, ST36, SP6, KI6 for at least 12 consecutive weeks. Primary evaluation included reduction of thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TGAb) titers, and secondary outcomes included improvement of thyroid function, ovarian function, rate of primary ovarian failure, and pregnancy success.
Conclusion: It seems that the acupuncture method can be one of the acceptable methods to improve thyroid function and fertility results in women with hashimoto's thyroiditis and can be successful in regulating the energy in the body and restoring the hormonal and the balance of the reproductive and endocrine system.
Keywords: Acupuncture, Effectiveness, Fertility, Hashimoto Thyroiditis

Pnm-5: Saliva Biomarkers for Non-invasive Diagnosis of Endometriosis

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Background: Endometriosis, affecting 2-10% of women globally, involves the growth of uterine-like tissue outside the uterus. Early diagnosis is hindered by vague symptoms and inconclusive exams, leading to an average 12-year diagnostic delay. This delay harms patients' health, increasing surgery risks, infertility, and disease progression. Non-invasive diagnostic methods are vital for early detection.
Materials and Methods: This abstract was generated by searching endometriosis, mi-RNA biomarkers, and saliva keywords in Google Scholar, PubMed, and Science Direct.
Results: Saliva biomarkers are gaining attention for endometriosis diagnosis due to their stability and tissue specificity. MicroRNAs (miRNAs) like let-7b, miR-125b-5p, miR-150-5p, miR-342-3p, miR-451a, and MiR-3613-5p show promise in distinguishing endometriosis from other gynecological conditions. Notably, hsa-mir-135a expression was elevated in plasma and saliva of endometriosis patients. Among 34 miRNAs in saliva, miRNAs 6818-5p, 498, 1910-3p, 3119, and 501-5p exhibited significant potential for endometriosis diagnosis.
Conclusion: While various miRNAs hold promise as non-invasive endometriosis biomarkers, further research is essential for validation and standardization. Developing accurate, non-invasive diagnostic tools using saliva biomarkers could transform early endometriosis diagnosis, enhancing care accessibility and global health outcomes.
Keywords: Endometriosis, mi-RNA Biomarkers, Saliva

Pnm-6: Reproductive Health: A Missing Concern in The Life of Iranian Women with Endometriosis

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Background: Little is known about reproductive health and experiences of women with endometriosis, despite a high prevalence of this disease among reproductive-age women. This study explored the experiences and concerns of women with endometriosis about reproductive health.
Materials and Methods: The study was a qualitative research with conventional content analysis approach which was sampled by purposive method among reproductive-age women with endometriosis. Twenty-five women were interviewed with semi-structured and in-depth face to face interviews. In analyzing the data, the Graneheim and Lund man models were used and through MAXQDA.10 software.
Results: The main categories which emerged in this study were “Marital unsatisfaction”, “Urinary dysfunction”, “Damaged sexual relationships”, “Weakness in fertility”, “Concern about being pregnant”, “limitation in contraception methods”.
Conclusion: This results showed that endometriosis can damage reproductive health, and education and support should be extended among women with endometriosis, from early stages after diagnosis.
Keywords: Endometriosis, Reproductive Health, Concern, Qualitative Study

Pnm-7: Evaluating Loss to Follow-up in Newborn Hearing Screening in Central Iran

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Background: Hearing loss is one of the most common developmental disorders. According to the latest World Health Organization (WHO) estimates, In the World report on hearing, the WHO estimates that “by 2050 nearly 2.5 billion people will be living with some degree of hearing loss, at least 700 million of whom will require rehabilitation services. Currently, this number is 430 million, which includes people with moderate or higher grades of hearing loss who are most likely to benefit from hearing rehabilitation services. The vast majority of these people live in low- and middle-income countries, where access to ear and hearing care (EHC) is often limited
Materials and Methods: The present study is a retrospective study of infants born in maternity hospitals in Khorramabad,

Lorestan province. We calculated the percent use of screening for infants born in March 2018 and February 2021 who did not pass hearing screening in one or two ears. Researcher after obtaining a license to conduct research from the University Ethics Committee with the code of ethics IR. IUMS. REC. 1399. 1153 The researcher, after obtaining permission from the hospital director and security officials entered the study environment to collect information.

To complete the questionnaires, first the demographic data of infants, parents and hearing screening of newborns who were completed in the hospital from March 2018 and February 2021 by a nurse and audiologist and were in the file were reviewed and the required data were extracted and in the first part of the questionnaire. Total number, demographic information about each of the infants who was not pass for hearing screening at birth, maternal characteristics, social and economic factors were extracted from the file. To complete the next part of the questionnaire and to complete the information that was not available in the electronic data available in the hospital, the contact number of the baby's parents was extracted and contacted, and after obtaining verbal consent, the information was asked. , and 10 infants due to parental unresponsiveness and file distortion due to problems including incorrect contact number, unwillingness to cooperate and s. Errors in the file were excluded. Then, in order to increase the information and confirm the accuracy of these statistics, who had referred for the second screening or not, they went to the tannery clinic in Khorramabad and the data related to the screening follow-up were evaluated. If infant information was not available at this clinic, we contacted to parents again and asked about follow-up screening to make sure they have not been screened at another clinic or hearing center

Results: Out of 13,710 neonates born between March 2018 and February 2021, 310 neonates in one of the central provenience in Iran, (2.26%) were not pass the first hearing screening. Of this group, 60 infants (20%) loss to follow up second screening and access to information of ten infants was not possible. Table 1 shows the demographic and clinical characteristics of the neonates. Among the causes of loss to follow-up, lack of necessity with 36.7% and fear of Covid disease with 26.7% had the highest frequency among the causes of loss to follow-up. Table two shows some related factor of loss to follow up

Conclusion: Findings of the present study and similar studies in Iran and other countries, the need for a comprehensive neonatal hearing screening plan for timely diagnosis and intervention of hearing loss is essential. Based on this study, it points to the acute role of socio-economic factors in determining the status of auditory predation follow-up and determines the commitment to improve economic indicators in the field of neonatal health

Keywords: Hearing Loss, Hearing Screening, Neonates, Loss To Follow-up

Pnm-8: Relationship between Polycystic Ovary Syndrome and Lifestyle in Reproductive Age: A Systematic Review

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Background: Although Polycystic Ovary Syndrome (PCOS) is a common endocrine disease. The present study was conducted with the aim of determining relationship between PCOS and lifestyle in reproductive age.

Materials and Methods: In this study, 143 articles through electronic search in data base ISI Web of Knowledge, PubMed central, MEDLINE and EMBASE and Scopus, Iran Medex, SID to identify relevant articles with MeSH terms, up to 2024. Finally, examined 15 articles.

Results: Studies found that factors associated with higher risk for incident PCOS included the following: obesity (compared with nonobese) class I–II (body–mass index [BMI], 30–40 kg/m2; odds ratio [OR], 3.8; 95% confidence interval [CI], 3.4–4.2), Class III (BMI > 40 kg/m^2; OR, 7.5, 95% CI, 6.5–8.7), weight gain (compared with weight loss or maintenance) of 1–10% (OR, 1.7, 95% CI, 1.3–2.1), 10–20% (OR, 1.9; 95% CI, 1.5–2.4), and >20% (OR, 2.6; 95% CI, 1.9–3.6), prediabetes (OR, 2.7; 95% CI, 2.1–3.4), and metabolic syndrome (OR, 1.8: 95% CI, 1.5–2.1). Among the risk factors related to lifestyle, sleep disorder and its effect on mental parameters, oxidative stress and inflammation that lead to diabetes, infertility and cardiovascular disease.

Conclusion: Excessive weight gain and obesity and metabolic disorder may play a key role in the expression of the PCOS phenotype. Therefore, measures should be taken to prevent weight gain in the early years of fertility by modifying the lifestyle and choosing the right food and physical activity to reduce this syndrome.

Keywords: Polycystic Ovary Syndrome, Risk Factors, Obesity, Metabolic Disorder

Pnm-9: Relation of Preconception Eating Behaviors and Exercising and Lifestyle with Gestational Weight Gain: A Systematic Review

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Background: The global prevalence of overweight and obesity in pregnancy is rising and this represents a significant challenge for the management of pregnancy and delivery. This study aims to investigate to preconception eating behaviors with longitudinal dietary patterns from preconception to late pregnancy as well as Gestational Weight Gain (GWG) is limited.

Materials and Methods: To conduct this research, a systematic of descriptive observational studies by review was conducted searching databases, i.e. PubMed, Iran Medex, SID and Google Scholar up to 2024, using the related keywords. The quality of the extracted articles was evaluated based on the STORBE checklist of contents and finally 20 articles were analyzed.

Results: In the studies, a three-factor eating questionnaire was used, which showed that women with bulimia nervosa and uncontrolled eating have a higher chance of consuming fast food and fried foods and snacks [Odds Ratio (OR): 1.25, 95% Confidence Interval (CI): 1.03, 1.51], and women with cognitive restriction in food consumption and emotional eating have a higher chance of gaining weight GWG [Relative Risk Ratio (RRR): 1.35, 95% CI: 1.02, 1.80]and also obese pregnant

women had significantly greater odds of reducing discretionary foods ((OR) = 6.69 95% (CI) 2.13–21.00, p = 0.001) and using structured diets (adjusted odds ratio (AOR) = 9.13 95% CI 2.90–28.81, P<0.001) compared to normal-weight women. Studies have shown that the chance of exercising and using folic acid before pregnancy is lower in overweight and obese women (overweight: AOR = 0.40 95% CI 0.18–0.90, p = 0.01, obese: AOR = 0.38 95% CI 0.16–0.91, P= 0.03.)
Conclusion: The results showed that there may be a need for eating behavior interventions in pregnant women before pregnancy to improve the food pattern. More research is needed to investigate women's lifestyle before pregnancy and in order to increase their awareness and use effective strategies to promote health, so that we can see the health of the pregnancy and the favorable outcome of the baby.
Keywords: Obesity, Pregnancy, Eating Behaviors, Lifestyle

Pnm-10: Evaluation of The Effect of Endometriosis and Educational on The Quality of Lfe, Sexual Function, Anxiety and Depression of Women: A Systematic Review

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Background: Endometriosis is one of the most common gynecological diseases and is defined as a chronic disorder characterized by the ectopic presence of functional endometrial tissue, glands, and stroma outside the uterine cavity. The aim of this review is evaluation of the effect of endometriosis and educational on the quality of life, sexual function, anxiety and depression of reproductive age women.
Materials and Methods: Research data were obtained by searching Scopus and Web of Science databases, Google Scholar, PubMed. It was done with the endometriosis، quality of life، sexual function ، anxiety and depression ، educational intervention and their English equivalent from 2011 to 2024. Thus, the quality of the articles was evaluated by Consort and Jadad's checklist and finally 25 articles were examined.
Results: Studies showed that women with pain had higher anxiety and depression scores and worse quality of life than women without pain ($p < 0.001$). Regarding sexual function, all the groups were at risk for sexual dysfunction ($p = 0.671$). The group of women with pain and infertility have worse anxiety scores (25.31 ± 15.96) and depression (18.81 ± 11.16) than the other groups. There was no statistically significant difference between both groups regarding demographic and obstetrical characteristics ($p > 0.05$). Before implementation of educational intervention, the mean scores of total EHP-30 and SHOW-Q showed impaired quality of life and sexual function in the both groups ($p > 0.05$). After one and two months of educational intervention implementation, mean total score of EHP-30 was significantly lowered in the study group compared with the control.
Conclusion: It is recommended to provide a health education program for women with endometriosis in order to change their lifestyle and thus improve the quality of life and sexual performance and reduce depression.
Keywords: Endometriosis, Sexual Function, Anxiety and Depression

Pnm-11: Impact of Endometriosis on Quality of Life in Adolescents: A Literature Review

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Background: While endometriosis is a gynecological disease that has undesirable impact on quality of life in adult patients, its effects may be understudied in adolescents. However, the purpose of this study was to determine whether endometriosis has a significant impact on quality of life for adolescents.
Materials and Methods: Search was performed in some databases like PubMed, Scopus, Google Scholar, and Science Direct. 11 full-text articles in English from 2014 to 2024 were found in which their topic was similar to our topic. Participants under 20 years were included in the study. Quality of life parameters were measured by SF-36 or EHP-5 questionnaires in all studies.
Results: The results of the study showed that adolescents with endometriosis had significantly lower physical component summary and lower work productivity. Also, the mental component summary is significantly lower in this group. Mental health problems were more prevalent in this teenager. Overall, adolescents with endometriosis showed a poorer quality of life in this study.
Conclusion: Endometriosis is associated with significantly worse quality of life in adolescents. Also, younger age is associated with lower quality of life in patients carrying endometriosis.
Keywords: Endometriosis, Quality of Life, Adolescents, SF-36

Pnm-12: Timing of Bilateral Salpingectomy and Fertilization and Embryo Transfer Outcomes

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Background: Hydrosalpinx can reduce Fertilization and Embryo Transfer (IVF-ET) success rates by up to 50% in both implantation and pregnancy rates, potentially doubling the spontaneous abortion rate. Pretreatment is crucial for patients with severe hydrosalpinx prior to IVF-ET-based fertility treatment. The salpingectomy is recommended for effective tubal infertility treatment and minimizing infection risks during oocyte retrieval. The timing of ovulation induction following salpingectomy and its impact on transplant results continues to be

a subject of curiosity for healthcare professionals and patients alike. The present study aimed to displaying time interval between salpingectomy and IVF-ET outcomes in patients with a history of hydrosalpinx undergoing infertility treatment.
Materials and Methods: In the retrospective study, 177 women was recruited with a history of hydrosalpinx undergoing infertility treatment. After salpingectomy, they were followed until and IVF-ET outcomes (pregnancy and live births).
Results: In the first IVF cycle, of 76 cycles followed, 7 live births (9.21%) were reported. The majority of live births occurred at 15 months after salpingectomy. In the second IVF cycle after salpingectomy, of 18 cycles followed, 2 live births (11.1%) were recorded; the first live births occurred 11 and 12 months after salpingectomy to the second IVF cycle. In the first FET cycle after salpingectomy, of 38 cycles followed, 4 live births (10.53%) were recorded; the first live birth occurred 11 months after salpingectomy.
Conclusion: Findings of the present descriptive study clearly showed there were no uniform trends for time interval between surgery and live birth as the main outcome of infertility treatment by type of cycle. The variations in success rates observed in ART after salpingectomy can be attributed to several factors. This variability in outcomes could be due to chance or individual differences (such as tubal function and healing time, sample size and statistical variability, individual patient characteristics and embryo quality and transfer timing).
Keywords: Hydrosalpinx, Salpingectomy, Infertility

Pnm-13: A Review of The Effect of Acupuncture on The Treatment of Infertility Due to Endometriosis

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Background: Endometriosis is one of the common causes of infertility. Endometriosis is a chronic inflammatory disease that affects fertility. Acupuncture has been shown to be an effective and safe method to relieve dysmenorrhea, shorten the duration of pain, and improve well-being and quality of life in women with endometriosis pain. Therefore, the present study was conducted with the aim of reviewing the effectiveness of acupuncture on infertility related to endometriosis.
Materials and Methods: Academic and step-by-step search according to the purpose of study of PubMed, Googol Scholar and SID, Magiran were investigated during the years 2014-2024. Then the articles were selected based on the inclusion criteria. Articles published in English and Farsi with the same research topic and purpose they started studying. Studies that specifically discussed theories or case reports or quality were included in the exit qualities.
Results: Endometriosis is a complex disease and needs effective treatments. Endometriosis is a complex disease with no pathogenic factors. Acupuncture may be an effective treatment for Encefalite Autoimune (EAI) and there are no Randomized Controlled Trials (RCTs) proving its efficacy up to now. Acupuncture has been proven to be a safe and effective treatment for relieving dysmenorrhea, shortening the duration of pain, and improving health and quality of life in women with endometriosis pain. Our previous research also shows that acupuncture can use endometrial recurrence to prevent surgery and improve menstrual conditions and quality of life. However, its therapeutic effect needs careful scientific evaluation.
Conclusion: The results of this study will help to investigate the effectiveness and safety of acupuncture in increasing the pregnancy rate of infertile women with endometriosis. It also offers a developer-related treatment for infertility with potential benefits and fewer complications. Therefore, considering the importance of complementary medicine, it is better to use this treatment together with fertility treatments.
Keywords: Endometriosis, Infertility, Acupuncture

Pnm-14: Active Versus Passive Confrontation: How Do Iranian Gamete and Embryo Donors Overcome Their Donation-Related Concerns

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Background: Gamete and embryo donors go through a complex process of decision-making for donation, in which they will have to face obstacles and concerns. In order to be able to guide gamete donors in overcoming these challenges and provide them with the services and care they need; it is important to understand the experience of gamete donors about the concerns they face and the ways they deal with them. This study aimed to explore how Iranian gamete and embryo donors overcome their donation-related concerns.
Materials and Methods: A descriptive qualitative study was conducted in three fertility centers, each located in a different city in the central and northeast regions of Iran. Participants including three embryo donors, nine egg donors, three sperm donors, and four family members of donors entered the study through purposeful sampling within 14 months. Data were collected via semi-structured interviews, and analysed adopting conventional content analysis based on the Graneheim and Lundman approach.
Results: Reproductive donors to overcome their concerns went through one, or both of the following paths: “Active confrontation” or “passive encountering”. In active confrontation donors used diverse approaches of “purposeful information searching”, “dealing with clinical problems”, “counseling with an expert/experienced person”, “trying to fill the legal gaps”, “coping with feelings towards the donor-conceived child”, and “selective disclosure”. Passive encountering included strategies of “appeal for divine help”, “acceptance and surrendering”, as well as “avoidance and evasion”.
Conclusion: Donors use different approaches to overcome their donation-related concerns. It is important to notice that some of the approaches employed by donors may temporally help them to deal with their concerns but do not solve all their problems. Fertility centers must counsel donors about their concerns and help them choose the appropriate approach to overcome their concerns.
Keywords: Third-party reproduction, Embryo Donation, Oocyte Donation, Sperm Donation, Concerns

Pnm-15: From Happiness to Bitterness and Regret: A Qualitative Study Exploring the Experiences of Iranian Gamete and Embryo Donors

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Background: Gamete and embryo donation can be a physically, mentally, and even socially challenging experience for donors. It is important to understand how donors perceive their experience and what conditions contribute to their gratification and discontent, in order to be able to improve the donation process. The aim of this study was to explore the donation experiences of Iranian gamete and embryo donors.
Materials and Methods: A qualitative study was conducted in three fertility centers, one in the Northeast and two in the central region of Iran. 15 individuals including three embryo donors, two known egg donors, seven commercial egg donors, and three commercial sperm donors were recruited through purposive sampling between October 2022 and April 2024. The data were collected using semi-structured interviews and analyzed by the Graneheim and Lundman approach using MAXQDA 2020 software.
Results: Although most donors were satisfied with their donation experience, some have regrets about parts of their donation journey, and a few regretted all of it. Donors described their sources of satisfaction as "being able to help a person in need", "solving their problems", "making their family happy", and "producing a high number of retrieved eggs or embryos". On the other hand, "donating to the wrong people", "receiving little to no care", "experiencing side effects of therapeutic procedures for donation", "financial disputes with recipient couples", and "inability to cope with feelings toward the donor-conceived child" were identified as donors' sources for regret.
Conclusion: In order to fulfill donors' expectations and resolve their dissatisfaction, and also to provide overall better care for donors; infertility treatment centers must take lessons from former donors' experiences. Also, those centers must provide donors with long-term counseling and care to deal with their senses of regret or loss.
Keywords: Third-party Reproduction, Embryo Donation, Egg Donation, Sperm Donation, Experience

Pnm-16: Qualitative Exploration of Iranian Reproductive Donors' Needs in The Donation Process

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Background: Gamete and embryo donors' needs and desires are often neglected in third-party reproduction treatment. Identifying donors' needs is an important step in planning a comprehensive care plan for reproductive donation. The aim of this study was to explore Iranian reproductive donors' needs in the donation process.
Materials and Methods: A descriptive qualitative study was conducted with 21 individuals including 15 reproductive donors (nine oocyte donors, three sperm donors, and three embryo donors), and six fertility care providers/researchers (two infertility specialists, and one key informant from each of the urology, midwifery, reproductive health, and reproductive biology disciplines) in three Iranian fertility care centers in the central and northeast regions of Iran. Participants were recruited through purposive sampling between October 2022 and April 2024. The data were collected using semi-structured interviews. Using MAXQDA 2020 software data analysis was done based on the conventional content analysis approach.
Results: The overarching category emerging from the data was "multifaceted perceived needs", which included five subcategories of "counseling and support needs", "requirements for the provision of information", "demand for proper regulation and supervision", "need to maintain respect and dignity", and "necessity of clinical care improvement".
Conclusion: Donors have complex perceived needs that can affect the donation process. By taking donors' needs into account and employing a donor-based care plan; fertility centers could provide more appropriate care, not only for the donors but also for all those involved in third-party reproduction procedures.
Keywords: Reproductive Donation, Oocyte Donation, Sperm Donation, Embryo Donation, Needs

Pnm-17: The Effect of Infertility on Women's Mental Health

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Background: Infertility is defined by the World Health Organization as the inability to conceive after 1 year of unprotected sex. Infertility has a profound effect on the mental health of women and the whole person. The physical, emotional, sexual, spiritual and financial aspects of every person's life are affected by this disease of the reproductive system. The current study was conducted with the aim of investigating the effect of infertility on women's mental health.
Materials and Methods: To conduct this research, a search method was used in foreign articles. The search was done in PubMed, Elsevier, Scopus, Google Scholar and with the keywords of mental disorders, infertility and women. Relevant articles in Persian language were included in the study. Finally, the obtained results are classified so that a clear understanding of the effects of infertility on women's mental health and its dimensions can be obtained.
Results: The results show that the symptoms of anxiety and depression, along with social, psychological and cultural im-

portance, have caused infertility to be classified as one of the biggest stressful factors in life. As the medical treatments of patients increase physically and emotionally, the symptoms of anxiety, social and cultural pressure and society's norms, depression and emotions such as anger, betrayal, guilt, sadness and even despair are reported. Infertility can also affect a person's self-esteem, desire and sexual performance. Infertile women report high levels of psychological distress and depression and stress, and this depression may reduce their chances of conceiving. Depression and anxiety disorders in infertility patients, in societies that usually consider women to be the cause of couple's inability, by not getting pregnant, the possibility of women suffering from mental problems increases. Depression affects infertility by these disorders in hypothalamus axis, pituitary gland, adrenal gland, thyroid dysfunction, abnormality in prolactin level. The increasing age of parents also causes high-risk fertility, but many women did not know about the relationship between age and fertility. One of the most difficult emotional consequences of infertility is losing control over life. Many couples have sex as a way to bond emotionally. When sex is associated with failure and disappointment, couples may lose this emotional connection. Pressure to have or avoid sex due to infertility treatments can isolate partners and tear couples apart. Age, gender, duration of infertility, education, cause of infertility and occurrence of previous infertility treatment failures are strongly related to it.

Conclusion: It can be concluded from the present study that mental health problems such as anxiety, depression, loss of behavioral-emotional control, and mental distress are common among infertile women. The relationship between mood disorders and fertility is complex and a socially necessary approach to diagnosis and management. Teaching positive coping skills and teaching communication skills is especially helpful because of the stress that infertility places on marital and non-marital relationships. This training gives patients a framework for identifying negative cycles of distance and conflict and teaches them positive cycles.

Keywords: Infertility, Women, Mental Disorders

Pnm-18: The Effect of Cognitive-Behavioral Therapy on Anxiety, Depression and Marital Satisfaction of Infertile Women in Iran and The World

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Background: Infertility is a biological, psychological and social disorder that threatens the mental health of infertile couples and causes anxiety and depression and subsequent marital dissatisfaction in them. Cognitive-behavioral therapy is one of the effective treatments in this field, whose effectiveness in infertile women is unclear; Therefore, the current study reviews the studies conducted in Iran and the world with the aim of the effect of cognitive-behavioral therapy on anxiety, depression and marital satisfaction of infertile women.

Materials and Methods: In this systematic review study, all articles published in English and Persian languages were searched in Magiran, Iranmedex, SID, PubMed, Scopus, Google Scholar, Embase, Cochrane Library and Web of Sciences databases until April 2024. 20 clinical trial studies that investigated the effect of cognitive-behavioral therapy on anxiety, depression and marital satisfaction were investigated. Studies that had unclear sample size and method of implementation were excluded from the study process.

Results: 16 studies were included in the systematic review. Of which 12 studies were related to the effectiveness of cognitive-behavioral therapy on anxiety and depression and 4 studies were related to investigating the effect of this method on marital satisfaction. The studies included intervention and control groups, in which the intervention group, received cognitive-behavioral therapy and the control group only received routine care. The results of most of the studies showed that the mean score of anxiety and depression in the group receiving cognitive-behavioral therapy was significantly lower than the control group and the mean score of marital satisfaction was significantly higher in the group receiving cognitive-behavioral therapy compared to the control group.

Conclusion: The results of most studies have shown the effect of cognitive-behavioral therapy on improving anxiety and depression and increasing marital satisfaction in infertile women.

Keywords: Infertility, Cognitive-Behavioral Therapy, Anxiety, Depression, Marital Satisfaction

Pnm-19: Investigating The Relationship Between Hematoma and Bleeding in The First and Second Trimester of Pregnancy with The Occurrence of Postpartum Bleeding or Placental Adhesion: A Pilot Study

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Background: Placental adhesion is one of the major problems during pregnancy that can cause postpartum bleeding and maternal complications. The causes of placental adhesion or the spectrum of placenta accreta are different and are often related to the history of previous cesarean section or uterine surgeries. Most of the observed cases are related to the history of previous repeated cesarean section, but there are cases where the mother has frequent and prolonged bleeding in the first trimester as well as the second trimester, or there is a history of hematoma.

Materials and Methods: In this small study, the number of 50 mothers who had bleeding in the first or second trimester of pregnancy or hematoma were evaluated. These mothers had no history of uterine surgery or caesarean section and had no risk factors for adhesions. The control group included 50 mothers with a history of previous cesarean section, whose only risk factor was previous cesarean section. All the cases of singleton pregnancy and the age of the mothers were between 18 and 40 years old. All cases were followed up until delivery and were compared in terms of the incidence of postpartum bleeding or vaginal bleeding or adhesions.

Results: Out of 50 cases of patients under study, 40 cases underwent cesarean section as desired and 10 cases underwent natural delivery. Out of these patients, 8 cases equal to 16% suffered from postpartum hemorrhage or placental adhesion, and these 8 cases were all cesarean cases. Out of 50 cases of control patients, all of them had a history of at least one previous caesarean section. None of them suffered from postpartum bleeding or adhesions.

Conclusion: Bleeding in the first and second trimesters and he-

matoma can be an important reason for the occurrence of placental adhesions, especially in a mild form, and bleeding from the uterine floor during, giving birth which we must pay special attention to it.
Keywords: Adhesion, Postpartum Hemorrhage, Hematoma

Pnm-20: To Evaluate The Effect of Apple Cider Vinegar on Metabolic Syndrome and Insulin Resistance in: Polycystic Ovary Syndrome Patients

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, affecting 5-18% of women. This disease is characterized by a combination of hyperandrogenism (clinical or biochemical), chronic anovulation, and polycystic ovaries. This syndrome leads to a range of symptoms caused by "hyperandrogenism", such as weight gain, abdominal fat, and insulin resistance. Women with PCOS are also prone to metabolic syndrome. Due to the complications caused by the use of old therapies, today more and more new treatments have been taken into account such as apple cider vinegar. Consumption of apple cider vinegar can improve plasma lipid profile, glycemic indices (HOMA-IR, HOMA-B, QUICKI), blood pressure, and inflammatory biomarkers.
Materials and Methods: A literature search was conducted through PubMed and Science Direct to identify the effect of apple cider vinegar on metabolic syndrome and insulin resistance
Results: According to studies, apple cider vinegar can improve lipid profile including reducing total cholesterol, VLDL, LDL-C, and Triglycerides and increasing HDL-C. It also reduces FBS, insulin resistance, and BMI. One of the studies has shown that apple cider vinegar was associated with a reduction in LH/FSH ratio in PCOS patients.
Conclusion: Since apple cider vinegar affects metabolic syndrome and insulin resistance, we can with more studies use it as an adjunct to other drugs in the treatment of PCOS.
Keywords: Polycystic Ovary Syndrome, Apple Cider Vinegar, Metabolic Syndrome, Lipid Profile, Insulin Resistance

Pnm-21: Complementary and Alternative Medicine for The Management of Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a prevalent, complex endocrine disorder characterized by polycystic ovaries, chronic anovulation, and hyperandrogenism leading to symptoms of irregular menstrual cycles, hirsutism, acne, and infertility. Several pharmaceutical treatments have been proposed for PCOS however they have disadvantages, such as adverse effects, low compliance of patients with long-term pharmaceutical treatments, low efficacy, and contraindications in some cases. Therefore, in recent years, more and more attention has been paid to complementary and alternative medicine (CAM), which has been widely used in clinical practice. The aim of this study is to investigate herbal medicine used in PCOS.
Materials and Methods: PubMed, Embase, Cochrane, and Scopus databases were searched based on related keywords.
Results: According to studies, a wide spectrum of herbs can be used to improve different aspects of PCOS. Herbs such as Cimicifuga racemose, Vitex agnus-castus, Paeonia lactiflora, Trigonella foenum-graecum L, Cinnamomum verum and apple cider vinegar can impact on ovulatory dysfunctions, obesity, insulin resistance, metabolic syndrome, and hyperandrogenism
Conclusion: Some plants as natural remedies may have useful effects on improving various aspects of PCOS, but more studies are needed.
Keywords: Polycystic Ovary Syndrome, Complementary and Alternative Medicine, Herbal Medicine

Pnm-22: Determining The Sexual Health Status of Women Referred to The Infertility Center of Imam Khomeini Hospital in Sari During The Covid 19 Pandemic

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Background: Difficulty in social life, decrease in income and stressful lifestyle are factors that can affect women's sexual desire and the number of times they have intercourse. This study was conducted with the aim of determining the status of women's sexual performance during the covid-19 pandemic.
Materials and Methods: This descriptive and analytical study was conducted on 215 women referring to Imam Sari Infertility Center who completed the demographic information form and Female Sexual Function Index (FSFI) sexual performance index questionnaire. After collecting information, data analysis was done using SPSS version 24 software.
Results: The results showed that the frequency of sexual dysfunction in barren women was 93(%, and the frequency of the disorder was in the Desire area (22.7%), in the Arousal area (28.3%), and in the Lubrication area (59.5%)., in the area of Orgasm (31.1(%, in the area of Satisfaction (19.5% (and in the area of Pain (1.78). A significant relationship was observed between people with average economic status and sexual dysfunction (P: 0.000).
Conclusion: The frequency of sexual dysfunction in these women is reported to be relatively high. The middle class of society and people with average economic status have more sexual dysfunction, which can indicate the relationship between unfavorable economic status and sexual dysfunction.
Keywords: Covid-19, Sexual Health, Sexual Dysfunction, Infertility

Pnm-23: Relationship between Serum Levels of Vitamin D and The Success Rate of Pregnancy in An Fertilization Cycle: A Prospective Cohort Study.

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Background: Vitamin D deficiency in women may play a role in the pathogenesis of infertility and menstrual dysfunction. Due to the high percentage of insufficient vitamin D serum levels in women of reproductive age, recently the role of vitamin D in reproductive physiology has also been considered. The present study was performed with aim to determine the relationship between vitamin D serum level and pregnancy success rate in a cycle of Fertilization (IVF).
Materials and Methods: In this prospective cohort study conducted in 2022, 116 reproductive-aged women diagnosed with both primary or secondary infertility in their first IVF cycle were studied. Based on the serum level of vitamin D in the blood sample measured by the Elisa method 7 days before embryo transfer, the participants were divided into two groups: deficiency or insufficient and sufficient and were evaluated at the gestational age of 7 weeks in terms of the presence of intrauterine gestational sac and the presence of heartbeat. Data collection tools included socio-demographic, infertility, and nutrition questionnaires, and checklists for recording test results and pregnancy outcomes. Data were analyzed by SPSS statistical software (version 24) and chi-square, independent t-test, and multivariate logistic regression. P <0.05 was considered statistically significant.
Results: The mean of vitamin D serum level in people with successful pregnancy and unsuccessful pregnancy was 60.3 ± 26.8 and 66.5 ± 37.4, respectively (P=0.361) and the frequency of pregnancy success in vitamin D sufficient and insufficient group was 23.3% and 13.6%, respectively (P=0.247). Based on multivariate logistic regression and adjusting for confounding variables, there was no statistically significant difference between the groups in terms of pregnancy success rate odds ratio: 2.08; 95% (CI): 0.45 to 9.5; (P=0.346).
Conclusion: Although vitamin D serum level in infertile women with successful pregnancies was more than in women with unsuccessful pregnancies, however, this difference was not significant.
Keywords: Fertilization , Vitamin D, Tate of Pregnancy

Pnm-24: Endometriosis and Endocrine-Disrupting Chemicals

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Background: Endometriosis is a chronic gynecological disease that affects a growing number of women of childbearing age, being present in more than 176 million women worldwide according to recent estimates. The etiology of endometriosis has yet to be fully elucidated; however, it is known to be multifactorial and hormonal, genetic, lifestyle, and environmental risk factors have been implicated in recent decades. Increasing evidence has been published over recent years on the implication of endocrine-disrupting chemicals including parabens and benzophenones on endometriosis.
Materials and Methods: A literature search was conduct through PubMed, google scholar, ProQuest, Scopus, Springer and Science Direct to identify the relationship between endometriosis and endocrine-disrupting chemicals including paraben and benzophenone.
Results: Our findings indicate that the frequency of cosmetics and personal care products utilization is a strong predictor of exposure to certain benzophenone and paraben congeners. The results of the studies on exposure to parabens, benzophenones are inconclusive.
Conclusion: The studies were mostly well-designed epidemiological studies, using biomarkers of exposure, where the outcome (endometriosis) was based on a confirmed diagnosis. Additionally, the statistical models were adjusted for potential confounding factors. Due to the insufficient evidence, further epidemiological studies are needed to confirm these findings.
Keywords: Endometriosis, Endocrine-disrupting Chemicals, Benzophenone, Paraben

Pnm-25: Recurrent Pregnancy Loss and Endometriosis

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Background: Recurrent pregnancy loss (RPL) is a distressing pregnancy disorder experienced by ~2.5% of women trying to conceive. Recurrent pregnancy loss (RPL) is defined as the failure of two or more clinically recognized pregnancies before 20–24 weeks of gestation and includes embryonic and fetal losses. Implantation and pregnancy development require a functional and optimal interplay between a good quality embryo and a receptive endometrium. Endometriosis is a chronic inflammatory disease, characterized by the growth of endometrium-like tissue outside the uterine cavity.
Materials and Methods: A literature search was conduct through PubMed, google scholar, ProQuest, Scopus, Springer and Science Direct to identify the relationship between endometriosis and RPL.
Results: The results showed that endometriosis to be associated with RPL.
Conclusion: RPL represents an unresolved problem for contemporary gynecology and obstetrics. In fact, it is not only a relevant complication of pregnancy, but is also a significant reproductive disorder affecting some of couples desiring a child. The current knowledge on RPL is largely incomplete, since nearly 50% of RPL cases are still classified as unexplained. Pathologies resulting in chronic endometrial inflammation in case of endometriosis have been associated with increased risk of RPL.
Keywords: Endometriosis, Recurrent Pregnancy Loss, Pregnancy

Pnm-26: Physical Psychological Disorder in Women with Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus (SLE) is an au-

toimmune disease can have multi-organ conflict in women of the reproductive age. This study was conducted with the aim of assessing the related factors of physical-psychological disorder (PPD) in women with SLE.
Materials and Methods: This cross-sectional study was conducted on 235 women of reproductive age (10-49 years) diagnosed with SLE. The data were collected randomly through an online questionnaire, which was completed by women attending the rheumatology clinic. The data were analyzed using statistical tests such as simple and multiple liner regression tests by SPSS 26.
Results: Findings showed mean Age of women was 36.21 years (SD: 5.98). Age of women (β : 0.25; 95% CI: 0.23 - 1.97; P= 0.014), Mild or mode grading of SLE (β : 0.71 ; 95% CI: 59.7 - 21.61; P= <0.001), sever grading of SLE (β : 0.63 ; 95% CI: 50.44 - 21.61; P=<.001) can predict 34 percent of PPD score changes in women with SLE (Rs: 0.34, P<0.001).
Conclusion: The findings clearly discovered higher age of women, higher Mild or mode grading of SLE, and higher sever grading of SLE in comparison silent degree increased PPD scores in women with SLE.
Keywords: Systemic Lupus Erythematosus, Women, Physical Psychological Disorder

Pnm-27: Spirituality in women with Systemic Lupus Erythematosus

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Background: Spiritual health empower women to tolerate chronic disease easily. This study was conducted with the aim of assessing the related factors of spirituality in women with Systemic Lupus Erythematosus (SLE).
Materials and Methods: This cross-sectional study was conducted on 235 women of reproductive age (10-49 years) diagnosed with SLE. The data were collected randomly through an online questionnaire, which was completed by women attending the rheumatology clinic. The data were analyzed using statistical tests such as simple and multiple liner regression tests by SPSS 26.
Results: Findings showed number of delivery after contracting SLE (β: -0.43; 95% CI: -44.24 - -3.61; P= 0.02), duration of SLE (β: 0.43; CI95%: 9.75 - 37.46; P=<0.001), and sever grading of SLE (β: 0.44; 95% CI: 1.76 - 9.17; P= 0.005) could predict 38 percent of spirituality (Rs: 0.38, P: 0.03).
Conclusion: The findings clearly discovered lower number of delivery after contracting SLE, higher duration of SLE, and higher sever grading of SLE in comparison silent degree increased spirituality scores (spirituality tendency) in women with SLE.
Keywords: Systemic Lupus Erythematosus, Women, Spirituality

Pnm-28: Fertility Challenging in Women with Systemic Lupus Erythematosus: A Qualitative Study

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Background: Systemic lupus erythematosus (SLE) is a multisystem disease that mostly affects women of the reproductive age. Since women's reproductive health is all aspects related to the reproductive system. The effect of SLE on the reproductive health has been largely ignored in the clinical performance and life of these individuals. This study was conducted with the aim of exploring the perceptions of women with SLE regarding challenging fertility.
Materials and Methods: This qualitative research was conducted using 27 semi-structured deep interviews with 19 married women suffering from SLE (15-49 years old) selected through purposive sampling in the referral Rheumatology Center in Iran. Data analysis was performed with a content analysis approach using the conventional method proposed by the Zhang and Wildemuth (2016) by 10 MAXQDA.
Results: The women's perceptions about Challenging fertility were categorized in 2 subcategories included bothersome pregnancy (According to the women talking, women with SLE experienced pregnancy problems such as frequent abortions, increased fetal disorders, seizure during pregnancy, lack of will for making decisions about pregnancy, psychological problems, activation of the disease after delivery, and inability to use preventive methods.) and impaired parenting (according to the women perception, disturbed parenting occurs due to loss of a sense of motherhood, impatience in taking care of the child, fear from death with the child being left alone, and concerns about the child's future).
Conclusion: The findings clearly suggested the negative effects of SLE on the reproductive health experience of these women. Also emphasized, women with SLE requires strategic and multidisciplinary management.
Keywords: Systemic Lupus Erythematosus, Women, Qualitative Study

Pnm-29: The Effect of Video-Based Mindfulness-Based Counseling on Perceived Stress and Salivary Cortisol of Infertile Women Undergoing Fertilization Treatment

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Background: Infertility is known to be a stressful experience. Although Fertilization (IVF) has given hope to infertile couples, it has also made them more stressed. One of the most effective approaches in the field of stress reduction is cognitive therapy based on mindfulness (2), but some clients refuse to attend the sessions due to financial limitations and waste of time in commuting. To enhance society's knowledge, the third wave civilization requires a powerful tool that is affordable, quick, and reliable. Due to the increase in the rate of infertility, the existence of stress in infertile women, and according to the latest government policies regarding population growth, infertile women should be under It is desirable to replace the method that can be provided with high access in front of low cost. Therefore, this study was conducted with the aim of determining the effect of video counseling based on mindfulness on

perceived stress and salivary cortisol of infertile women undergoing fertilization treatment.
Materials and Methods: This study is a randomized clinical trial that was conducted in 1402 on 140 women referred to the Milad Infertility Center in Mashhad who were treated with fertilization. The research units were selected using the available sampling method and randomly divided into intervention and control groups (70 people in each group). The intervention group received mindfulness video counseling in 8 15-minute sessions filmed by the researcher. The control group was not given counseling care. Before and after the intervention, saliva collection was done along with questionnaires (Newton's infertility stress, five aspects of mindfulness, Dass21). To analyze data, SSPS16 software and descriptive and analytical statistical tests will be utilized
Results: The findings indicated that video-based mindfulness-based counseling can lower perceived stress and salivary cortisol levels (p0/001).
Conclusion: The findings indicate that video counseling based on mindfulness is effective in decreasing perceived stress and salivary cortisol levels among infertile women undergoing IVF treatment. Findings of our study indicate the need for the specific psychological interventions for all infertility infertile women, to improve IVF success rate.
Keywords: Stress, Video Counselling, Mindfulness, Infertile Women, Fertilization
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Pnm-30: Effects of Physical Activity and Exercise on Endometriosis

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Background: Endometriosis is a benign disease of women, a condition in which abnormal cells similar to endometrium cells are placed outside the uterine cavity; Their main clinical symptom is severe pain during menstruation, pain during intercourse, chronic pelvic pain is also common. There is no definitive treatment for endometriosis. International clinical guidelines focus on the role of physical activity and exercise as part of the treatment approach for endometriosis. Women who suffer from symptoms related to endometriosis suggested. The present study was conducted with the aim of investigating the effects of physical activity in improving the symptoms of endometriosis.
Materials and Methods: To conduct this research, the search method was used in foreign articles, the search was done in google scholar and PubMed database with the keywords "endometriosis and physical exercises", "endometriosis and lifestyle" and "chronic pelvic pain".
Results: Studies show that women who exercise regularly have a significantly lower risk of endometriosis than women who do not exercise. Accordingly, more physical training had a protective effect for women who had started exercising at least 2 hours per week before the age of 26; But based on recent studies, there is little information about the effect of Physical Activity (PA) and exercise on the improvement of symptoms in women with this disease. Women with premature menstruation less than 12 years old and menstrual period of 8 days or more are at higher risk of endometriosis. Avoiding vigorous exercise during menstruation is a preventive factor for endometriosis, in general some women prefer not to exercise during their period due to dysmenorrhea. This study also draws attention to the possibility that pain can negatively affect physical exercise in women with endometriosis. The use of painkillers can be less effective in endometriosis patients who exercise regularly. The main focus is on pain management with hormonal suppression of the disease or surgical excision. Hormonal therapy can have intolerable side effects or become ineffective over time, while the effects of surgery are often short-term; Advances in the understanding of endometriosis have expanded the focus on non-invasive, non-pharmacological treatments. One of the common symptoms of endometriosis is chronic pelvic pain. The prevalence of chronic pelvic pain (CPP), a debilitating condition, is high in women of reproductive age. CPP has a negative impact on their quality of life, social, professional and marital relationships. Dysmenorrhea is one of the common causes of persistent pelvic pain; when these two symptoms are present together, even if the ultrasound scan or magnetic resonance imaging is normal,The diagnosis of endometriosis should be suspected. In fact, endometriosis is strongly associated with pelvic pain in women, however, there is no clear relationship between the extent of the disease and the severity of the pain. In women with suspected or confirmed endometriosis through laparoscopy, pain threshold and symptoms of central sensitivity are lower, which indicates that persistent pain from the presence of endometriosis is visible in causing central sensitivity. Recent studies have shown that physical exercise can reduce clinical pain, this is also true in women with endometriosis or dysmenorrhea.
Conclusion: Physical activity may have a wide range of beneficial effects on endometriosis-related symptoms, but unfortunately these effects cannot be definitively confirmed, and this beneficial role of PA and exercise should be communicated to women with endometriosis symptoms. Women with endometriosis symptoms had a lower pain threshold than healthy women.
Findings:
Keywords: Endometriosis, Physical Exercises, Lifestyle, Chronic Pelvic Pain

Pnm-31: New Understanding of Diagnosis, Treatment and Prevention of Endometriosis

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Background: Pelvic endometriosis is associated with mild pain, cystic ovarian endometriosis with severe and deep endometriosis with very severe pain. However, some 50, 25% and 5% of these women are pain-free, respectively. Endometriosis is associated with infertility, but it is unclear whether endometriosis causes infertility, except in cystic ovarian endometriosis with severe adhesions. The purpose of this study was to investigate new understanding of diagnosis, treatment and prevention of endometriosis.
Materials and Methods: The study was conducted by searching the PubMed, Elsevier, Scopus, google scholar databases as well as the Iranian SID and Iran-doc databases with the keywords endometriosis, diagnosis, treatment and prevention. The articles from 2000 to 2024 with Persian and English were included in the study. All studies in the field of remote intensive care at the time of accidents and disasters, which were in Persian and English and the full article was available, were includ-

ed in the study. Selected articles were reviewed with a prism evaluation checklist of Prisma articles
Results: The higher risk of initiating endometriosis after puberty was indirectly confirmed by the incidence of laparoscopies for endometriosis in the UAE, France, Belgium and the USA. These similar observations in Arabic countries, Europe and the USA, suggest a fundamental mechanism involving estrogens and the peritoneal microbiome, less affected by food intake or climate or environment. Although not yet investigated or proven, repetitive vaginal infections and severe dysmenorrhea in women with a hereditary risk seem to deserve more clinical interest. Medical therapy of endometriosis needs to be reconsidered since endometriosis lesions are heterogeneous, with variable progesterone resistance and since superficial endometriosis is affected mainly by peritoneal fluid concentrations of steroid hormones. More specifically, it needs to be investigated whether and in which lesions a specific progesterone effect can be expected. Virtual colonoscopy, colonoscopy and ultrasound seem less performant. Deciding to do bowel resection before surgery based on imaging and the depth of infiltration is associated with a higher percentage of bowel resections than deciding during the laparoscopy. However, the latter is impractical unless the gynecologist/pelvic surgeon can perform the bowel resections
Conclusion: The management of endometriosis changes if considered initiating following a series of cumulative genetic-epigenetic incidents, with a subsequent self-limiting growth and fibrosis. The risk of initiating endometriosis will be highest after menarche, mainly in predisposed women, and will decrease thereafter, becoming low after 30 years.
Keywords: Endometriosis, Fertility, Diagnosis, Treatment

Pnm-32: The Effect of Diet and Nutritional Factors on Male Fertility

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Background: Infidelity in men is defined as failure to conceive after 12 months or more of regular unprotected intercourse by couples. In general, human fertility is affected by several factors, including female factors (uterine disorders and ovulation), male factors (abnormal sperm production and function), medical factors (such as pelvic inflammatory diseases and cancer) and other factors such as genetics, gender and age. The most important of all changeable lifestyle factors such as physical activity, overweight, alcohol and smoking, stress and long-term use of contraceptives are placed. The present study was conducted with the aim of investigating the effect of diet and nutritional factors on male fertility.
Materials and Methods: To conduct this research, a search method was used in foreign articles. The search was done in PubMed, Elsevier, Scopus, Google Scholar databases with the keywords of nutritional factors, semen quality, antioxidants, infertility and men. Relevant articles in Persian language were included in the study. Finally, the obtained results are classified so that a clear understanding of the effects of nutrition on male fertility and its dimensions can be obtained.
Results: Despite the other variables based on the aforementioned couple, the quality of semen is considered as a representative of male infertility, and over the past 50 years, factors such as developmental genetic factors, lifestyle and, most importantly, nutritional factors have caused the quality Semen has decreased significantly. Most studies have shown that the saturated fats found in animal foods such as red meat, processed meats, and high-fat dairy products have a negative effect on fertility and sperm quality. In contrast, antioxidant molecules, which are abundantly found in fruits and vegetables, have a positive effect on male fertility and overall health conditions by counteracting the activity of active oxygen species. In fact, by damaging the plasma membrane and mitochondrial function, it has a significant effect on sperm motility. Other factors, including chronic alcohol consumption, can significantly decrease the number, progressive motility, and vitality of sperm. Some studies showed a possible connection between sperm DNA damage and coffee and caffeine consumption. Contamination of food and water: In general, food contaminants such as herbicides often cause endocrine disorders and have a negative effect on sperm quality. Also, smokers have an unusually high concentration of heavy metals in their semen, which has a negative effect on the concentration and mobility of semen and affects its inherent quality. Low levels of vitamin E have been observed in the semen of infertile men, so it can be said that vitamin E can improve sperm quality. Phytoestrogens are herbal compounds of plant origin that are most abundant in soybeans and its products, and it is suggested that these substances can be a substitute for hormonal replacement for menopausal women. Also, a series of anti-mutagenic and antioxidant properties are known from these substances.
Conclusion: Nutrition and lifestyle are considered as the main factors in reproduction and fertility, and male obesity, especially obese men with type 2 diabetes and insulin resistance (hyperglycemia has a negative effect on sperm motility and the fertilization process) and Also, lifestyle factors such as smoking and alcohol consumption have a negative effect on sperm quality. Proper diet plays a key role in improving the quality of sperm, especially the Mediterranean diet, which is rich in fatty acids and omega-3, antioxidants, vitamins, etc., in contrast to the consumption of trans and saturated fats, which is associated with low quality of semen
Keywords: Nutritional Factors, Male Infertility, Semen Quality, Antioxidant

Pnm-33: The Review of The Principles Of Medical Ethics in Assisted Reproductive Technology In Elderly Women

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Background: Currently, there are no international guidelines for age limits for assisted reproductive technology (ART), but many centers limit these procedures to women of reproductive age. On the contrary, according to the Declaration of Human Rights, the limitation of this method based on age is controversial. It is believed that women should not pursue motherhood after a certain age and society should not pay for expensive and often unsuccessful ART for age-related fertility problems. In this review article, the ethical issues for and against the access of elderly parents to ART services will be discussed.
Materials and Methods: This review article was prepared by

studying 20 articles such as Google, Pub Med, and Scopus sites with keywords like Advanced Motherhood - Advanced maternal age- Reproductive Ethics- Age Limits- ART.

Results: Many studies have highlighted the challenges that older mothers face in promoting the well-being of their children, including the physical, psychological, emotional, and social dimensions. Advanced maternal age may increase the risk of pregnancy loss and maternal mortality. However, the management of many of these pregnancy risks in older women is similar to the management of high-risk pregnancies in younger women. It is proposed that in carefully investigated cases, pregnancy in advanced age is considered high risk, and informed consent, screening, and perinatal management are required. Those who oppose the reproductive rights of postmenopausal women claim that children raised by older parents are more likely to assume the caregiving role of their older parents at a young age, and may even experience the death of a parent before puberty. This belief is based on the principle of beneficence for the future child. Therefore, those who consider the physical limitations and reduced life expectancy of women as a factor for prohibiting the use of assisted reproductive methods should not support the use of ART in women with disabilities or chronic diseases. Furthermore, some ethicists reject the notion of restricting access to ART based on potential harm to the child. They claim that the concerns are rarely serious enough to justify a restriction on reproductive freedom. According to the principle of reproductive freedom, every human being has the right to make reproductive decisions free from discrimination, coercion, or violence. Delayed motherhood is typically discussed in the literature in the context of four main ethical principles: beneficence, nonmaleficence, autonomy, and justice. Based on the principles of autonomy and beneficence, ART services should be available to older women. According to the principle of autonomy, informed consent must be obtained based on adequate and accurate information about ART risks, success rate, and benefits. Under the principle of justice, age should not be a reason for excluding older women from ART services.

Conclusion: According to the above ethical conflicts, age should not be a criterion for receiving ART services, and there is a need to develop a comprehensive set of guidelines for the use of ART services.

Keywords: Advanced Motherhood, Advanced Maternal Age, Reproductive Ethics, Age Limits, ART

Pnm-34: The Relationship Between Weight Loss and Health-related Quality of Life in Obese Women

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Background: Obesity represents one of the most significant health concerns of the contemporary era. Previous research has demonstrated that obesity increases the risk of chronic disease and decreases Health-related Quality of Life (HRQOL). In studies examining the impact of dietary interventions on generic HRQL, the beneficial effect of weight loss on subscales of HRQL has differed across studies. Some studies have reported improvements in the physical and mental aspects of HRQL following dietary interventions, while others have found improvements in various subscales of HRQL. A weight loss of between 5% -10% of initial body weight is associated with a reduction in the risk of cardiovascular disease and other comorbidities. However, it remains unclear whether this level of weight loss may influence the likelihood of improvements in HRQOL, due to the limited number of studies and the varying findings across these studies. This study aims to examine the relationship between the amount of weight loss and changes in HRQOL.

Materials and Methods: 158 overweight and obese women (age 29 ± 7.2 years, BMI= 31.6 ± 3.8) participated in 24 weeks of an energy-restricted diet to cause 0.5 kg of weight loss per week. The Short Form Health Survey (SF-36) was used to assess HRQOL. It has 8 dimensions physical functioning (PF), role limitation due to physical problem (RP), bodily pain (BP), general health perception (GH), vitality (VT), social functioning (SF), role limitation due to the emotional problem (RE) and mental health (MH). These eight domains can be summarized as follows; the physical (PCS) and mental component scales (MCS), domains that range from 0 (minimum health status) to 100 (maximum health status). Participants were classified according to the percentage of weight change: 0%-4.99% weight loss (category 1), and 5-10% weight loss (category 2). HRQOL and anthropometric variables were assessed at baseline and 24 weeks. For each group (categories 1 and 2), the percentage change from baseline to six months was calculated for each domain of HRQOL. To compare differences between groups, the independent t-test or Mann-Whitney U test was used.

Results: At the end of the intervention, the average percentage of weight loss was 6.5 ± 2.4 %. Of the 158 participants, 68% (n=107) lost 5-10 % of their baseline weight, and 32 % (n=51) lost less than 4.99 % of their baseline weight. Participants who lost 5-10 % of their initial weight, compared to those who lost <4.99 % of their initial weight, had greater improvement in domains of PF (28.4 vs, 5.1%), RP (18.7 vs, 4.1%), BP (12.5 vs, 1.1%), GH (21.8 vs, -2.6%), and PCS (18.8 vs, 1.1%) (P<0.001). There was no significant enhancement in the mental aspects of HRQOL.

Conclusion: In women with obesity, modest weight loss improves HRQOL. The most notable improvement was observed in the physical functioning domain of HRQOL.

Keywords: Weight loss, HRQOL, Obesity

Pnm-35: Evaluating The Health of Children Resulting from Assisted Reproductive Technology: A Review Study

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Background: The increasing number of infertile couples benefits from a range of assisted reproductive technologies, like Fertilization (IVF), which poses risks to children including cardiovascular issues, high blood pressure, asthma, and frequent hospitalization. Adverse effects stem from assisted reproductive technology (ART) treatment, multiple pregnancies, and characteristics of infertile parents, with risks like low birth weight and birth defects. This study evaluates children's health

due to assisted reproductive technology.
Materials and Methods: A review was conducted independently by two people based on PICO criteria. Articles were searched in Google Scholar, PubMed, Medline, Web of Science and SID databases using Boolean operators. The search was conducted between the years 2005 and 2024 using the assisted reproductive technology, children, and health.
Results: Among over 85 articles, 13 met inclusion criteria. Results suggest similar health outcomes in ART-born children, with limited evidence indicating higher risks of cancer, diabetes, blood pressure changes, and cardiovascular issues. Women conceiving through ART face increased risks compared to natural conception, potentially influenced by laboratory techniques used.
Conclusion: The study revealed that using ART for infertility treatment raises risks such as non-chromosomal fetal defects. Advanced midwifery care is essential due to infertility treatments' characteristics. Long-term monitoring of children born through ART is vital for their health.
Keywords: Assisted Reproductive Technology, Children, Health

Pnm-36: Predicting The Severity of Premenstrual Syndrome Symptoms based on Sleep and Anxiety Patterns in Married Women with Children

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Background: The examination of women and issues related to them has always been one of the most important human topics. Women, as wives and then in the role of mother, are considered as one of the key elements in the family and society. Menstruation and the associated problems have been one of the numerous issues that many women have faced throughout history. Premenstrual syndrome (PMS) is an important issue that is associated with the menstrual cycle and occurs during the luteal phase, usually disappearing with the onset of menstruation. The aim of this study is to predict the severity of PMS symptoms based on sleep patterns and anxiety in married women with children.
Materials and Methods: The present research was descriptive and correlational in design. The study population consisted of all married women with children aged 25 to 35 residing in Tehran. Among them, 168 individuals were selected using convenience sampling method, and they completed the Premenstrual Screening Symptoms Tool (PSST), Beck Anxiety Inventory, and Pittsburgh Sleep Quality Index (PSQI) questionnaires. The data were analyzed using SPSS-20 software at two descriptive and inferential levels.
Results: The results of the regression analysis showed that there is a significant relationship between all components of the present study ($P<0.05$). Additionally, 44% of the variance premenstrual syndrome symptoms is due to changes in other variables. According to the results, anxiety, with a beta coefficient of 0.539, sleep duration, with a beta coefficient of 0.159, sleep quality, with a beta coefficient of 0.190, use of sleep medications, with a beta coefficient of 0.145, and daily functional impairments, with a beta coefficient of 0.171, indicate their positive effects on premenstrual syndrome symptoms in women.
Conclusion: Analysis and investigation of the results of this research showed that anxiety and sleep patterns are significantly related to the severity of premenstrual syndrome symptoms and can predict the severity of premenstrual syndrome symptoms. In other words, the higher the level of anxiety and the lower the quality of sleep, as well as higher daily functional sleep problems, the higher the predicted severity of premenstrual syndrome symptoms.
Keywords: Anxiety, Sleep Pattern, Married Women with Children, Premenstrual Syndrome

Pnm-37: Nutritional Factors and Treatment of Fibroids: A Literature Review

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Background: Uterine fibroids (UFs) are the most common gynecological tumors and a major cause of gynecological morbidity in reproductive-age women. Although UFs are benign tumors, they can cause a myriad of symptoms and outcomes, including pelvic pain, abnormal uterine bleeding, bladder dysfunction, and even infertility. Nowadays numerous methods of UF treatment are available-from conservative treatment to invasive surgeries. Several natural compounds have been found to help treat UFs and relieve their symptoms. In this study, we reviewed the currently available data on possible natural compounds that are useful for patients with UF, especially those who want to preserve their future fertility.
Materials and Methods: Searching was performed in some databases like PubMed, Scopus, and Web of Science. From 2000 to 2024, 8 full text articles in English were found which their topic was similar to our topic. So, we reviewed data on fibroid treatment and diet, as well as studies that looked at natural components
Results: A link between Vitamin D deficiency and UFs formation is strongly indicated, making it a potent compound in fibroma therapy. Other natural substances, such as curcumin, can reduce oxidative stress and protect against inflammation in fibroma. Trace elements such as selenium can also contribute to anti-inflammatory and antioxidant properties of a recommended diet. Studies on epigallocatechin gallate showed its apoptosis-promoting and antifibrinolytic effect in fibroid cells.
Conclusion: The results of this study showed that it is possible to look at nutrition as a treatment for fibroids, so encouraging people to modify their diet seems necessary. Randomized trials and further investigations are needed to finally confirm the existing findings and to draw robust evidence-based conclusions about the effects of diet and nutrients on the treatment of uterine fibroids.
Keywords: Fibroma, Diet, Nutrition, Treatment

Pnm-38: The Educational Role of Nurses in Relation to The Screening of Pregnant Mothers and The Prevention of Fetal Abnormalities with The Ultrasound Method

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Background: Mid-second trimester gynecological ultrasound is a routine examination in many countries. It plays an important role in ensuring the health of the pregnant patient and the developing fetus. This non-invasive imaging technique, usually performed between 18 and 22 weeks of pregnancy, is a cornerstone of modern prenatal care and is primarily aimed at evaluating fetal anatomy and detecting any fetal abnormalities. Prenatal ultrasound is widely used in pregnancy to evaluate the growth and anatomy of the fetus.
Materials and Methods: A review of scientific articles available on SID-GOOGLE CHROME-PUB MED-ISC.
Results: Although ultrasound screening is now an integral part of routine pregnancy care, recommendations for providing obstetric ultrasound vary from country to country. Obstetric ultrasound reports the number of fetuses present, gestational age, and placental location. It provides an opportunity to detect congenital anomalies, soft markers of aneuploidy, and maternal pelvic pathology. Harmonic imaging may visualize fine anatomic details, especially in patients with They are weak, increase. High-frequency ultrasound transducers increase spatial resolution but decrease sound beam penetration. Several factors influence transducer selection and optimal operating frequency, including maternal position, fetal position, and the chosen method, e.g., transvaginal and/or transabdominal, using from a half-lying position with the head up, especially in late pregnancy. The lithotomy position is used for transvaginal examination. In obese people, imaging can be improved by placing the transducer on the side instead of the midline while the patient is lying on the side. Transvaginal probe is also useful in these patients.
Conclusion: Clinical researchers have achieved technological advances such as instant imaging, color and power Doppler, transvaginal ultrasound, and three-dimensional and four-dimensional imaging to improve the examination and management of patients in various areas such as assessment of fetal growth and health, screening. Fetal anomalies, and ultrasound-guided procedures are an essential component in fetal treatment. (trisomy 18), early growth delay and bradycardia can be detected in 11-13 weeks. Also, the nasal bone is absent in 55% of cases and 75% have a single artery in the umbilical cord. In Pato's syndrome (trisomy 13), 70% of fetuses have tachycardia. The purpose of this screening is to diagnose congenital abnormalities and other symptoms of chromosomal abnormalities and other syndromes. Therefore, this ultrasound examination is called "genetic screening".
Keywords: Training of Nurses, Screening, Sonography

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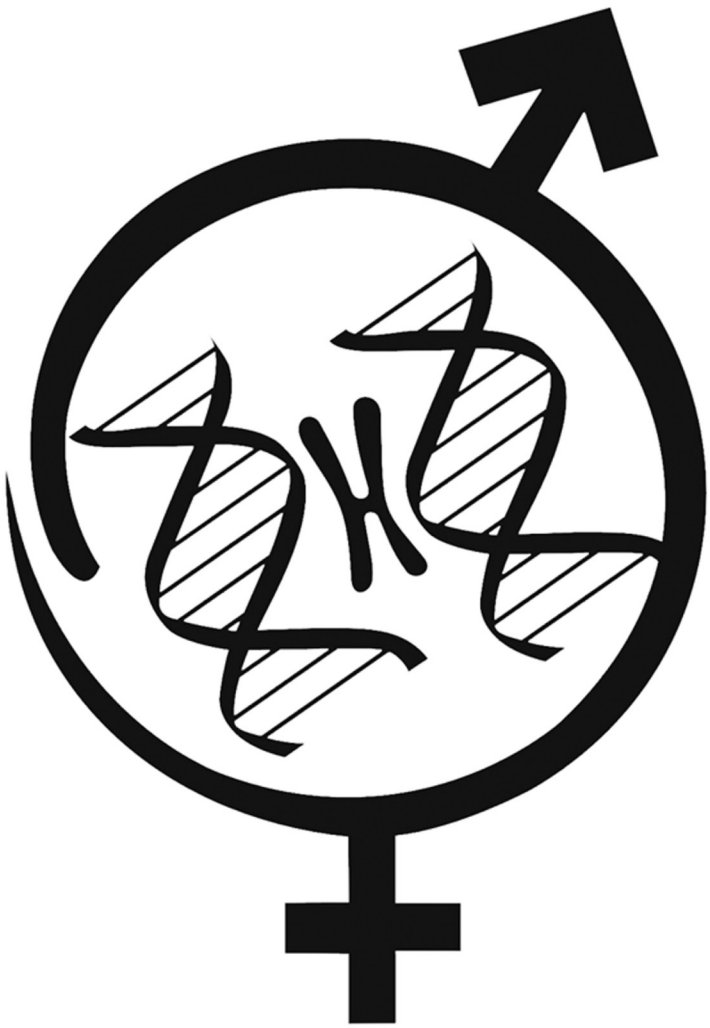

Royan Institute

Cell Science Research Center

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Congress Chairperson

Leila Taghiyar

On behalf of the organizing committee, it is my great pleasure to welcome you to the "20th International hybrid Congress on Stem Cell Biology and Technology (ICSCBT), 28-30 August 2024, Tehran, Iran".

The ICSCBT is an international annual gathering in stem cell research and regenerative medicine covering both basic and translational contents in Iran. So, the brightest thoughts in stem cell research from worldwide, and disciplines will discuss impediments, and innovation, and share recent cutting-edge research in the field of stem cells.

Royan Institute was established in 1991 by the late Dr. Kazemi Ashtiani. As a pioneer Institute, the Royan Institute for Stem Cell Biology and Technology (RI-SCBT) embraced stem cell basic and translational studies, developmental biology, and regenerative medicine.

We kindly invite you to join us and invited scientists from around the world at ICSCBT 2024 for a 3-day program featuring the year's most significant new developments in the field, in the historical/ multicultural city of Tehran.

Kind regards
Leila Taghiyar, PhD
Chairperson of 20th Royan International Virtual Congress on Stem Cell Biology & Technology

Invited Speakers

Is-1: Empowering Parents for Early Childhood Development: A Journey with Kidora

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Kidora is a pioneering startup committed to empowering parents and enhancing early childhood development through scientific solutions. The first five years of a child's life are crucial for brain development, and Kidora aims to bridge the gap between busy parents and their children's need for enriched experiences during this formative period. By collaborating with experts and offering expert-backed programs and resources, Kidora provides parents with the knowledge, tools, and confidence to nurture their child's mental development effectively.

Is-2: Immune Cell Therapy: Side Effects, Risks and Limitations in Cancer

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Immune cell therapies, particularly Chimeric Antigen Receptor T-cell (CAR-T) therapies, have revolutionized the treatment landscape for various hematological malignancies and hold promise for solid tumors. Despite their remarkable efficacy, these therapies are accompanied by significant side effects, risks, and limitations that necessitate careful consideration and management. CAR-T cell therapy has demonstrated substantial success in treating certain cancers, but it also poses risks of severe toxicities. Common adverse effects include cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). CRS, characterized by fever, hypotension, and multi-organ dysfunction, occurs due to massive cytokine release following CAR-T cell activation and proliferation. ICANS, presenting as encephalopathy, seizures, and cerebral edema, represents another major toxicity that can significantly impact patient outcomes. Additionally, CAR-T cell therapy is associated with unique cardiac toxicities, as detailed in recent studies. Adverse cardiac effects, such as arrhythmias, cardiomyopathy, and heart failure, necessitate vigilant cardiac monitoring and management strategies. The long-term outcomes of CAR-T cell therapy also reveal persistent cytopenias, hypogammaglobulinemia, and secondary malignancies, highlighting the need for ongoing surveillance and supportive care. The review further explores the limitations of CAR-T cell therapy, including antigen escape, tumor heterogeneity, and limited efficacy in solid tumors. The identification of target antigens and the development of strategies to overcome these challenges remain critical for expanding the applicability of CAR-T cell therapies. Emerging data from clinical studies underscore the importance of balancing efficacy with toxicity management. Strategies such as optimized CAR design, early intervention for toxicities, and combination therapies are being investigated to enhance the safety and effectiveness of CAR-T cell treatments.

Is-3: Liver Gene Therapy Platform for Therapeutic Induction of Immunological Tolerance in Type 1 Diabetes

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The liver gene therapy platform for Type 1 Diabetes (T1D) leverages the liver's inherent tolerogenic properties by targeting hepatocytes with lentiviral vectors (LVs) to induce antigen-specific tolerance. By delivering genes encoding the insulin B chain 9-23 (InsB9-23), an immunodominant T cell epitope, to hepatocytes, the therapy promotes the generation of both effector T cells and FoxP3(+) regulatory T cells (Tregs), which prevent islet immune cell infiltration and protect against T1D. Combining this gene therapy with a suboptimal dose of anti-CD3 monoclonal antibody (mAb) has shown efficacy in reversing T1D in nonobese diabetic (NOD) mice. The antigen-specific nature of the protection is confirmed by the ability of splenocytes from treated mice to halt diabetes development in other mice. Integrase-defective LVs (IDLVs) offer a safer alternative by mitigating concerns about insertional mutagenesis while still effectively protecting against T1D. This approach aims for long-term immune tolerance without continuous therapy, presenting a promising strategy for managing autoimmune diseases. Future directions include optimizing vector design and delivery methods for broader applications in other autoimmune diseases, allergies, and organ transplantation, with ongoing research focused on facilitating the transition from preclinical models to human trials.

Is-4: Biomimetic Smart Theranostic Platforms

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Tailored drug delivery system-based nanocarriers have been intensively studied and developed for use in clinical oncology. Currently, the fundamental mechanism of tumor-specific nanocarrier uptake is achieved through increased permeability and retention effects. However, the immune system recognizes and eliminates the majority of nanoparticles, thereby limiting their therapeutic use. Therefore, it is crucial to develop safer and more effective methods. One of the most promising platforms in this context is biomimetic nanoparticles decorated with bioactive membranes. These nanoparticles versatility as well as the adaptability of NPs along with the intricacy and utility of cell membranes could lead to significant changes to the tumor microenvironment. In our laboratory, we actively worked on biomimetic drug delivery systems. For instance, we fabricated a platform by combining a cationic lipid, 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP) with mesenchymal stem cell membrane (MSCM) to produce a positively charged

hybrid vesicle. The prepared hybrid vesicle was used to condense BIRC5 CRISPR/Cas9 plasmid for survivin (*BIRC5*) gene editing against melanoma. In another study, MSCs-derived exosomes were used as carrier for oxygen and doxorubicin delivery to tumor site. As an approach, oxygen delivery could provide tumor oxygenation, enabling ultrasound imaging. In this regard, the surface of the exosomes was also coated with a Sgc8-c aptamer against protein tyrosine kinase (PTK-7). Furthermore, by different cells membrane coating strategies, multimodal inorganic nanoparticles were prepared for cancer treatment and imaging. Based on our investigation, the application of biomimetic vehicles provides a potentially safe strategy for cancer therapy and has great potential for clinical translation.
Keywords: Biomimetic, Exosome, Mesenchymal Stem Cell Membrane, Nanobubble

Is-5: Glioblastoma Gene Therapy: Using Cocktail Constructs

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Glioblastoma multiforme (GBM) is a highly aggressive and deadly brain cancer that remains incurable with standard treatments. MicroRNAs (miRNAs) hold significant promise for gene therapy due to their ability to regulate multiple target genes simultaneously. Our research identified several miRNAs, which inhibit the cell cycle and induce apoptosis in glioblastoma cells. These miRNAs have predicted target genes across various signaling pathways, analyzed through bioinformatics tools, and validated by experimental methods such as luciferase assays, Real-Time PCR, and western blotting. Treating brain tumors, particularly GBM, remains challenging due to the lack of efficient drug delivery systems. However, the combined use of microRNA and the suicide gene (SG), along with the prodrug, induced cytotoxicity in glioma cells in vitro. Overexpression of SG/miR in the presence of prodrug significantly enhanced apoptosis and caused cell cycle arrest in these cells. Our findings demonstrate the effectiveness of drug delivery using engineered apoptosis-inducing exosomes and highlight the improved apoptosis achieved through this combination approach. In animal models, apoptosis induction and tumor growth inhibition were significantly greater in those carrying SG/miR compared to the control group.
Keywords: Glioblastoma Multiforme, microRNAs, Gene Therapy, Cytosine Deaminase

Is-6: Stem Cells, Nanotechnology and Regenerative Medicine

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Integration of new developing sciences like biomedicine with other engineering and biomaterial sciences promoted the development of nanotechnology and regenerative medicine with potential to revolutionise modern medicine leading to non-invasive and more efficient treatments.
Nanotechnology plays a crucial role in enhancing the effectiveness of stem cell therapies. Nanomaterials can be engineered to create scaffolds that mimic the natural extracellular matrix, providing a supportive environment for stem cells to grow and differentiate. These scaffolds can be designed to release growth factors and other signalling molecules in a controlled manner, promoting the development of specific cell types needed for tissue regeneration.
Nanoparticles can also be used to deliver drugs directly to stem cells or the site of injury, improving the efficiency of regenerative treatments. For example, nanoparticles can be loaded with drugs that promote stem cell survival and differentiation, ensuring that the stem cells function optimally once they are transplanted into the body.
Anyhow nanotechnology also enhances the ability to image and track stem cells in the body. Nanoparticles can be designed to be visible under various imaging modalities, such as MRI or fluorescence microscopy. This allows researchers and clinicians to monitor the distribution, migration, and integration of stem cells in real-time, providing valuable insights into the effectiveness of regenerative therapies.
In regenerative medicine, tissue engineering involves creating bioengineered tissues and organs that can replace damaged ones. Nanotechnology contributes to this field by providing materials that can be used to construct these tissues. For instance, nanofibers can be used to create scaffolds that support the growth of stem cells into functional tissues, such as skin, cartilage, or even more complex organs.
As conclusion, the integration of stem cells, nanotechnology, and regenerative medicine is a rapidly evolving field with immense potential. Future research may focus on developing more sophisticated nanomaterials that can precisely control stem cell behaviour, creating more effective and personalized regenerative therapies. Additionally, advances in nanotechnology may lead to new ways to deliver stem cells and therapeutic agents to specific tissues, further improving the outcomes of regenerative treatments. These interconnected fields are paving the way for innovative medical treatments that could revolutionize healthcare and improve the quality of life for many patients.

Is-7: Bioceramics-Based 3D Printed Scaffold for Bone Tissue Engineering

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Tissue engineering has a special place in the field of bone damage reconstruction. Meanwhile, scaffolds are one of the most important parts of a bone tissue engineering system. Although there are several conventional methods for the fabrication of bone scaffolds; recently, 3D printing techniques have been dramatically considered for this purpose due to their capabilities such as the creation of a controllable complex internal and external structure at the micro-scale. Among the 3D printing methods, the robocasting technique as an extrusion-based method is highly regarded especially for designing ceramic-based scaffolds. In this research, a three-dimensional 45S5 bioactive glass (BG) doped by zinc (3, 9, and 15%) and cobalt ions (1, 3, and 5%) /β-tricalcium phosphate (TCP) composite (50/50 wt. %) scaffold was prepared by the robocasting method. For this aim, BG and TCP were synthesized and characterized. The scaffolds were prepared by extruding the ink composed of a proper amount of BG, TCP, and required additives, then analyzed in

terms of physicochemical and biological properties. The fabricated 3D-printed scaffolds showed a hierarchical structure with regular and uniform porosities with a higher range of mechanical properties compared with the scaffolds with the same composition prepared with the foam casting method. In addition, the scaffolds doped with Zinc and cobalt showed improved mechanical strength, bioactivity, cell viability, and cell attachment compared to the undoped scaffolds. It also showed the important role of zinc and cobalt ions in improving osteogenic properties and, as a result, the expression of osteogenic genes and proteins. This study proved that the prepared scaffolds hopefully may be used for bone tissue engineering even in load-bearing defects.

Is-8: Personalized Medicine: from Molecular Phenotyping to Regenerative Medicine

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Personalized medicine has emerged as a promising approach in the treatment of a wide range of human diseases, aiming to tailor medical decisions to the individual characteristics of each patient. Initially, the focus lies on molecular phenotyping techniques which have revolutionized diagnosis by unraveling the molecular intricacies underlying diseases. These techniques, mainly next-generation sequencing (NGS), enable the identification of specific molecular signatures and biomarkers, paving the way for targeted therapies tailored to individual patients. It is worth noting that the integration of bioinformatics and artificial intelligence will cast a flash of light on the complex molecular datasets to predict treatment responses with unprecedented accuracy.
Moreover, the advent of regenerative medicines has opened new horizons in personalized medicine, encompassing innovative strategies such as cell-based therapies, gene therapies, tissue engineering, and organoids. These approaches hold immense potential for generating patient-specific models to elucidate disease mechanisms, screen drug candidates, and ultimately, engineer personalized therapies with enhanced efficacy and safety profiles. In summary, this presentation underscores the transformative impact of personalized medicine in human diseases, highlighting its journey from molecular phenotyping to the frontier of regenerative medicines, and emphasizing its profound implications for improving patient outcomes in the era of precision medicine.

Is-9: Requirements of stem cell-mediated skeletal muscle regeneration

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Contraction of skeletal muscles is mediated by syncytial myofibers, containing a highly specialized contractile apparatus maintained by large numbers of post-mitotic myonuclei. Adult skeletal muscle fibres are terminally differentiated and cannot be replenished without the help of muscle stem cells (MuSC), closely attached to myofibers and situated below the basal lamina. We have studied the transcriptional networks necessary to generate and maintain muscle cells from uncommitted mesodermal progenitor cells during embryonic development. Furthermore, we investigated the requirements of stem cell-mediated skeletal muscle regeneration. We discovered that the H4K20 methyl transferase Kmt5b is essential to maintain a high level of heterochromatin in muscle stem cells (MuSCs), whose depletion promotes exit of MuSCs from quiescence. We also found that loss of heterochromatin in Kmt5b-deficient MuSCs leads to aberrant transcription during S-phase, facilitating transcription replication collisions and genome instability. We concluded that transcriptional regulation of chromatin modifiers controlling heterochromatin formation is decisive to keep MuSCs in a quiescent state and to maintain nuclear architecture. Thus, we searched for transcription factors or transcriptional co-factors that may play a role in the regulation of chromatin modifiers in MusCs, Surprisingly, we identified TAF4A, primarily known as a subunit of the general transcription factor TFIID, as critical for genome stability and quiescence of MuSC. TAF4A is necessary for expression of Kansl2, which together with MOV is part of the non-specific lethal (NLS) complex, acetylating nuclear lamin A/C. Impaired acetylation of lamin A/C decreases stiffness of MuSC nuclei and disrupts the nuclear architecture. The subsequent loss of heterochromatin and MuSC activation, in combination with pronounced genomic instability, activates MuSCs and impairs MuSC proliferation. Furthermore, we found that Piezo1, a mechanosensitive ion channel, keeps MuSCs in a quiescent state and prevents senescence. Inactivation of Piezo1 results in compensatory up- regulation of T-type voltage-gated Ca2+ channels, leading to increased Ca 2+ influx, which strongly induces NOX4 expression via cPKC. Elevated NOX4 expression increases ROS levels and DNA damage, causing P53-dependent cellular senescence and cell death of MuSCs. Pharmacological inhibition of P53 in Piezo1-deficient mice abrogates increased senescence of MuSC and normalizes muscle regeneration. Reduced mechanosignaling due to decreased physical activity during aging may contribute to the increase of senescent cells and the decline of MuSC numbers in geriatric mice and humans.

Is-10: Reprograming the heart for regeneration

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Ischemic cardiomyopathy is the single largest cause of death in countries of all income groups. Remarkable progress in the treatment of acute myocardial infarctions has improved the outcome but the loss of myocardial tissue eventually leads to heart failure, showing an alarming increase in recent years. Unfortunately, the heart is a notorious non-regenerative organ, lacking dedicated stem cells, which complicates regenerative therapies. Terminally differentiated adult cardiac muscle cells only show a very low renewal rate in mice and man, insufficient for efficient organ regeneration after massive insults. In contrast, cardiomyocytes are still dividing during fetal and neonatal stages, permitting heart regeneration. This capacity that is lost shortly after birth concomitant with cardiomyocytes maturation, including a profound switch of the cellular metabolism. Mammalian cardiomyocytes become filled with sarcomeres and mitochondria, essentially creating pseudo-crystalline structures that

prevent cytokinesis. Reversal of this process, essentially a re-winding of the developmental program, may re-enable division of cardiomyocytes for replacement of lost contractile tissue and successful organ regeneration.
We have pursued several different strategies to rewind the development program for stimulation of heart regeneration. We found that microRNAs of the miR-1/133a family are instrumental for suppression of two crucial regulatory circuits controlling postnatal cardiomyocyte proliferation and dedifferentiation, the FGFR and OSMR pathways. Concomitant inactivation of both miR gene clusters in postnatal cardiomyocytes activates expression of cell cycle regulatory genes and cell-cycle re-entry of adult cardiomyocytes. We also found that heart-specific expression of OSKM (Oct4(O), Sox2(S), Klf4(K) and c-Myc(M)) converts cardiomyocytes to a fetal-like state, conferring regenerative capacity to adult hearts. Short-term OSKM expression before and during myocardial infarction ameliorates myocardial damage and improves cardiac function, demonstrating that temporally controlled dedifferentiation and reprogramming of cardiomyocytes facilitates heart regeneration. Inactivation of the miR-1/133a gene family as well as short-term cardiomyocyte-specific expression of OSKM reprograms heart metabolism from oxidative phosphorylation to more anaerobic energy-producing pathways. We therefore asked whether abrogation of fatty acid oxidation (FAO) in cardiomyocytes is sufficient to induce cardiomyocyte de-differentiation, proliferation and eventually heart regeneration. We discovered that inhibition of FAO after ischemic injury nearly full restores cardiac function, which is caused by accumulation of α-ketoglutarate, leading to activation of the α-ketoglutarate-dependent lysine demethylase KDM5. Activated KDM5 demethylates broad H3K4me3 domains in genes that drive cardiomyocyte maturation, lowering their transcription levels and shifting cardiomyocytes into a less mature state, promoting proliferation. Our studies provide fascinating perspectives for future clinical applications.

Is-11: Computational and Experimental Cardiac Flow Assessment: *In vitro*, *In vivo*, Clinical

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Cardiovascular diseases often manifest under disturbed hemodynamics conditions. Hence it is important to assess disturbed hemodynamics under clinical settings as well as for *in vitro* and *in vivo* experiments as part of cardiovascular research. In our studies, we focus on mechanobiology of severe cardiac diseases such as aortic aneursyms, aortic valve disease and cardiomyopathy, where we combine several bioengineering approaches including computational fluid dynamics modeling, and machine learning, to investigate progression of the conditions as well as for advancement of therapies. For clinical investigation, we investigate rupture risk assessment of aortic aneursysms, and predictive modeling of transcatheter aortic valve replacements. For *in vitro* studied, we develop micro pump systems enabling dynamic culture of cardiac cells within microfluidics system. For *in vivo* experiments, our main animal model is Zebrafish embryo due to its transparent nature enabling visualization and assessment of blood flow. In this lecture, I will be presenting our latest findings on cardiovascular mechanobiology.

Is-10: Nanostructures as Novel Drug Delivery Systems: Fundamental Issues and Market Perspectives

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Rassoul Dinarvand, received his Pharm.D. from University of Tehran in 1988. He then successfully completed his Ph.D. in the field of Controlled Drug Delivery Systems at the University of Manchester in 1993. He then joined Faculty of Pharmacy, Tehran University of Medical Sciences as a faculty member. He was promoted to full professorship in Pharmaceutics in 2005. He then became the Dean of the Faculty of Pharmacy at Tehran University of Medical Sciences in 2009 for 4 years.
Prof. Dinarvand has been active in the harmaceutical administration in both industry and government at the highest levels: seven years as CEO of several Pharmaceutical companies and twice as Deputy Minister of Health and Head of Iran Food and Drug Administration (8 years). He has also been involved in pharmacoeconomy and national drug policy research. However, his main research interest is in the area of nanomedicine and polymeric drug delivery systems and has published over 450 international papers in this field. In recent years he has focused on the design and application of nano-structures as targeted drug delivery systems mainly in the field of cancer treatment. His group have established several knowledge-based companies active in this field. He is the founder and director of Nanotechnology Research Centre, Tehran University of Medical Sciences.

Is-12: Checkpoint Adaption by Cyclin-Like Proteins Promotes Susceptibility for more Aggressive, Drug-Resistant Breast Tumours

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Senescence and quiescence are mechanisms used to maintain proper balance of cell populations and to protect cells from overgrowth or growth during unfavourable conditions. Overriding this protective event permits uncontrolled cell growth in cancer. At a molecular level senescence/quiescence is enforced through the inhibition of the cell cycle at well-established checkpoints. Healthy mammary cells overcome checkpoint arrest to re-enter the cell cycle and expand cell populations throughout development and during changing environmental conditions. Dissecting the reversibility of this checkpoint may reveal essential molecular mediators in the initiation and progression of cancer. Our lab has demonstrated that an atypical cyclin-like protein, Spy1 is capable of bypassing senescent and quiescent checkpoints throughout development, following DNA damage and during aging in a number of different cell types. Using transgenic mouse models we have established that elevated levels of this protein expands normal stem cell populations and renders the organism susceptible to breast, brain and liver cancers. Human patient data demonstrates that this protein is found at

elevated levels in each of these cancers. Manipulating levels in patient derived samples we demonstrate that even advanced tumours can be sensitized to chemotherapy approaches dependent upon cell cycle arrest. This presentation will describe a negative feedback loop between Spy1 and the tumour suppressor p53; whereby p53 keeps overall levels of the Spy1 protein in check. When elevated, Spy1 is capable of overriding the cell cycle and apoptotic effects of p53. In the face of DNA damage, damage will accumulate. Interestingly, in stem cell populations elevated levels of Spy1 promotes the reprogramming of cell populations to a more primitive state – even demonstrating hallmarks of pluripotency.
This presentation will also highlight the importance of understanding the role of these atypical proteins in driving breast cancer stem cells and contributing to the aggressiveness of disease. We find that reducing levels of this protein in human breast cancers reduces stemness properties and sensitizes breast cancers to select drug treatments. This presentation will focus on our data to date to discover the mechanism by which Spy1 aids in breast cancer stem cell expansion and drug resistance and to demonstrate a potential strategy by which we may target this pathway therapeutically.

Is-13: Harnessing The Potential of SATB Family Proteins as A Novel Molecular Target for Cancer Therapy Using Statins

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Colorectal cancer is the second leading cause of cancer-related deaths worldwide, highlighting the need for improved treatments and advanced molecular research. A recent therapeutic approach focuses on repurposing drugs to target dysregulated pathways involved in tumorigenesis. Among these, statins, commonly known for lowering cholesterol, have attracted attention for their potential anti-cancer properties. Here, we provide direct evidence for the same by assessing the impact of statin treatment on lipid, transcript, and protein levels. Our findings reveal that statins specifically target key components of the Wnt/β-catenin pathway, a major factor in adenoma formation, including the Special AT-rich Binding protein (SATB) family proteins. While SATB1 is recognized as a regulator of tumorigenesis, particularly under Wnt signaling, SATB2 appears to exert an opposing role. We demonstrate that statin treatment reciprocally alters the expression pattern of these proteins. Furthermore, a human clinical trial evaluating statins as an anticancer therapy supports the hypothesis that differential expression of SATB proteins is crucial in tumorigenic outcomes. In conclusion, this modulation by statin treatment suggests promising new therapeutic avenues through drug repurposing.

Is-14: Development of Adoptive NK Cell Therapy for Cancer Treatment

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Recently T cell therapy has been added to the standard treatment of cancer. CAR-T cells direct against CD19 or BCMA are being administered to patients suffering from hematological tumors. Although the clinical success is clear, it comes at the cost of serious side effects that need additional treatment to contain them. We have been aiming to develop adoptive Natural Killer cell therapy as due to their different activation mechanism they are expected to cause less (severe) side effects. Over the years we have developed a GMP compliant NK cell expansion protocol using feeder cell technology in which we can obtain 30-40 billion NK cells from a starting population of 10-20 million (present in 400 ml peripheral blood). These NK cells have a activated phenotype, including CD16 expression allowing for Antibody Dependent Cellular Cytotoxicity. We have shown in cell lines, mice, human cancer organoids and primary human tumor cells that these expanded NK cells have a powerful killing capacity. They can also be genetically modified (NKG2A knock-out or CAR introduced) and show an improved killing over unmodified NK cells.

Is-15: RNA-Mediated Epigenetic Attenuation of The Cell Senescence via Locus-Specific Induction of Endogenous SIRT1

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SIRT1, a known regulator of cellular senescence, is a therapeutic target for age related disorders and its upregulation is a strategy to improve the cell therapeutic potentials of human mesenchymal stem cell (MSCs). Knockdown of natural antisense transcripts via small activating RNAs (RNAa) is an emerging approach for safe and locus specific gene regulation. We have recently identified a natural antisense transcript at human SIRT1 locus (SIRT1-NAT), the expression of which shows a negative correlation with that of SIRT1. To test the hypothetic upregulation of SIRT1 via knockdown of SIRT1-NAT, in this study we designed a single stranded oligonucleotide (SIRT1-antagoNAT) against the antisense transcript, transfection of which efficiently knocked down the SIRT1-NAT and induced SIRT1 transcription in human MSCs. In addition, activation of SIRT1 transfection via knockdown of SIRT1-NAT in human MSCs enhanced their proliferation and differentiation potentials, reduced senescence associated β-galactosidase activity and reversed the senescence associated molecular alterations. Our findings introduce an RNAa mediated approach for epigenetic induction of endogenous SIRT1 and the consequent attenuation of senescence. Further studies should evaluate the therapeutic potentials of this approach against various age related disorders.

Is-16: Boost Brittle Bones Before Birth: A Clinical Trial on Stem Cell Transplantation for Treatment of Osteogenesis Imperfecta

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Objectives: Severe osteogenesis imperfecta (OI) can be diagnosed before birth. No efficient therapy is available for OI, but pre-clinical and initial clinical data show that mesenchymal stem cells (MSCs) could be used to ameliorate OI. BOOSTB4 is an open label phase I/II trial evaluate the safety and efficacy of postnatal or pre- and postnatal intravenous administration of first trimester human allogeneic fetal liver MSCs as a treatment of OI type 3 or severe type 4 with glycine substitutions in COL1A1/COL1A2.
Materials and Methods: The trial's primary endpoint is safety and tolerability of intravenous administration of MSCs to the infant and fetus, and safety for the pregnant woman. The secondary efficacy endpoints are fracture frequency, time to fracture, growth, bone mineral density, biochemical bone turnover and clinical OI status. Exploratory endpoints are quality of Life, paracrine and immune cell effects, and donor cell engraftment. The treatment groups are compared to matched historical controls (EudraCT-number: 2015-003669-60, ClinicalTrial.gov: NCT03706482).
Results: The trial is performed in Sweden (dosing and follow-up) and the Netherlands (follow-up) and includes children from 7 European countries. Each child received 4 doses at 4-month intervals. In total 67 postnatal doses and 3 prenatal doses have been administered to 18 children. The subjects underwent inpatient monitoring at each dose until 48 (dose 1–2) or 24 (dose 3–4) hours. Until the 12-month follow-up no significant short-term adverse reactions have been identified in the infant, pregnant woman or fetus. The last primary follow-up at 6 and 12 months after the last dose was performed mid-January 2024, and the efficacy will be evaluated under 2024. The subjects are followed yearly for 8 further years.
Conclusions: To date, the subjects have received all doses of MSCs with no short-term complications recorded. The primary follow-up until 12 months after the last dose has been performed, and the efficacy is now summarised. The BOOSTB4 project has received funding from the EU Horizon 2020 research and innovation programme (Grant No 681045), the Swedish Research Council and Region Stockholm.

Is-17: Industrial Development of Advanced Therapy Medicinal Products

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Advanced therapy medicinal products (ATMPs) are divided into four distinct types: GTMPs, SCTMPs, TEPs, and the combined ATMPs (cATMPs). GTMPs are products directly related to therapeutic, prophylactic, or diagnostic effects with a recombinant nucleic acid sequence. SCTMPs are products that contain substantially manipulated cells or tissues, or the cells or tissues not intended to be used for the same essential function(s) in the recipient and the donor. TEPs are engineered cells or tissues that have the properties of regenerating, repairing, or replacing human tissue, all in accordance with the medicinal products general definition and finally, cATMPs comprise another type of these products and contain one or several medical devices that are an integral part of the GTMPs, SCTMPs, or TEPs. For Industrial development of advanced therapy medicinal products with specific quality criteria, in compliance with all necessary regulatory requirement, a quality management system that includes quality assurance (QA) and quality control (QC) plan should be applied in all operations. The establishment and maintenance of a quality management system could assure the long- term sustainability of the mentioned products. Furthermore, quality systems are necessary for the delivery of high-quality products to the end-user. In this regard, some quality assurance and quality control activities are interrelated, the two are defined differently. Quality assurance is an integrated system of management activities involving planning, implementation, documentation, assessment, and improvement to ensure that quality is embedded in every step of the production. Quality control is the system of technical activities that measures the attributes and performance of a process or product against defined standards to verify that the stated requirements are fully met. An essential component of quality management is a formal system for documentation and record-keeping. The documents of the quality management system ensure that the industrial production infrastructure is in compliance with regulatory and safety requirements.

Is-18: Customizable Bone Composite Scaffold Incorporating Inductive Molecules: A Novel Approach

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Bone grafting, particularly after blood transfusion, is one of the most commonly performed procedures globally, driven by the need for bone volume enhancement in dental implant surgeries. Adequate bone regeneration is often necessary before implant placement, especially in the maxillary and mandibular regions. Techniques like the sausage technique, titanium mesh, non-absorbable membranes, and the use of autogenous, allogeneic, and xenogeneic bone blocks are common methods for augmenting jawbone height and width. However, addressing large defects, particularly within the jaw, often requires extensive surgical time and can lead to complications.
Recent advances in digital dentistry and molecular biology have revolutionized bone defect reconstruction. Customized bone grafts, designed from 3D images using CB-CT scans and DICOM files, offer a significant innovation in treatment. These grafts, traditionally made from allogeneic bones, are now being complemented by customized xenogenic and synthetic blocks, optimized through 3D printing and milling technologies.
The key benefits of using customized bone blocks include reduced surgical time, decreased morbidity, and improved accuracy in graft fitting. Computer-aided design and manufacturing (CAD/CAM) technology stands out by being applicable to a wide range of grafting materials, offering advantages over 3D printing. Designing these customized structures requires careful consideration of geometric and biological factors, including the patient's jawbone volume, systemic health, medications, and defect specifics.
Moreover, the integration of additive materials such as collagen, absorbable polymers, and inductive autogenous materials

like PRF (Platelet-Rich Fibrin) enhances graft success. These materials support healing by preventing soft tissue infiltration into hard tissue and providing necessary growth factors, ultimately contributing to the effectiveness of the bone grafting process.

Is-19: How to overcome the regulatory challenges of CAR T cell therapy?

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CAR T-cell therapy has become a promising treatment for cancer, especially for hematologic malignancies, with several FDA approvals. However, this cutting-edge cancer therapy faces some significant challenges and regulatory concerns. One of the primary challenges is the complexity of the manufacturing process. CAR T-cell therapy involves extracting T cells from a patient, genetically modifying them to express chimeric antigen receptors (CARs), and then expanding these modified cells before reinfusing them into the patient. Ensuring consistency and quality can be challenging due to the complex and multi-step process, which can result in product batch variations. Furthermore, the production and distribution logistics of these therapies are complicated and require specialized facilities and strict quality control procedures Another major challenge is managing the severe side effects associated with CAR T-cell therapy. Patients may develop neurotoxicity and cytokine release syndrome (CRS), which can be life-threatening if not treated properly. These side effects require close monitoring and immediate intervention, making treatment more complex.
The FDA has raised new concerns regarding the long-term safety of CAR T-cell therapies. Recent studies have highlighted the potential risk of secondary cancers developing following treatment. The genetic modification required to produce CAR T cells raises this concern because it can inadvertently lead to genetic changes that promote the development of new cancers. Due to this, the FDA now requires lifelong monitoring of patients treated with CAR T-cell therapies and has mandated boxed warnings to inform patients of this rare but serious risk.
Moreover, the high cost of CAR T-cell therapies represents a significant barrier to their widespread adoption. Due to the high cost of production and administration, many patients are unable to receive these therapies. The FDA regulatory framework offers detailed instructions for the creation, production, and clinical testing of CAR T-cell therapies in an effort to address these issues. However, ongoing updates and case-by-case assessments are necessary due to the quick advancements in this field, which frequently surpass the regulatory framework .
In summary, while CAR T-cell therapy offers promising benefits for cancer treatment, it also poses significant challenges in terms of manufacturing complexity, side effect management, long-term safety and cost. Ongoing efforts by international regulatory authorities to address these issues are critical to ensure the safe and effective implementation of these innovative therapies.

Is-20: Decoding Early Human Development with Blastoids

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One week after fertilization, human embryos undergo the critical process of implantation into the uterus, a fundamental event vital for both embryonic and adult life. However, the intricate nature of this process presents significant challenges for direct investigation. In our study, we elucidated how stem cells serve as a valuable tool for modeling early human development through the creation of blastoids. By culturing human pluripotent stem cells (hPSCs) in PXGL medium and inhibiting the Hippo, TGF-β, and ERK pathways, we successfully generated blastoids with an efficiency surpassing 70%. These blastoids faithfully represent the three lineages (trophectoderm, epiblast, and primitive endoderm). Blastoids spontaneously establish the first axis and show directional attachment capabilities to endometrial cells. Furthermore, they faithfully recapitulate the morphological and developmental trajectory of the blastocyst, generating transcriptionally accurate preimplantation-stage cells. As a reliable and efficient model, blastoids offer a viable and ethically sound alternative to the utilization of human embryos in the study of implantation and early development.

Is-21: The Potential Significance of Extracellular Vesicles in Osteoarthritis Treatment

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Osteoarthritis (OA) is the most prevalent rheumatic disease worldwide that leads to progressive articular cartilage destruction. Conventional therapies that include disease-modifying drugs and surgical interventions lack the capability to fully treat OA and regenerate functional articular cartilage. Although mesenchymal stem cell (MSC) therapy has been proposed to treat various degenerative diseases such as OA, abnormalities in cellular behaviors caused by in vitro cultures or donor characteristics, as well as undesired hypertrophy and ossification, limit their clinical use. Extracellular vesicles (EVs) are trophic factors secreted by various cell types in the extracellular environment that provide a novel therapeutic opportunity. The therapeutic effects of EVs have been identified as a significant factor in intercellular communication in different disease treatments, including OA. Compared to the conventional approaches in treating OA, EV therapy is a non-invasive and cell-free method. However, improving the yield of EVs and their therapeutic effects are the main challenges for clinical applications. In this talk, I will discuss EV engineering strategies in order to achieve an efficacious therapeutic outcome. The importance of co-culture engineering as an indirect EV engineering method that efficiently increases EV production and quality to achieve a more therapeutic effect for the restoration of cartilage defects with natural properties will be also covered.

Is-22: Engineering Tools to Interrogate the Host Immunity, Microbiome and Their Crosstalk

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The interplay between the commensal microbiota and the mammalian immune system development and function includes multifold interactions in homeostasis and disease. Nevertheless, many unknowns and challenges remain in disentangling microbiome-immunity interactions. Although animal models have been used extensively to analyze host-microbiome interactions and their contributions to pathophysiology, there are limited bioengineered systems available to recapitulate the complexity of the human organs and verify the host immune-microbiome interactions. Recent advances in tissue engineering, microfabrication and stem cell biology have enabled the development of more sophisticated systems, in particular organoid cultures and organ-on-chip microfluidic models, that can provide unparalleled independent control over biomechanical, biochemical, and cellular parameters, and faithfully recapitulate the cellular microenvironment and tissue function and advance personalized medicine. Such systems, along with recently emerged sampling microneedle approaches that enable the longitudinal, in situ immune monitoring of human patients, are great tools to identify the immune-microbiome interactions in different human conditions. Here, I highlight the human intestine chip we have developed to establish stable complex community of living human aerobic and anaerobic commensal gut microorganisms in direct contact with living, mucus producing human intestinal epithelium in vitro. In addition, I will explore the application of our developed sampling microneedle skin patch platform, which allows for noninvasive and longitudinal monitoring of immune responses in various scenarios. While there are currently limited advanced bioengineered methods exist to study the human complex host-microbiome crosstalk, I present novel concepts for harnessing these technologies to construct advanced models that effectively simulate and capture the complex dynamics of long-term host-microbiome interactions. We will use these engineered tools to further understand the mechanistic causal relationships between commensal microbiota and host innate and adaptive immune systems in infectious diseases, autoimmunity and cancer.

Is-23: AI in Healthcare: Bridging the Gap Between Innovation and Clinical Impact

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In this 15-minute talk, I will explore the transformative potential of AI in healthcare, focusing on its role in early disease detection, personalized medicine, and operational efficiency. The discussion will highlight how AI-driven tools are not just improving patient outcomes but are also enhancing the capabilities of healthcare professionals. Drawing on real-world examples, including Connectivity's cognitive assessment technology, I will address the challenges of integrating AI into clinical practice, such as data privacy, regulatory hurdles, and the need for robust clinical validation. The talk will conclude with a vision for the future, emphasizing the importance of collaboration between AI developers, clinicians, and policymakers to ensure that AI innovations translate into meaningful improvements in healthcare delivery.
Key Topics to Cover:
1. The Role of AI in Early Disease Detection: How AI is enhancing early diagnosis, with examples from brain health and other domains; The potential for AI to detect subtle changes that may be missed by traditional methods.
2. Personalized Medicine: AI's impact on tailoring treatments to individual patients; How AI models can analyze vast amounts of data to predict patient responses to therapies.
3. Operational Efficiency: AI applications in streamlining hospital operations, reducing errors, and optimizing resource allocation. Case studies on how AI is reducing the burden on healthcare providers.
4. Challenges and Opportunities: The importance of data privacy and security in AI healthcare applications; Navigating regulatory landscapes to bring AI innovations to market; The need for rigorous clinical validation to build trust and adoption among clinicians.
5. Future Outlook: The evolving role of AI in healthcare and its potential to democratize access to quality care.

Is-24: Transposon-Mediated Genetic Methods in Zebrafish and Their Applications to The Study of Neuroscience

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For the past decade, we have developed Tol2-transposon mediated genetic methods, including transgenesis, gene trapping, enhancer trapping and the Gal4FF- UAS system in the model vertebrate zebrafish. By using these methods, we have performed a large-scale genetic screen, and generated more than 2,000 transgenic lines that expressed Gal4FF, a synthetic Gal4 transcription activator, in specific cells, tissues, and organs. Further, we constructed a web-based database zTrap (https://ztrap.nig.ac.jp/ztrap/), in which transgenic fish can be searched based on their expression patterns and transposon integration sites. Thus, our transgenic fish resource has been powerful for the study of developmental biology, neuroscience, and disease modeling.
We have investigated functional neuronal circuits controlling the larval and adult behaviors by employing the transgenic fish resource. First, we aimed to disclose how visual stimuli evoke appetite during the larval prey capture behavior. By using brain-specific Gal4FF fish and the UAS:GCaMP effector fish, we successfully imaged the neuronal activity during prey capture in real-time. We found that the visual stimulus recognized by the optic tectum activates the hypothalamic feeding center through prey detector neurons in the pretectum. Second, we aimed to identify functional equivalents of the mammalian amygdala and hippocampus, that play crucial roles in processing emotional memory or episodic and spatial memory, respectively. We constructed fish carrying UAS:botulinum-neurotoxin gene, and inhibited the activity of various Gal4-expressing neurons in the brain. We found a neuronal population in Dm (dorsomedial telencephalon) is essential for fear conditioning in zebrafish, and a

neuronal population in Dl (dorsolateral telencephalon) is essential for the episodic (trace fear conditioning) and spatial learning. Thus, we propose these neuronal populations are functional equivalents of the amygdala and hippocampus in teleost.

Is-25: Impact of Culture Conditions on Cytokine Expression in Mesenchymal Stromal Cells: Implications for Regenerative Medicine

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Objective: Multipotent mesenchymal stromal cells (MSCs) have garnered significant interest in regenerative medicine due to their remarkable immunomodulatory properties and potential therapeutic applications across a spectrum of pathological conditions. Emerging evidence suggests that the efficacy of MSCs-based therapies is intricately linked to the cellular microenvironment, including the tissue source from which MSCs are derived and the culture conditions in which they are maintained. In this study, we aimed to compare secretome among MSCs derived from different sources and cultured in different conditions.
Materials and Methods: MSCs were isolated from diverse tissue sources, including adipose tissue (AT), bone marrow (BM), gingiva (G), placenta (PL), and umbilical cord (UC). These MSCs were cultured either as monolayers (2D) or cell spheroids (3D). Immunophenotype and osteogenic, chondrogenic, adipogenic differentiation potential were evaluated. Cytokines and growth factors in MSCs-conditioned media (CM) samples were analyzed utilizing xMAP technology (Luminex, USA).
Results: MSCs expressed the mesenchymal markers (CD105, CD73, CD90, CD44) and demonstrated trilineage differentiation capacities. Analysis of cytokine expression in MSCs-CM unveiled distinct cytokine profiles among MSCs derived from different sources and culture conditions. Notably, UC-MSCs-CM exhibited elevated levels of cytokines compared to CM from other MSC sources. Moreover, comparison between 2D and 3D cultures of UC-MSCs revealed differences in cytokine expression, with 3D cultures demonstrating higher levels. These findings emphasize the influence of culture conditions on the cytokine profile of MSCs.
Conclusion: The observed differences in cytokine expression highlight the importance of optimizing culture conditions for MSCs-based therapies. UC-MSCs, particularly from 3D cultures, show promise as a potent source of cytokines with potential applications in treating inflammatory and tissue repair disorders. Further research into the specific mechanisms underlying these cytokine differences and their therapeutic effects is warranted to harness the full potential of MSCs-based therapies. The study was supported by the Academic leadership program Priority 2030 proposed by Sechenov University.
Keywords: Conditioned Media, Cytokines, Mesenchymal Stromal Cells, Secretome, 3D Culture

Is-26: Synthesis of Cell-based Chronic Wound Dressing with Gelatin Methacrylate (GelMA) Hydrogel for Skin Regeneration

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Objective: Effective wound healing requires quick wound closure, less scarring, and accelerated angiogenesis. Hydrogels stand out among dressing materials and offer a number of benefits that promote wound healing because of their high biocompatibility, structural resemblance to the extracellular matrix (ECM), and highwater content. Despite the fact that hydrogel is frequently employed in tissue engineering, it is not the best option because of its weak mechanical and vascularization capabilities. Here, we coated GelMA hydrogel on silicone polymer. GelMA hydrogel mixed with amniotic membrane extract increased angiogenesis properties of the wound area.
Materials and Methods: A 1:1 mixture of pharmaceutical-grade two-component A&B silicone was prepared. It was then put into polymethyl methacrylate (PMMA) molds, heated for 5 minutes at 120 °C to form a silicone film. Pharmaceutical grade GelMA (10% concentration) was prepared, then 0.1 mg/ml amniotic membrane extract, Irgacure 2959 were added, the resulting solution was poured onto the silicone film and exposed to UV (at 365 nm) light for 1 minute. Structural tests including SEM and tensile test were carried out to prove the strength of the scaffold used. The amount of tube formation was examined in order to study the angiogenesis.
Results: The results of this study showed that the silicone structure has no pores and its surface is completely smooth and has no cracks in the structure. The GelMA 10% structure has suitable pores with a size of 65 micrometers. Biodegradability tests showed that the thin layer of GelMA starts to degrade and release the amniotic membrane extract at the wound site after 24 hours. The in vitro tests showed that the blood cells in the vicinity of the prepared dressing had a very good tube formation after 48 hours compared to the sterile gas control sample.
Conclusion: Because of the lengthy treatment times, delays in healing of chronic wounds, they have an impact on quality of life and on costs. Today, silicone wound dressings are widely used in the repair of chronic wounds. It can be concluded that skin tissue engineering using GelMA to the silicone surface and amniotic membrane extract can speed up the healing of wound while preventing exudate buildup and the formation of scar tissue.

Is-27: Will Be Announced

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Is-28: 3D Intestinal Organoid Platform to Test Epithelial Recovery Potential of Lead Bioactives

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We present a novel 3D intestinal organoid microarrayed high-throughput platform designed to assess the epithelial recovery potential of various lead bioactive compounds under conditions simulating intestinal barrier disruption. The platform leverages human- and canine-derived small intestinal organoids to model inflammatory conditions, such as those present in inflammatory bowel disease (IBD). Through the application of inflammatory challenges (e.g., CytoMix and IFN-γ), the system allows for real-time observation of barrier disruption and subsequent epithelial recovery. Recovery interventions with bioactives, such as NAD+ precursor and dexamethasone, were tested, showing differential effects on the restoration of barrier integrity. RNA sequencing in single-organoid resolution revealed the involvement of key signaling pathways, including WNT/β-catenin, in the recovery process. The platform's high-throughput capability, coupled with hallmark pathway analysis, enables precise quantification of bioactive efficacy, providing a robust tool for evaluating therapeutic potentials aimed at epithelial repair and recovery.

Is-29: Novel Micrornas Enabling The Maintenance and Derivation of Embryonic Stem Cells from Blastocysts

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Cells of the inner cell mass (ICM) acquire a unique ability for unlimited self-renewal during transition into embryonic stem cells (ESCs) in vitro, while preserving their natural multilineage differentiation potential. Several different pathways have been identified to play roles during ESC maintenance and derivation but the function of non-coding RNAs in this process is poorly understood. Here, we describe several microRNAs (miRNAs) that are crucial for the efficient maintenance and generation of mouse ESCs from ICMs. Using small-RNA sequencing, we identify critical miRNAs in cultured ESCs and characterize dynamic changes in miRNA expression profiles during ICM-ESC transition in a high-resolution, time-course dependent manner. We report several waves of miRNA transcription during ESC formation, to which miRNAs from the imprinted Dlk1-Dio3 locus contribute extensively. In silico analyses followed by functional investigations reveal that Dlk1-Dio3 locus-embedded miRNAs (miR-541-5p, miR-410-3p, and miR-381-3p), miR-183-5p, and miR-302b-3p promote, while miR-212-5p and let-7d-3p inhibit ESC formation. Moreover, the same miRNAs from the Dlk1-Dio3 locus promote the maintenance of the so-called ground-state ESCs by targeting differentiation-associated genes. Collectively, these findings offer new mechanistic insights into the role of miRNAs in ESC maintenance and during ESC derivation.
Keywords: microRNA, pluripotency, stemness, early embryo, ES cells

Is-30: Artificial Intelligence Biology and Reciprocal Disruption

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Advances in artificial intelligence (AI) and biology are frequently highlighted in both scientific and popular media, as each field has significantly impacted our lives. However, the future holds even greater potential due to the emerging reciprocal disruption between AI and biology. Disruption in the biological side roots in using AI tools for biological experiment automation and implicit modeling of biological systems, in addition to hypothesis generation and testing. Interestingly, the opposite direction is also originated in AI, where we have faced high energy consumption of silicon intelligence at scale, need for big data, complexity of learning tools, and most importantly have remained behind solo-AI. These limitations have triggered research in Bio-AI and Neuro-AI where we seek biological and social solutions to overcome the current limitations of AI toward creation of artificial general AI.

Is-31: Prefrontal Control of Visual Signals

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Visual attention is an integral part of our daily life. A network of cortical and subcortical structures are involved in control of visual attention. To date, how these areas interact with each other and the chain of events giving rise to visual attention are still unclear. I will present our findings regarding the neurons and neuromodulators involved in attention and will discuss how these findings help us untangle the neural circuitry of attention.

Is-32: Do *In Vitro* Fabricated Cardiac Microtissues Replace Experimental Models in Cardiovascular Research?

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The current knowledge on the cardiovascular biology and pathology was mostly obtained by using animal models. However, there are considerable interspecies differences which hinder the application of results for human. Therefore, the two-dimensional (2D) culture systems of human cardiovascular cells intervened, in order to resolve these difference. While, conventional 2D cultures proved effective in studying cardiac function using both primary culture as well as human pluripotent stem cell-derived cardiovascular lineage cells, these systems exhibit inherent limitations such as lack of three-dimensional (3D) cell-cell and cell-extracellular matrix interaction. To address these challenges, tissue engineering technology offered the integration of biomaterials as substrates alongside cardiac lineage cells, thus creating an environment more suitable to conduct precise in vitro cardiovascular research. Natural and synthetic biomaterials were employed to establish scaffolds, forming a 3D platform for generating heart microtissue. Today, simultaneous applica-

tion of electromechanical stimulation as well as biochemical cues considerably improved the formation of a cardiac tissue-like platform for related studies. These platforms were verified with respect to structure and function in order to be used as alternatives of animal models for cardiovascular research. However, there are some challenges remaining such as appropriate vascularization and incorporation of immune cells in order to mimic the native cardiac microenvironment. These challenges are being carefully investigated and will be the priority for the next few years.

Key words: Cardiac Microtissue, *In Vitro* 3D Culture, 2D Culture

Is-33: Stem Cell-Based Human Embryo Models

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Modelling early human embryogenesis with naïve pluripotent stem cells. In early mammalian embryos, cells with identical genomes exhibit distinct gene expression patterns. In addition, human embryo development leads to the formation of complex structures by day 14 of development. Development relies on precisely orchestrated gene regulatory programs and epigenetic processes that determine cell identity and tissue architecture. The tight control of gene expression is critical throughout an organism's lifetime. In this talk, I will present our efforts to understand the role of chromatin regulators in early human development and pluripotency. In addition, I will explore the regulation of gene dosage, using X-chromosome dosage compensation as a paradigm. I will present novel data challenging existing notions about mammalian dosage compensation regulation. Additionally, I will also discuss recent advances achieved using human naïve pluripotent stem cells to model the initial two weeks of human development, including emerging stem cell-based embryo models. I will discuss how these advances revolutionize the way we study developmental epigenetics.

Is-34: Early-Stage Brain Stem Cell Therapy for Treating Progressive Multiple Sclerosis

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Advances in stem cell biology have raised great expectations that diseases of the central nervous system may be ameliorated by the development of non-hematopoietic stem cell medicines. Yet, the application of stem cells as therapeutics is challenging and the interpretation of some of the outcomes ambiguous. The initial idea that stem cell transplants work only via structural cell replacement has been challenged by the observation of consistent intercellular information exchange between the graft and the host. Sustained stem cell graft-to-host exchange of signals has led to remarkable trophic effects on endogenous brain cells and beneficial modulatory actions on innate and adaptive immune responses that ultimately promote the healing of the injured CNS. Among a few promising candidate stem cell sources, neural stem/precursor cells (NSCs) are being extensively investigated for their capacities to signal to the immune system upon transplantation in experimental CNS diseases.

The rationale for NSC therapeutics efficacy studies in people with progressive MS is based on (i) our progress in developing cell isolation and expansion technologies 1,2; on (ii) the successful demonstration of the effectiveness of syngeneic and xenogeneic NSC grafts in a variety of transplant models of MS-like disease and lesions in small rodents and non-human primates 3-6; and (iii) the completion of the first and largest phase I clinical trial employing allogeneic, single donor, hNSC-based ACTs from a homogeneous cell source in people with SPMS paving the way for the administration of the same standardized stem cell medicinal product in future clinical trials of efficacy 7. Here I will discuss new mechanisms of cellular licensing by which transplanted NSCs counteract CNS-compartmentalised chronic inflammation in mice and potentially also in humans. By understanding the mechanisms of intercellular (neuro-immune) signalling, chronic diseases of the brain and spinal cord that include progressive MS may be treated more effectively, and significant neuroprotection and restoration of key functions may be achieved with new tailored molecular therapeutics.

Is-35: The Pantetheinase VNN1 Regulates Energy Metabolism in Acute Myeloid Leukemia Stem Cells By Controlling The Availability of Coenzyme A

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To enhance the clinical outcomes for patients with acute myeloid leukemia (AML), targeting leukemic stem cells (LSCs) is essential. These cells are at the top of the cellular hierarchy and are the main cause of disease relapse. LSCs mainly use oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) to meet their energy needs, both of which depend on coenzyme A (CoA). Our study shows that the enzyme pantetheinase Vanin1 (VNN1) is crucial for maintaining LSCs. VNN1 is more abundant in LSCs than AML blasts and healthy hematopoietic stem and progenitor cells (HSPCs). Genetic and pharmacologic interventions revealed that VNN1 is necessary for LSC function but not for healthy HSPCs. Inhibiting VNN1 specifically targets immature leukemic cells, and high VNN1 expression is a strong predictor of poor prognosis in AMLs with primitive cellular structures. Mechanistically, reducing VNN1 levels or inhibiting its activity lowers intracellular CoA levels, leading to a shift from OXPHOS and FAO to lactate production. This change makes cells more sensitive to BCL-2 inhibition by Venetoclax. Our findings suggest that targeting the VNN1-CoA pathway could effectively disrupt LSC function, especially in some AML subtypes, and when combined withVenetoclax.

Is-36: The Evolution of Human Blastocyst Development and Implantation

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Embryogenesis is the vehicle of evolution through which genetic variations turn into heritable innovations. Human embryos differ from those of primates in their implantation strategy, exhibiting deep but fragile uterine invasion. Our laboratory has developed an embryo model known as the blastoid, which is unique in two ways: it is complete and represents the pre-implantation stage, enabling the modelling of implantation in utero. Here, we leveraged the versatility and throughput of blastoids to study the genetic properties underlying a specific features of primate clades, namely superficial and deep invasion. We used single-cell RNA and ATACseq data to reconstruct the GRN of human tissue that mediates invasion, identified essential TFs that were validated by genetic knockout in blastoids and subsequent in vitroattachment and invasion assays. Using deep learning and convolutional neural networks based on transfer learning, we identified enhancer sequences and DNA motifs that regulate these TFs. Using a comparative approach, we pinpointed clade-specific differences in enhancers that correlate with phenotypes. This functional exploration reveals aspects of genome evolution that have impacted early human embryogenesis.

Is-37: Blastoids: Modeling Blastocyst Genesis and Implantation with Stem Cells.

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The blastocyst is the early mammalian organism before implantation inthe uterus. We have promoted the self-organization of stem cells intomodels of mouse and human blastocysts, which we have namedblastoids (Nature 2018, Nature 2021). Blastoids are morphologically andtranscriptionally similar to the blastocyst and contain analogs of all threecell types that would eventually develop into the complete organism(embryonic and extraembryonic). Because blastoids are complete andmodel the preimplantation stage, they can be introduced into the uterus(mouse model) or combined in vitro with uterine cells (human model) to recapitulate aspects of the normally hidden implantation processes. Unlike blastocysts, blastoids come in large numbers and facilitate a more systematic modulation and analysis of development. As such, they represent both a scientific and ethical alternative to the use of embryos for research. Using this approach, we are investigating the genome evolution underlying species-specific aspects of blastocyst development and implantation, with the long-term goal of understanding the evolutionary basis of suboptimal human pregnancy (50% of fertilized eggs never develop). This knowledge could help solve the global health problems of family planning and developmental origin of health and disease.

Is-38: Electrically Conductive Biohybrid Hydrogels for Maturing hiPSC-Derived Cardiomyocytes and Protecting The MI-Heart Against Arrhythmia

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Introduction: Myocardial infarction (MI) causes cell death, disrupts electrical activity, triggers arrhythmia, and results in heart failure, whereby 50-60% of MI patients die from sudden cardiac death (SCD). Due to a limited renewal capacity of adult cardiomyocytes the MI-induced cardiomyocyte loss proceeds to the formation of fibrotic tissue and subsequent ventricular wall dilation. The fibrotic tissue is electrically insulating, and such a low conductivity, together with hetero-cellular interactions in the scar border zone, contribute to electrical decoupling of the remaining viable cardiomyocytes in the infarcted region, thereby promoting asynchronous ventricular contractions. The most effective therapy for SCD prevention is implantable cardioverter defibrillators (ICDs). However, ICDs contribute to adverse remodeling and disease progression and do not prevent arrhythmia. Moreover, there is a gap in designing an electrically conductive hydrogel that prevents post-MI arrhythmia and has the potential to be combined with cardiomyocytes. We developed an injectable collagen-PEDOT:PSS hydrogel that protects infarcted hearts against ventricular tachycardia (VT) and can be combined with hiPSC- cardiomyocytes to promote partial cardiac remuscularization.
Materials and Methods: We have generated an injectable collagen-PEDOT:PSS hydrogel and studied gelation kinetics, electrical conductivity as well as the hydrogel micromorphology by scanning electron microscopy. Moreover, we generated engineered cardiac tissues based on hiPSC-derived cardiomyocytes and these hydrogels and studied cell survival, microcellular organization of contractile machinery and calcium handling of these cells in the tissues for them up to 40 days. Moreover, RNA-sequencing analyses revealed a shift towards more mature cardiomyocytes within electrically conductive hydrogels without any external stimuli. Eventually we have transplanted the hydrogels in a cryoinfarction model of MI in mouse. We have assigned five groups, including sham, MI, collagen, collagen-PEDOT:PSS, and collagen-PEDOT:PSS containing hiPSC-derived cardiomyocytes.
Results: PEDOT:PSS improves collagen gel formation and results in a faster gelation. It transforms single-fibrillar collagen morphology into entangled thick helical microfibrous morphology in collagen-PEDOT:PSS and increases electrical conductivity from ~41 mS/cm for collagen to ~65 mS/cm for collagen-PEDOT:PSS. Compared to cells in collagen hydrogels, hiPSC- cardiomyocytes in collagen-PEDOT:PSS hydrogels exhibit near-adult sarcomeric length (~2 μm), improved contractility (~500%), enhanced calcium handling, and conduction velocity (~700%). RNA-sequencing data revealed an upregulation of genes associated with conduction, structure, energetics, oxidative phosphorylation and fatty acid oxidation, calcium handling, and cell-ECM interactions, indicating enhanced maturation and improved cell-matrix interactions in hiPSC- cardiomyocytes within collagen-PEDOT:PSS hydrogels. Injecting collagen-PEDOT:PSS hydrogels in infarcted mouse hearts results in a ~400% decrease in ventricular tachycardia occurrence compared to MI, reaching to the levels of healthy hearts. Furthermore, hiPSC-cardiomyocyte implementation results in improved heart function (fraction shortening: 25% vs. ~18% in MI).
Conclusion: Collectively, the here-developed hydrogel showed great potential to promote stem cell derived cardiomyocyte maturation and to protect the infarcted heart against ventricular tachycardia. Finally, collagen-PEDOT:PSS hydrogels offer a versatile platform for treating injuries in hearts and other electrically sensitive tissues.

Is-39: Mechanisms of Embryo Failure at Implantation

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Aneuploidy, the presence of an abnormal number of chromosomes, is a major cause of early pregnancy loss in humans. Yet, the developmental consequences of specific aneuploidies remain unexplored. We have explored the extent of post-implantation development of human embryos bearing common aneuploidies using a recently established culture platform. We have seen that while trisomy 15 and trisomy 21 embryos develop similarly to euploid embryos, monosomy 21 embryos exhibit high rates of developmental arrest, and trisomy 16 embryos display a hypo-proliferation of the trophoblast, the tissue that forms the placenta. Using human trophoblast stem cells, we show that this phenotype can be mechanistically ascribed to increased levels of the cell adhesion protein E-CADHERIN, which lead to premature differentiation and cell cycle arrest. We identify three cases of mosaicism in embryos diagnosed as full aneuploid by pre- implantation genetic testing. Our results present the first detailed analysis of post-implantation development of aneuploid human embryos.

Is-40: Mitochondria-Incorporated Electroconductive Alginate-Based Hydrogel

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Background Here, the angiogenesis properties of mesenchymal stem cell (MSC) mitochondria were investigated in a rat model of myocardial infarction. Material and Methods Mesenchymal stem cells were pre-treated with mitochondria-induction compounds metformin (Met; 50 μM), and dichloroacetic acid (DCA; 40 μM). After the isolation of mitochondria, the isolated particles were characterized and incorporated (~2 × 107 particles) inside the alginate (3%)-gelatin (1%) hydrogel containing 1 μM pyrrole in the presence of FeCl3. The physicochemical properties of the final composite were monitored using several assays such as FTIR, swelling, biodegradation, porosity tests, and SEM. Mitochondria-loaded hydrogels (~2 × 107 particles/100 μl) were injected into the border zone of infarcted myocardium induced by LAD ligation. After two weeks, rats were euthanized and mitochondrial uptake and angiogenesis status were monitored using histological examination. Results Data showed appropriate physicochemical (swelling rate, biodegradation, porosity) and cytocompatibility properties of alginate-gelatin pyrrole hydrogel evaluated by SEM, FTIR, and MTT assays. Flow cytometry analysis indicated high-rate purity and functionality of isolated mitochondria. Masson's Trichrome staining and ECG analysis indicated appropriate myocardial infarction in the control rats. Immunohistochemistry analysis showed the stimulation of angiogenesis in the infarcted area (vWF+ capillaries↑, and α-SMA+ arterioles↑) in rats that received mitochondria-loaded hydrogel compared to mitochondria and hydrogel groups ($P<0.05$). Data showed a significant increase in anterior wall thickness in the mitochondria-loaded hydrogel group ($P<0.05$). Conclusion Mitochondria-loaded hydrogels are suitable delivery platforms for the induction of angiogenesis and reduction of fibrosis in ischemic myocardium.
Keywords: Mesenchymal Stem Cells, Mitochondria, Electroconductive Alginate-Gelatin/Pyrrole Hydrogel, Rat Myocardial Infarction, Angiogenesis

Is-41: Dissecting The Molecular Mechanisms of Mammalian Germline Development Using Single Cell Omics Sequencing Approach

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Meiotic recombination is initiated by programmed DNA double-strand breaks (DSBs). Limited by the scarcity of fetal germ cells, the dynamics and epigenetic regulatory mechanisms of meiotic DSBs in females remain elusive. We generate the genome-wide map of mouse female DSBs at single-cell resolution. The number and high dynamic range of meiotic DSBs in individual cells rewrite previous perceptions. We prove that the sequence-specific binding of PRDM9 directly induces chromatin remodeling rather than a permissive environment around DSBs that had been created in advance. Our study covers multiple histone modifications and reveals their unique enrichment patterns and regulatory functions during DSB formation. Our results comprehensively elucidated the distribution and epigenetic regulation of meiotic DSBs in female mammals.

Is-42: Bioprinting of the made-to-order organs

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Background: The area of personalized organ fabrication is highly important in the fields of healthcare and biotechnology, providing groundbreaking solutions for challenges in organ transplantation, personalized medicine, and the management of diverse health conditions. Bioprinting is a leading-edge technology in organ engineering, presenting novel prospects in this field. The current work summarizes recent developments, challenges, and future prospects in bioprinting for personalized organ production.
Results: To ensure successful tissue production, scaffolds must mimic the specific microenvironment necessary for cell spreading, proliferation, and differentiation. The microarchitecture of the scaffold should correspond to the mechanical signals required by cells, depending on the type of tissue. Laser-based

techniques like two-photon polymerization (2PP) offer a wide range of materials for creating customized scaffolds that resemble native tissue structures, guiding cell behavior. Bioprinting methods are crucial for precise cell distribution within scaffolds, with techniques like Laser-Induced Forward Transfer (LIFT) enabling high-resolution manipulation of cells. Spheroids, the units of future tissues, can be organized into specific patterns. Extrusion bioprinting has enabled the creation of complex bioequivalents on a larger scale, specifically with spheroids, gaining traction as ideal building blocks due to their regenerative potential, physiological mimicry, and self-organizing abilities, surpassing traditional 2D cultures and cell suspensions in efficacy.

Conclusion: While significant advancements have been achieved in bioprinting technologies in recent years, each approach has its own set of constraints, such as limitations in process resolution, scalability, and the availability of compatible bioinks. Nevertheless, current progress suggests that the strengths of different methods can offset their respective weaknesses. In the future, it is probable that technologies will merge various modalities into unified platforms, incorporating innovative techniques like cell aggregate bioprinting. This integration aims to address existing technological gaps and produce tissue constructs that are scalable, structurally sound, perfusable, and exhibit enhanced biomimicry and desired functionality. The study was supported by the Russian Science Foundation (№23-15-00481).

Key words: Bioprinting, Tissue Engineering, Scaffolds, Regenerative Medicine

Is-43: Potential of Freeze Dried-Secretome From Human Wharton's Jelly Mesenchymal Stem Cells for Wound Healing

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Background: Burn injury, caused by heat, chemicals, electricity, radiation, or friction, can rapidly disrupt body homeostasis, leading to multi-organ dysfunction and potentially life-threatening injuries. Indonesia has a high incidence of burn injuries, leading to the development of new therapies for non-healing wounds. Among the most promising is stem-cell-based therapy. The secretome from human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs-Sec) offers diverse biological activities that are particularly beneficial for skin applications and wound healing, making it a key alternative in regenerative medicine. Research objective: The research investigates the application of freeze-dried and freeze-dried gel from hWJMSCs-Sec on burn injured-rats by analyzing protein expression.

Materials and Methods: Male rats were subjected to burn injuries using an instrument probe applied to the dorsal area for 10 seconds. After burn induction, freeze-dried and gel freeze-dried hWJMSCs-Sec were applied to the dorsal area once or twice daily for 16 days. Epidermal protein expression of Interleukin-1β (IL-1β), Transforming Growth Factor-β (TGF-β), Keratinocyte Growth Factor (KGF), and Collagen (COL) was measured using Immunohistochemistry (IHC). Additionally, gene expression of Epidermal Growth Factor (EGF), Tissue Inhibitor of Metalloproteinases-1 (TIMP-1), Matrix Metalloproteinase-1 (MMP-1), and COL-1A1 was analyzed using quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). Epidermal inflammation and angiogenesis were assessed using Hematoxylin-Eosin (HE) staining.

Results: On day 16, during the proliferation phase of wound healing, treatment with freeze-dried and freeze-dried gel from hWJMSCs-Sec improved wound healing by reducing IL-1β and enhancing TGF-β, KGF, and COL expression. Additionally, during the proliferation phase, these treatments increased the epidermal gene expression of EGF, TIMP-1, MMP-1, and COL-1A1. Furthermore, freeze-dried and freeze-dried gel from hWJMSCs-Sec treatments also improved inflammation and angiogenesis during the proliferation phase of wound healing. Conclusions: Freeze dried- and freeze-dried gel from hWJMSCs could improve and accelerate wound healing process.

Is-44: Ipscs-Derived Venous Endothelial Cells for Modeling Vascular Malformation and Drug Discovery

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Venous malformations (VMs) represent prevalent vascular anomalies typically attributed to non-inherited somatic mutations occurring within venous endothelial cells (VECs). The lack of robust disease models for VMs impeded the discovery of new drugs. Here, we implemented heterozygous mutation into iPSCs and devised a robust protocol for the generation of iVECs. This protocol involved the deliberate manipulation of cell cycle dynamics mediated through the retinoid signaling pathway. The mutated iVECs exhibited aberrant TIE2 signaling and formed dilated blood vessels *in vivo*, thereby recapitulating the phenotypic characteristics observed in VMs. Moreover, utilizing a deep neural network and a high-throughput DRUG-Seq approach, we performed drug screening and identified Bosutinib that effectively rescued the disease phenotype in vitro and *in vivo*. In summary, by leveraging on the genome editing and stem cell technology, we generated VM models that enabled the development of new potential therapeutics.

Is-45: Design and Development of Nano Formulated Bioacteve Component for Molecular Targeted Therapy of Cancer Stem Cells

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The development of targeted treatments for cancer and cancer stem cells (CSCs) is crucial in overcoming the challenges of recurrence and resistance to conventional treatments. This research focuses on the design and development of a nano-delivering system for a bioactive compound that targets CSCs specifically. Using nanotechnology, the bioactive component was encapsulated in a nano-delivering system to increase its stability, bioavailability, and precise targeting of CSCs. The nanoformed compound was evaluated by in vitro and *in vivo* studies to assess its efficacy in inhibiting CSC growth and disrupting the major molecular pathways involved in their self-renewal and survival. The results indicate that this nanodispersion system significantly improves the therapeutic impact of the bioactive compound, reducing the effectiveness of the CSC and overcoming chemoresistance. This innovative approach highlights the potential of combining bioactive compounds with nanosystems to target cancer therapy. By providing a new method for targeting CSCs, this research contributes to ongoing efforts to develop more effective cancer treatments and pave the way for clinical applications aimed at reducing tumor recurrence and improving patient outcomes.

Is-46: Wnt Signaling in Stem Cell Differentiation and Tissue Regeneration

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Wnt family members are secreted-type glycoproteins that orchestrate major developmental processes in the embryonic state and regulate maintenance, self-renewal and differentiation of adult mammalian tissue stem cells. Wnt/β-catenin pathway has been recently identified as one of the key players in neuronal differentiation. We showed that during trabecular meshwork-derived mesenchymal stem cells (TM-MSCs) differentiation into neuron-like cells, β-catenin protein accumulation was increased in the nucleus, indicating the increased activity of Wnt/β-catenin pathway in the time-window of neuronal differentiation. Interestingly, DKK1 transcript level was reduced dramatically in TM-MSCs during this differentiation period. In consistent with the above results, treatment of neurally induced TM-MSC with CHIR (Wnt agonist) significantly elevated the expression level of neuronal markers while treatment with IWP-2 significantly decreased these markers. These results suggest that the neuronal fate in TM-MSCs is triggered specifically by Wnt/β-catenin activation and attenuated if it is antagonized. There are also increasing evidence that Wnt/β-catenin pathway is a key regulator of neural tissue regeneration. To address this question, we fabricated an electrospun nanofibrous scaffold (ENS) containing polylactide nanofibers and lithium ion (Li) as a Wnt/β-catenin signaling activator. Our results showed that the Li loaded ENSs gradually released Li during 11 days of culture, with the range concentration that upregulate the expression of Wnt/β-catenin target genes. Moreover, the expression levels of Schwann cell markers in human adipose-derived mesenchymal stem cells (hADMSCs) cultured on these scaffolds, were notably increased compared to those in the control. Our *in vivo* assay showed that implantation of Li loaded ENSs (with or without cells) in the rat model of crush injury improved behavioral features and electrophysiological parametres and also increased number of organized regenerating axons of injured sciatic nerve. From our results, it can be implied that Li is the main element of our fabricated scaffold contributing to sciatic nerve regeneration. Li may exert its effect by activating Wnt/β-catenin signaling pathway emphasizing the role of this signaling pathway in neural differentiation and regeneration.

Keywords: Wnt/β-catenin Signaling, Neuronal Differentiation, Regeneration, Nanofibrous Scaffold

Is-47: Human Retinal Organoids for Disease Modeling

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With the significant advances in human stem cell biology and organoid medicine, currently in vitro differentiation of human retinal organoids is well established. In this talk, I will introduce its application for disease modeling for retinitis pigmentosa and retinoblastoma.

Oral Presentation

Os-1: Establishment of Bladder Patient Derived Tumor Organoid for Drug Screening

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Objective: Bladder cancer (BLCa) is one of the most cost-intensive cancers with a poor prognosis due to high recurrence. Thus, there is an urgent need for unique preclinical models recapitulating patient-specific histopathological and molecular diversity that patients could benefit from a more personalized treatment approaches. To address this need we established bladder cancer organoid as an advanced organ culture model for predicting drug response which indicate the inter-patient heterogeneity.
Materials and Methods: In our study, we establish patient derived tumor organoid cultures from different muscle-invasive and non-muscle-invasive BLCa stages and grades. The tumor organoids were characterized based on culture efficacy, gene and protein expression by RT-PCR and immunofluorescence microscopy. PDOs preserve the histological and molecular heterogeneity of the parental tumors. Drug screening pipeline is implemented using PDOs, tested standard-of-care (cisplatin & gemcitabine) and FDA-approved compound (lapatinib) for other tumors.
Results: The tumor organoids showed high expression of CK14 (Basal marker) and LGR5, other genes (CK14, CK20, LGR5, UPK III, FOX1A, GATA3a, CK5 and CK44) didn't show significance difference in expression. Quantification of IF imaging showed 3D structure of tumoroid with high expression of KI67, CK20, β-actin, and UPK III. Drug screening showed the combination of cisplatin and lapatinib significantly decreased the viability of PDO.
Conclusion: By further assessing the pathological results and clinical history of cases, we determined that monitoring of tumor-drug responses via bladder organoid model has the potential to determine and optimize patient-specific treatment efficacy.
Keywords: Bladder, Drug Screening, Personalized Medicine, Tumor Organoid

Os-2: Impact of Culture Conditions on Cytokine Expression in Mesenchymal Stromal Cells: Implications for Regenerative Medicine

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Objective: Multipotent mesenchymal stromal cells (MSCs) have garnered significant interest in regenerative medicine due to their remarkable immunomodulatory properties and potential therapeutic applications across a spectrum of pathological conditions. Emerging evidence suggests that the efficacy of MSCs-based therapies is intricately linked to the cellular microenvironment, including the tissue source from which MSCs are derived and the culture conditions in which they are maintained. In this study, we aimed to compare secretome among MSCs derived from different sources and cultured in different conditions.
Materials and Methods: MSCs were isolated from diverse tissue sources, including adipose tissue (AT), bone marrow (BM), gingiva (G), placenta (PL), and umbilical cord (UC). These MSCs were cultured either as monolayers (2D) or cell spheroids (3D). Immunophenotype and osteogenic, chondrogenic, adipogenic differentiation potential were evaluated. Cytokines and growth factors in MSCs-conditioned media (CM) samples were analyzed utilizing xMAP technology (Luminex, USA).
Results: MSCs expressed the mesenchymal markers (CD105, CD73, CD90, CD44) and demonstrated trilineage differentiation capacities. Analysis of cytokine expression in MSCs-CM unveiled distinct cytokine profiles among MSCs derived from different sources and culture conditions. Notably, UC-MSCs-CM exhibited elevated levels of cytokines compared to CM from other MSC sources. Moreover, comparison between 2D and 3D cultures of UC-MSCs revealed differences in cytokine expression, with 3D cultures demonstrating higher levels. These findings emphasize the influence of culture conditions on the cytokine profile of MSCs.
Conclusion: The observed differences in cytokine expression highlight the importance of optimizing culture conditions for MSCs-based therapies. UC-MSCs, particularly from 3D cultures, show promise as a potent source of cytokines with potential applications in treating inflammatory and tissue repair disorders. Further research into the specific mechanisms underlying these cytokine differences and their therapeutic effects is warranted to harness the full potential of MSCs-based therapies. The study was supported by the Academic leadership program Priority 2030 proposed by Sechenov University.
Keywords: Conditioned Media, Cytokines, Mesenchymal Stromal Cells, Secretome, 3D Culture

Os-3: Wnt/Beta-Catenin Signaling Regulates Osteoblast Dedifferentiation During Zebrafish Bone Regeneration

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Objective: Cellular dedifferentiation is an important phenomenon that is invoked to form source cells for regeneration in various vertebrate species and organs. We define dedifferentiation as the process by which a differentiated cell reverts back to a less differentiated state and becomes proliferation-competent. In response to fin amputation, osteoblasts (OB) proximal to the amputation plane start to dedifferentiate, become proliferative and migrate to form part of the regeneration blastema. In zebrafish, only retinoic acid and NF-κB signaling have been de-

scribed as regulators of OB dedifferentiation. Our aim here is to better characterize the molecular changes occurring in dedifferentiating osteoblasts.

Materials and Methods: We have performed 10X Genomics Single cell RNA and Bulk RNA sequencing experiments at different timepoints by FAC-sorting OBs of all lineages using Zns5 antibody, and mature OBs only using a bglapl:eGFP reporter line respectively. For global inhibition of Wnt signaling pathway we used a hs:Axin1-YFP transgenic line, and for manipulating this pathway in epidermis and fibroblasts we used Cre-lox approach.

Results: We found that OBs are very heterogenous during de- and re-differentiation, and we discovered two trajectories where mature OBs and mesenchymal cells are major source of regeneration. Mechanistically, we found that Wnt/beta-catenin pathway is positively enriched within dedifferentiated OBs. Intriguingly, we found that ectopic Wnt signaling inhibition reduced OB dedifferentiation, and its inhibition in epidermis enhances, and in fibroblasts impairs dedifferentiation.

Conclusion: While our and other labs have described important roles for Wnt signaling at later stages of fin regeneration, including in OB re-differentiation, a potential function in the regulation of OB dedifferentiation has not been uncovered. Thus, it would be interesting to investigate Wnt signaling pathway regulation of OB dedifferentiation in more detail by finding targets and regulatory networks, and also delving deeper into the OB heterogeneity and interaction with other cells.

Keywords: Zebrafish Fin Regeneration, Wnt/Beta-Catenin Signaling, Single Cell RNA Sequencing, Intrinsic Regeneration, Cellular Dedifferentiation

Poster Presentations

Ps-1: Targeting Glioblastoma Stem Cells: A Comparative Study of NK Cell Cytotoxicity

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Objective: Glioblastoma (GBM), a prevalent primary malignant brain tumor, poses significant treatment challenges. One such challenge is the presence of cancer stem cells (CSCs) that exhibit resistance to chemotherapy and radiotherapy. The quest for advanced therapies to counter these cells has given rise to therapies based on immune cells. The selective transfer of immune cells, particularly Natural Killer (NK) cells, offers substantial therapeutic potential against tumor cells. Over the past decades, two-dimensional (2D) tumor models have been employed to study the efficacy of immune cell cytotoxicity. However, these 2D models lack structural complexity and fail to simulate the physiological conditions of the tumor microenvironment. Consequently, the effectiveness of immune cells against tumor cells, as demonstrated in these models, may not fully translate into clinical studies. To gain a more profound understanding of tumor-immune cell interactions, spheroid tumor models, which are more physiologically relevant, have been developed. These spheroid models can simulate dynamic cellular activities, thereby providing a more accurate representation of *in vivo* tumor profiles and cancer stem-like cells. Here, we aimed to investigate the cytotoxicity of NK cells in glioblastoma cancer stem-like cells (GCSLC) of the U251 GBM cell line in comparison to traditional 2D models.
Materials and Methods: Spheroid-forming assay in a serum-free culture medium was used to enrich the cellular population with stem-like properties. Spheroids were characterized by evaluating the stemness markers and clonogenicity. Moreover, spheroid and 2D models were co-cultured with NK cells and cytotoxicity was analysed using flow cytometry.
Results: Our findings, obtained through immunophenotyping, revealed that the expression of CSC markers in the spheroid model surpasses that in the 2D model. Furthermore, we evaluated the cytotoxicity of primary human NK cells, both activated with interleukin-15 and inactive, against these two models using flow cytometry. Our findings indicate that both inactive and activated NK cells exhibit higher cytotoxicity against 2D models compared to spheroid models.
Conclusion: Our study concludes that spheroids exhibit resistance to the cytotoxicity of NK cells activated with interleukin-15.
Keywords: Cancer Stem Cells, Glioblastoma, NK cells, Spheroid Culture, 2D Culture

Ps-2: Evaluation of NANOG Gene Suppression and Replacement of Let-7 in Breast Cancer Cell Line and The Effects on Stemness, Invasion and Apoptosis of Cells

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Objective: Failure and recurrence in breast cancer (BC) treatment because a significant obstacle in cancer therapy, and identification of cell population named cancer stem cells (CSCs) in the tumor can be help us to define these cells as target in novel therapeutic strategy. The goal of this research is to find a correlation between stemness and metastatic characteristics, to address whether CSCs are a potential target of therapy because of its developmental behavior and similarities with normal stem cells. Here, we focus on the expression of NANOG in breast CSCs, a key molecule in the physiological process of stem cells. Abnormally alteration in the miRNAs expression such as Let-7a has been demonstrated in several cancers, including BC.
Materials and Methods: SKBR3 cells have transfected by NANOG siRNA and Let-7a miRNA mimic by electroporation. Then, we have evaluated the effects of NANOG inhibition and the increase Let-7a on the apoptosis, invasion, migration, and stemness feature of the cells. Results: Our results showed that the inhibition of NANOG coincided with Let-7a increase contribute to significant decrease in stemness feature. The invasion factors such as MMP9 showed the low levels (2.8-fold) of invasion rate after transfection the cells. The apoptosis rate of the SKBR3 cells increased after transfection (35%) compared to control cells. As well as the increase in the BAX/BCL2 ratio (6-fold) confirmed the flow cytometery analysis of apoptosis. According to the results, the difference between the siRNA and miRNA mimic groups was not significant, but the differences between transfected cells with the control cells were significant.
Results: Our data showed the importance of Let-7a and Nanog in cancer progression. In conclusion, these findings showed that a combination of Let-7a miRNA mimic and Nanog siRNA could be exploited as a new treatment to enhance tumoricidal strategy treatment and to improve the cancer therapy outcome.
Conclusion: In this study, we showed that the using a combination of Let-7a increase and NANOG inhibition could possibly tumoricidal outcome.
Keywords: Breast Cancer, Let-7, NANOG

Ps-3: Harnessing The Power: Prenatal Decellularization for Tissue Repair

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Objective: The practice of prenatal decellularization has emerged as a promising avenue in regenerative medicine, with a specific focus on harnessing the unique capabilities of the placenta. This innovative approach involves the meticulous removal of cellular components from the placental tissue while preserving its extracellular matrix architecture and bioactive factors. By

unlocking the regenerative potential of decellularized placental scaffolds, we aimed to pave the way for revolutionary advancements in tissue engineering, organ transplantation, and wound healing therapies.
Materials and Methods: In the current study, the decellularized placenta powder was obtained using detergents such as sodium dodecyl sulfate and triton, finally it was freeze dried. *In vitro* characterization including total protein content, DNA content, and histology staining was deeply conducted. Furthermore, the biocompatibility of the prepared powder on fibroblast cell viability was investigated.
Results: This study delves into the innovative methods used to create decellularized scaffold powder, a versatile biomaterial that provides a structural framework for cell growth and tissue regeneration. According to our result, the decellularized scaffold showed retained extracellular matrix after decellularization, efficient retained extracellular matrix, and excellent biocompatibility *in vitro*.
Conclusion: Altogether, this study delves into the intricacies of harnessing the power of placenta decellularization, shedding light on its current applications, challenges, and future prospects in the realm of regenerative medicine.
Keywords: Perinatal, Placenta, Regenerative Medicine, Scaffold

Ps-4: Preparation and *In Vitro* Anticancer Evaluation of Etoposide Encapsulated Niosome Nanocarriers

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Objective: In today's world, nanomedicine-based therapeutics are used to treat breast cancer as the leading cause of death in females. Etoposide (ETO) is used in treatment of many malignancies including breast cancer, but its hydrophobicity cause hinderance to achieve high drug loading. Niosomes are novel drug delivery systems forms by self-assembly of nonionic amphiphiles in aqueous media. The aim of present study was to develop a drug delivery system based on niosome to encapsulate the ETO and investigate the safety and effectiveness of the newly developed system.
Materials and Methods: Introduced niosome has been prepared by thin layer hydration method. Particle size and zeta potential were evaluated using dynamic light scattering (DLS), and drug release rate from the system was investigated *in vitro*. Safety of the DDS and its gradient was checked using bone marrow mesenchymal stem cells (BMSCs) and red blood cells (RBC). MDA-MB-231 cell line was used to check the DDS+ DXR and CXR only.
Results: The newly formulated Niosome system NI showed excellent stability for up to 3 months at 4°C. The particle size was reported to be approximately 399.37 nm and its poly dispersity index (PDI) was 0.4. In addition, the drug loading rate was 89% and the releasing percentage of the drug after 24 hours through mouse skin and dialysis membrane was obtained as 37 and 52 %, respectively. The half maximal inhibition concentration (IC 50) of the drug for 48 and 72 hours of treatment was calculated to be 33.26 and 31.49 µg/ml respectively.
Conclusion: The newly developed NI-ETO system is suitable drug delivery system based on its high drug carrier capacity, releasing rate and its medicine safety.
Keywords: Breast Cancer, Drug Delivery, Etoposide, Niosome

Ps-5: Nebulization of Mesenchymal Stem Cells Secretome Attenuates Acute Respiratory Distress Syndrome Caused by SARS-CoV-2 Corona Virus

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Objective: Indeed, mesenchymal stem cells (MSCs) have shown promise in treating COVID-19 patients due to their immunomodulatory, anti-inflammatory, and regenerative properties. However, the limited lifespan of transplanted MSCs poses a challenge. Utilizing the secretome of MSCs, which comprises various bioactive factors such as cytokines, growth factors, and extracellular vesicles, presents a promising alternative. These factors collectively contribute to tissue repair and regeneration, making them valuable in combating the inflammatory response and tissue damage associated with COVID-19. Harnessing the therapeutic potential of MSC secretome could offer a more sustainable and effective treatment strategy for COVID-19 patients.
Materials and Methods: The randomized clinical trial conducted in Kerman, Iran, aimed to assess the efficacy of nebulized concentrated MSCs secretome as an additional therapy for severe COVID-19 pneumonia. Twenty patients in the severe phase of COVID-19 pneumonia were randomly assigned to either the investigation group (received standard therapy plus nebulized concentrated MSCs secretome) or the control group (received standard therapy alone). The investigation group received 2 mL of nebulized concentrated MSCs secretome twice daily for five consecutive days in addition to standard therapy. The study evaluated several parameters to assess the effect of MSCs secretome therapy, including the length of stay in the intensive care unit (ICU), oximetry readings, and paraclinical results. These parameters were assessed at baseline and 5 days after ICU admission to determine any improvements or differences between the investigation and control groups. This trial aimed to provide insights into the potential benefits of MSCs secretome therapy as an adjunctive treatment for severe COVID-19 pneumonia, with the goal of improving patient outcomes and reducing the duration of ICU stay.
Results: The study findings indicate that nebulized concentrated MSCs secretome therapy, administered as an adjunctive treatment for severe COVID-19 pneumonia, led to favorable outcomes without any observed adverse effects. Patients who received MSCs secretome exhibited a significant decrease in ICU stay duration compared to those who received standard therapy alone (10.9 ± 3.75 vs. 5.5 ± 1.64 days, P=0.04). Additionally, the MSCs secretome-treated group showed significant improvement in O2 saturation (60.65 ± 16.58 vs. 79.47 ± 15.36, P=0.02) compared to the control group. Furthermore, during the study period, the investigation group demonstrated significant decreases in C-reactive protein (CRP), prothrombin time (PTT), and international normalized ratio (INR), along with an upregulation in the lymphocyte fraction (P<0.05 for all). These findings suggest that nebulized concentrated MSCs secretome therapy may have immunomodulatory and anti-inflammatory effects, leading to improved clinical outcomes and potentially reducing the severity of COVID-19 pneumonia.

Conclusion: The results of this preliminary study highlight the potential benefits and safety of using MSCs secretome as a therapeutic option for COVID-19 patients. However, further validation of these findings through additional clinical trials across different stages of the disease is strongly recommended. This will help to confirm the efficacy and safety of MSCs secretome therapy and provide more comprehensive evidence for its use in managing COVID-19.

Keywords: COVID-19, Hospitalization, Mesenchymal Stem Cells, Oximetry, Secretome

Ps-6: Evaluating The Anticancer Effects of 5-Fluorouracil and Aspirin Combination Against Gastric Cancer Cell Line

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Objective: Gastric cancer (GC) is one of the cancers that is diagnosed in advanced stages and mostly causes death. The prevention of drug resistance in malignancies has been significantly improved by combination therapy. However, the significant obstacle for therapeutic medications appears to be an increase in drug resistance. In the present study, 5-fluorouracil (5-FU) and aspirin were investigated as a combination therapy in a gastric cancer cell line (MKN) to overcome 5-FU drug resistance.
Materials and Methods: The combined effects of 5-fluorouracil (5-FU) and aspirin at various concentrations on cell viability and proliferation of MKN cells were assessed using the MTT assay after 48 h. Additionally, apoptosis was investigated by evaluating the expression of *Bcl-2* and *Casp3* genes using real-time *RT-PCR*. The results of the MTT assay and real-time RT-PCR were evaluated using graph pad prism software.
Results: Aspirin showed an IC_{50} value of 3 mM in MKN cells, while 5-FU showed an IC_{50} value of 80 µM. The combined IC_{50} values of aspirin and 5-FU were 2.5 mM and 60 µM respectively. The real-time polymerase chain reaction (PCR) analysis revealed an upregulation of *Casp3* expression and a downregulation of *Bcl-2* expression.
Conclusion: In conclusion, the current research demonstrated that the combination of aspirin and 5-FU concurrently induced apoptosis, inhibited cell proliferation, and decreased drug resistance in gastric cancer cell line.

Keywords: Aspirin, Drug Resistance, Gastric Cancer, 5-Fluorouracil

Ps-7: Correlation between Sperm and DNA Damage and Severe Asthenozoospermia in Men: An In-Depth Investigation

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Objective: Sperm motility is vital for successful fertilization, as it enables the sperm to penetrate the oocyte. Asthenozoospermia, defined by sperm motility under 42%, is a common cause of male infertility. This retrospective cohort study investigates sperm DNA damage using the TUNEL and SCSA methods, and explores its correlation with sperm parameters, age, and body mass index (BMI) in individuals with severe asthenozoospermia (motility less than two percent) compared to those with normozoospermia.
Materials and Methods: The study involved 111 individuals with severe asthenozoospermia and 113 individuals with normozoospermia. Sperm parameters were evaluated according to the World Health Organization's 2010 guidelines, while TUNEL and SCSA methods were used to assess sperm DNA damage. Statistical analysis included the t-test for two independent samples, and Pearson's correlation coefficient to examine the relationship between sperm DNA damage and sperm parameters, age, and BMI. Significance was considered at a level of $P>0.05$.
Results: Subjects with severe asthenospermia exhibited significantly lower average seminal fluid volume, sperm concentration, total sperm count, overall sperm motility, and progressive sperm motility compared to those with normal asthenospermia ($P<0.001$). Additionally, the average percentage of sperm DNA fragmentation was significantly higher in individuals with severe asthenospermia compared to normozoospermic individuals ($P<0.001$). A positive and significant correlation was observed between the extent of sperm DNA fragmentation and the age of men with severe asthenozoospermia.
Conclusion: Sperm motility, sperm DNA fragmentation, and paternal age are all critical factors that influence successful conception, fetal development, and offspring health. Therefore, the evaluation of sperm DNA fragmentation is recommended as part of male fertility assessments, as it can help guide the selection of appropriate treatment approaches for men with severe asthenospermia.

Keywords: SCSA, Sperm DNA Damage, Sperm Parameters, TUNEL

Ps-8: Enriched Human Embryonic Stem Cells-Derived CD133+, CD24+ Renal Progenitors Engraft and Restore Function in A Gentamicin-Induced Kidney Injury in Mice

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Objective: Acute kidney injury (AKI) is a common health problem that leads to high morbidity and potential mortality. The failure of conventional treatments to improve forms of this condition highlights the need for innovative and effective treatment approaches. Regenerative therapies with Renal Progenitor Cells (RPCs) have been proposed as a promising new strategy. A growing body of evidence suggests that progenitor cells dif-

ferentiated from different sources, including human embryonic stem cells (hESCs), can effectively treat AKI.
Materials and Methods: We describe a method for generating RPCs and directed human embryoid bodies (EBs) towards $CD133^+$ $CD24^+$ renal progenitor cells and evaluate their functional activity in alleviating AKI.
Results: The obtained results show that hESCs-derived $CD133^+$ $CD24^+$ RPCs can engraft into damaged renal tubules and restore renal function and structure in mice with gentamicin-induced kidney injury, and significantly decrease blood urea nitrogen levels, suppress oxidative stress and inflammation, and attenuate histopathological disturbances, including tubular necrosis, tubular dilation, urinary casts, and interstitial fibrosis.
Conclusion: The results suggest that RPCs have a promising regenerative potential in improving renal disease and can lay the foundation for future cell therapy and disease modeling.
Keywords: $CD133^+$, $CD24^+$, Embryonic Stem Cells, Kidney Injury, Renal Progenitors

Ps-9: The Effect of Platelet-Rich Plasma Injection on Decreases of Apoptosis of Multiple Sclerosis in Cuprizone Induced Model in Rats

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Objective: Multiple sclerosis (MS) is one of the most common neurodegenerative diseases in which the myelin shath. Although apoptosis of oligodendrocytes and neurons has been observed in MS lesions, the contribution of this cell death process to disease pathogenesis remains controversial. Platelet-rich plasma (PRP) is a blood derivative that has Decreases inflammatory and Apoptosis cell-regenerating properties. It also contains various cytokines and growth factors that may be beneficial for healing. The purpose of this research is the effect of platelet-rich plasma injection on inhibition of apoptosis of multiple sclerosis in cuprizone-induced model in rats.
Materials and Methods: The demyelination model was induced using an oral regimen of cuprizon 0/2 for 6 weeks. After that, blood was obtained from Royan stem cells company and after centrifugation, PRP was injected into mic. Extension demyelination and Rmyelination investigated were evaluated luxel fast Blue (LFB). Also in order to movement changes of BBB and footprint was investigated.
Results: PRP significantly reduced demyelination in tissues prepared using LFB and reduced the resulting cellular damage. In addition, PRP significantly improved the status of BBB and Foot print motor tests and appeared to inhibit induced apoptosis.
Conclusion: The results of this study show that PRP significantly increased myelin repair, reduced apoptosis through neuroprotective properties, and improved motor dysfunction.
Keywords: Apoptosis, Cuprizon, Multiple Sclerosis, PRP

Ps-10: The Effect of Umbilical Cord Blood Serum on Improving Liver Fibrosis in Rats

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Objective: Liver fibrosis is a serious condition that affects liver function and can be caused by various factors. Treatment usually involves anti-inflammatory drugs, but there is no complete cure yet. Umbilical cord serum contains growth factors and cytokines that can be beneficial for tissue regeneration and cell culture techniques.
Materials and Methods: The present study involved the establishment of an animal model using male Wistar rats by injecting CCl4 for 7 weeks to induce liver fibrosis. Subsequently, the umbilical cord blood serum was injected into the rats every day for two weeks intraperitoneally. Finally, after sacrifice, blood and liver tissue samples were collected. The final evaluation of the results included measuring the serum levels of ALT, ALP, and AST as well as histological tests using H&E and Masson's trichrome staining. Furthermore, gene expression changes are assessed using RT-PCR and statistical analysis is conducted using ANOVA.
Results: The serum levels of ALT, ALP, and AST showed a notable decrease, indicating a reduction in liver damage. Histological analysis revealed a decrease in fibrotic tissue and an improvement in liver architecture. Gene expression analysis showed a downregulation of fibrosis related genes. Statistical analysis confirmed the significance of these findings.
Conclusion: This study suggests that umbilical cord blood serum, improved liver function and reduced fibrotic tissue in a rat model of liver fibrosis. The results showed a decrease in liver enzymes, improved liver histology, and downregulation of fibrosis-related genes, indicating potential therapeutic benefits. Further research and clinical trials are needed to confirm these findings.
Keywords: Liver Dysfunction, Liver Regenerations, Umbilical Cord

Ps-11: Anti-Proliferative Activity of JQ for Melanoma and Underlying Mechanism

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Objective: Aggressive cancers such as melanoma have a high mortality rate and a high degree of resistance to conventional therapies. Inhibitors targeting growth factor receptors, which are frequently overexpressed and associated with melanoma progression, are among the intriguing new options for targeted therapy against this malignancy that have recently emerged. The lack of success of targeted therapy alone has led us to investigate the efficacy of treatment *in vitro* models with an bromodomain and extra-terminal domain (BET) inhibitor, JQ1.
Materials and Methods: Given their respective mechanisms of actions, we hypothesized the effect assessed by viability assay MTT could be due to the target genes affected. Hence the target genes in corresponding mechanism were characterized for their

expression by qRT-PCR in the A375 cell line to prove the effect of JQ1. For this purpose, different concentrations of JQ1 (10 nmol/mL - 2 Umol/mL) were tested and ERBB2, BRAF, CDKN2A, MITF and P38 evaluated to find the mechanism of action.
Results: As a result, we found the 2 μM of JQ1 can inhibit proliferation significantly in 48hr of treatment, A375 cells were treated by DMSO taken as control. Besides, treated cells with 2 μM of JQ1 showed significantly downregulated the CDKN2a (P16).
Conclusion: JQ1 inhibits proliferation and results in selective gene regulation. Effects of JQ1 suggesting its potential suitability as an anti-cancer drug targeting BRD4-mediated transcriptional regulation.
Keywords: Bromodomain-Containing 4 Inhibitor, Drug Screening, Melanoma, Small Molecule, JQ1

Ps-12: Angiogenic Properties of An Antimicrobial Lithium Chloride-Loaded Silk Fibroin/Alginate 3D Porous Scaffold for Promoting Diabetic Wound Healing: *In Vitro* and *In Vivo*

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Objective: Healing diabetic ulcers with chronic inflammation is a major challenge for researchers and professionals, necessitating new strategies.
Materials and Methods: To rapidly treat diabetic wounds in rat models, we have fabricated a composite scaffold composed of alginate (Alg) and silk fibroin (SF) as a wound dressing that is laden with molecules of lithium chloride (LC). The physicochemical, bioactivity, and biocompatibility properties of Alg-SF-LC scaffolds were investigated in contrast to those of Alg, SF, and Alg-SF ones. Afterward, full-thickness wounds were ulcerated in diabetic rats in order to evaluate the capacity of LC-laden scaffolds to regenerate skin.
Results: The characterization findings demonstrated that the composite scaffolds possessed favorable antibacterial properties, cell compatibility, high swelling, controlled degradability, and good uniformity in the interconnected pore microstructure. Additionally, in terms of wound contraction, re-epithelialization, and angiogenesis improvement, LC-laden scaffolds revealed better performance in diabetic wound healing than the other groups.
Conclusion: This research indicates that utilizing lithium chloride molecules loaded in biological materials supports the best diabetic ulcers regeneration *in vivo*, produces a skin replacement with a cellular structure comparable to native skin.
Keywords: Alginate, Angiogenesis, Diabetic Wound, Lithium Chloride, Silk Fibroin

Ps-13: Photobiomodulation Stimulates the Survivability of 3D Bioprinted MSC-Based Spheroids

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Objective: The emerging field of tissue engineering and 3D bioprinting necessitates innovative methods to enhance cell survival, proliferation, and differentiation. Photobiomodulation emerges as a promising approach due to its non-invasive nature, ability to penetrate highly hydrated structures, and demonstrated capacity to modulate cell physiology under stress conditions. This study aimed to evaluate the impact of photobiomodulation on mesenchymal stromal cells (MSCs) spheroids within 3D environments.
Materials and Methods: Mesenchymal stromal cells (MSCs) sourced from human gingiva were utilized to form spheroids. Photobiomodulation in the red (633 nm) and near-infrared (840 nm) spectra was administered using nonmonochromatic LED emitters (IPT RAS, Russia). A 3D cell culture was established using a hydrogel based on modified fibrinogen фтв пудфешт, and 3D bioprinted constructs were created with the CellInk Bio X 3D bioprinter (CellInk, USA). Cell physiology analysis encompassed cell viability assessment (Live/Dead staining), metabolic activity evaluation (AlamarBlue assay, ATP assay), proliferation rate determination (PicoGreen assay), ICC, and morphometric analysis.
Results: The bioprinting process was observed to impact cell viability. Both types of photobiomodulation were found to stimulate proliferative activity. Specifically, photobiomodulation at 840 nm wavelength was noted to enhance metabolic activity and ATP synthesis. Moreover, 3D printed structures exposed to near-infrared photobiomodulation exhibited slightly increased spheroid activity in terms of cell migration.
Conclusion: Conclusively, this study demonstrates the potential of photobiomodulation in promoting cell metabolic activity and proliferation within 3D tissue-engineered structures, suggesting new avenues for applying photobiomodulation in the field of bioprinting. The study was supported by Russian Science Foundation (№22-75-10120)
Keywords: Bioprinting, MSCs, Photobiomodulation, Spheroids

Ps-14: Preparation of Solid Lipid Nanoparticles Containing Docorubicin Using Microemulsion Method and Investigating Its Effect on Cancer Cells

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Objective: Doxorubicin (DOX), one of the most effective anticancer drugs frequently is used in breast cancer treatment. However, its clinical use is restricted by toxicity to normal cells and its low specificity against cancer cells. As an alternative, nanoparticle drug delivery systems such as Solid Lipid nanoparticles (SLN) is used to overcome these limitations in cancer therapy. This work is intended to develop and evaluate a SLN system that can efficiently load and release DOX and enhance its efficacy against MDA-MB-231 cancer cell line.
Materials and Methods: The SLN drug delivery system has been prepared by a new strategy based on microemulsion sys-

tem. Particle size and zeta potential were evaluated using dynamic light scattering (DLS), and drug release rate from the system was investigated *in vivo* and *in vitro*. Safety of the DDS and its gradient was checked using bone marrow mesenchymal stem cells (BMSCs) and red blood cells (RBC). MDA-MB-231 cell line was used to check the DDS+ DXR and CXR only.
Results: The particle size was reported to be approximately 145 nm and its poly dispersity index (PDI) was 0.238. It was observed that the system and its components are completely non-toxic. In addition, the drug loading rate was 93% and the releasing percentage of the drug after 24 h through mouse skin and dialysis membrane was obtained as 38 and 56 %, respectively. The half maximal inhibition concentration (IC_{50}) of the drug for 48 and 72 hours of treatment was calculated to be 1.19 and 0.94 µM respectively.
Conclusion: Safeness, drug carrier capacity and its releasing rate of the newly developed SLN showed that this system is a suitable carrier for doxorubicin.
Keywords: Cancer, Doxorubicin, Solid Lipid Nanoparticles, Targeted Drug Delivery

Ps-15: Mesenchymal Stem Cells Derived Exosome Transfected as a Therapeutic Vehicle for Mir-486 as a Novel Complementary Treatment for Glioblastoma

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Objective: Mesenchymal stem cells (MSCs) are integral components of the most essential reservoir of mature stem cells that facilitate tissue regeneration in both physiological and pathological contexts. Exosomes, small extracellular vesicles with diameters of 40–150 nm, are discharged through the fusion of multivesicular bodies with the plasma membrane. Exosomes originating from MSCs possess notable benefits such as enhanced stability, diminished immunogenicity, favorable biocompatibility, prolonged circulation duration, and a homing mechanism resembling that of Mesenchymal stem cells. MSC-exosomes have the potential to serve as suitable vehicles for delivering bioactive compounds or therapeutic agents in order to modulate the extracellular milieu and impact cancer pathophysiology for the purpose of treating tumors safely. MicroRNAs (miRNA) are a class of non-coding RNAs that significantly contribute to gene regulation. Exosomes released externally contain numerous miRNAs, enabling their transfer between cells through exosome release and absorption, thereby facilitating intercellular gene regulation. The impact of MSCs is closely linked to the significant release of exosomes containing functional miRNAs. Recent studies have shown MSC-exosomes carrying miRNA can enter tumor cells, alter miRNA levels, impact pathways, affect tumor progression, and contribute to tumor therapy. miRNAs have reportedly been demonstrated to be capable of being transferred into MSC-exosomes via electroporation. Moreover, it has been observed that the membrane of MSC-exosomes has the ability to generate transient pores by means of short electrical pulses. These pores facilitate the entry of miRNAs into MSC-exosomes. Glioblastomas (GBM), the most aggressive primary brain tumors, often exhibit poor responses to existing treatments. Numerous studies have demonstrated the potential of individual microRNAs in inhibiting angiogenesis, migration, and metastasis in glioblastoma. Many studies have illustrated that the predominant mechanism of resistance to Temozolomide (TMZ) is attributed to the overexpression of O6-methylguanine-DNA methyltransferase (MGMT).
Materials and Methods: In the course of our investigation, we carried out an exhaustive examination of data and studies in this particular area, employing data analysis tools to guide our research. Through this method, we were able to acquire a thorough comprehension of the topic and establish a robust basis for our investigation, guaranteeing that our conclusions are well-supported and based on current scientific insights.
Results: The expression profiles of miRNA in recurrent glioblastoma a significant reduction in the levels of miR-486-3p. The identification of O6-methylguanine-DNA methyltransferase as a direct target of miR-486-3p has been established, and it has been observed that reduced levels of miR-486-3p lead to an increase in the protein translation of MGMT.
Conclusion: Overall, MSCs driven exosomes can be an attractive therapeutic vehicles for miR-486 which have great potential to act as supplements to chemotherapy in the clinic.
Keywords: Exosome, Glioblastoma, Mesenchymal Stem Cells, MicroRNA

Ps-16: A Novel Protocol for Generation of Off-The-Shelf Growth Factor-Enriched Lyophilized Platelet-Rich Fibrin (Ly- PRF)

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Objective: PRF is a novel biological product containing growth factors and anti-inflammatory cytokines, suitable for inflammation regulation in regenerative medicine. In order to increase the growth factor and cytokine concentrations in the obtained product, we tried to optimize the centrifugation process. Moreover, to maintain the functionality of the product during the cryopreservation process, different storage procedures were applied and compared based on the functionality of the product.
Materials and Methods: Peripheral blood samples were isolated from four volunteers after obtaining their informed consent and thereafter, were centrifuged to form PRF. Different centrifugation speeds and temperatures were utilized in this step to figure out the optimum procedure. Moreover, different storage groups were considered to evaluate the impact of storage items on the functionality of the product. In this regard, one group was immediately lyophilized and stored in a -80°C freezer, one group was stored in a -80°C freezer and then, was lyophilized, and the third group was sorted in 4°C after lyophilization. Bradford assay and ELISA technique were used to assess the quantity and release of PDGF-BB, TGF-β, and VEGF in freshly prepared or long-term stored Ly-PRF samples at four different time points of 1, 24, 48, and 72 hours. Finally, the effect of our different groups on the tube formation capacity of endothelial and the polarization of macrophages was assessed.
Results: Bradford and ELISA results showed that all three assessed growth factors were highly enriched in all our groups although the fresh samples had higher amounts compared to the long-term stored samples (not significant). Moreover, time-dependent release of growth factors was observed in all groups.

Furthermore, freshly prepared Ly-PRF samples could significantly increase the tube formation capacity of endothelial and the polarization of macrophages compared to the stored Ly-PRF samples although the stored samples could also show angiogenic and anti-inflammatory properties.
Conclusion: Taken together, we tried to optimize the Ly-PRF generation procedure to obtain highly growth factor-enriched products. Moreover, we showed that although the long-term storage could affect the amount and functionality of proteins in Ly-PRF samples, the cryopreserved samples still had angiogenic and immune-modulatory properties, which could be utilized as off-the-shelf biological products in regenerative medicine.
Keywords: Growth Factors, Platelet-Rich Fibrin, Regenerative Medicine, Storage

Ps-17: Chick Embryo Extract Enhances Proliferation and Glial Differentiation of Dental Pulp Stem Cells for Neurodegenerative Therapy

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Objective: Dental pulp stem cells (DPSCs) are promising candidates for cell-based therapies in neurodegenerative diseases due to their ability to differentiate into neural lineages. However, their clinical application is hindered by limitations such as the need for extensive ex vivo expansion to achieve adequate stem cell numbers and efficient differentiation into neural lineages. This study aims to investigate the impact of chick embryo extract (CEE) preconditioning on the characteristics of DPSCs during *in vitro* culture.
Materials and Methods: DPSCs were isolated from the pulp region of rat teeth and cultured in a growth medium supplemented with either 10% fetal bovine serum (FBS) alone (control group, CTRL) or 10% FBS plus 10% CEE. Stem cell proliferation was assessed in both experimental groups after the first subculture by counting the cells and at passage 4 using the MTT assay. The mesenchymal stem cell identity of the DPSCs was confirmed at passage 4 through flow cytometry analysis of CD44 and CD90 markers. Additionally, the expression of genes related to stemness, neuronal, and glial differentiation was evaluated in both groups using real-time polymerase chain reaction (PCR).
Results: Our results demonstrated that CEE supplementation enhances the proliferation rate of DPSCs. Furthermore, CEE-treated stem cells expressed the mesenchymal markers CD44 and CD90 similarly to the CTRL group. However, CEE preconditioning significantly downregulated the expression of Nestin, a neural crest stem cell marker. The expression of neuronal markers BIII tubulin and MAP2 significantly decreased in CEE-treated stem cells, whereas the expression of the glial marker PDGFR-alpha increased.
Conclusion: These findings suggest that CEE preconditioning can confer desirable characteristics to DPSCs during *in vitro* culture, potentially enhancing their therapeutic application. Specifically, CEE preconditioning promotes stem cell proliferation, facilitating the production of sufficient stem cells for future transplantation, and biases the differentiation of these stem cells towards a glial lineage.
Keywords: Dental Pulp Stem Cells, Glial Marker, Preconditioning, Proliferation

Ps-18: Evaluating The Potential of Exosome-Encapsulated MicroRNA 149-3p in Developing Novel Endometriosis Therapy

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Objective: Endometriosis, as a chronic inflammatory disease brings remarkable social, public health, and financial consequences. Based on two theories of retrograde menstruation and stem cells, menstrual blood-derived stem cells (MenSCs) play a significant role in endometriosis since key genes of critical cellular processes are differentially expressed in MenSCs of endometriosis (E-MenSCs) and non-endometriosis women (NE-MenSCs). Since microRNAs (miRNAs) can regulate gene expression, we find the proper miRNA and assay its effects on modulating reactive oxygen species (ROS) and the gene expression level of inflammatory factors of E-MenSCs using NE-MenSCs-derived exosomes as the miRNA carriers.
Materials and Methods: After using publicly accessible algorithm-based databases, in Silico selection of miRNA regulating the most number of upregulated genes during endometriosis was done. E- and NE-MenSCs were cultured as controls, and the other experimental groups were as follows: E-MenSCs transfect with empty and miRNA vectors (E-MenSC+BB and E-MenSC+miR), and E-MenSCs treat with exosomes derived from non-transfected and miRNA-transfected NE-MenSCs (E-MenSC+Exo and E-MenSC+T-Exo). Then, the ROS amount and the expression level of interleukin (IL)-6, -8, -10 and 1β was evaluated through Elisa and real-time polymerase chain reaction (PCR), respectively.
Results: We found that ROS level was significantly lower in the E-MenSCs+ Exo and E-MenSCs+T-Exo group compared to the E-MenSCs. In addition, there was a significant increase in the gene expression levels of IL-6, -8, -10, and 1β in E-MenSCs compared to the NE-MenSCs. Although both E-MenSCs+T-Exo and E-MenSC+miR showed decreased IL-6, -8, and -10, none had decreased IL-1β level.
Conclusion: Since exosome-encapsulated mir-149-3p showed better modulating effects on E-MenSCs, the potential of exosomes as miRNA carriers could be considered in developing novel endometriosis therapies.
Keywords: Endometriosis, Exosome, Mesenchymal Stem Cells, Menstrual Blood, MicroRNA-149-3p

Ps-19: The Effects of Resveratrol on The Migration and Progression Ability of Menstrual Derived-Stem Cells in Endometriosis Patients

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Objective: Ten percent of women who are of reproductive age experience endometriosis, an inflammatory gynecological and hormone-dependent disease. Owing to the deficiencies of current therapeutic approaches, it is imperative to utilize novel additional, and alternative medications to improve endometriosis treatments. Due to its numerous biological actions, such as antineoplastic, anti-oxidant, anti-angiogenic, anti-proliferative, anti-inflammatory, and pro-apoptotic properties, resveratrol, a phytochemical, received a lot of attention. It has been demonstrated that the gene expression profile of menstrual blood-derived stem cells (MenSCs) differs between endometriosis and non-endometriosis women, supporting two theories regarding the pathophysiology of endometriosis: retrograde menstruation and stem cells theories. Therefore, in this study, we tried to evaluate the effects of resveratrol on cell migration and the expression level of VEGF, KRAS, and IDO1 -as cell progression-related factors- in MenSCs of endometriosis patients.
Materials and Methods: Healthy and endometriosis derived-MenSCs were purchased and cultured to reach 70% confluence. Endometriosis MenSCs were treated with 100 M of resveratrol (resveratrol group) for 3 days, while in positive (healthy) and negative (endometriosis) control groups, cells did not receive any treatment during 3 days of culture. To test the migration ability and gene expression level of cells, scratch test (assessed in time points of 12, 24, and 36 hours), and real-time polymerase chain reaction (PCR) were applied, respectively.
Results: The obtained results showed that at the time point of 36 hours, cells in resveratrol group had a statistically significant lower rate of wound closure than in endometriosis group (P= 0.047). Moreover, a significantly lower expression of *KRAS* and *VEGF* genes in Resveratrol group was observed comparing to the Endometriosis group (P=0.048 & P=0.02, respectively). However, treating with resveratrol could not decrease the IDO1 expression level of resveratrol group in compare to endometriosis cells (P=0.064).
Conclusion: According to the findings, resveratrol may ameliorate endometriosis progression through reducing the migratory ability of cells and decreasing the expression of VEGF and KRAS in endometriosis MenSCs.
Keywords: Endometriosis, Menstrual Blood-Derived Stem Cells, Migration Resveratrol

Ps-20: The Effect of miR-149-3p Encapsulated in Exosomes Isolated from Menstrual Blood-Derived Stem Cells in Inhibiting Proliferation and Cell Cycle (G1/S) of Breast Cancer

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Objective: Breast cancer is the most frequent cancer in women. Today, it is well acknowledged that common cancer treatments are inefficient and ineffective. Therefore, numerous investigations on new treatment alternatives with fewer side effects have yielded encouraging results. Stem cells are recognized as a possible source of cancer treatment and can be used as a tool in gene therapy. Stem cells secreted exosomes are the main agents in their therapeutic effects. In cancer treatment, exosomes can be utilized as carriers of medicines and functional RNAs. The purpose of this study was to explore the influence of mir-149-3p encapsulated in exosomes isolated from menstrual blood derived Stem cells (MenSCs) on the proliferation and cell cycle of the MDA-MB-231 cell line.
Materials and Methods: MenSCs were isolated and cultured until third passage. Then Cells were transfected with miR-149-3p, and the exosome isolation was done from both transfected and non-transfected MenSCs. The MDA-MB-231 cells was then treated with miR-containing exosomes (T-Exo)/ Exosome (Exo), and the subsequent changes in the mRNA and protein levels of AKT2 and ESR1 were investigated using real-time polymerase chain reaction (PCR) and western blotting, respectively. The amount of apoptosis was also assessed using AnnexinV/PI.
Results: Based on the data, AKT2 and ESR1 mRNA level were significantly decreased in T-Exo and Exo groups comparing to the control (P≤0.05). ESR1 protein level was also significantly lower in both treated groups than the control group, but T-Exo group showed significantly lower level comparing to the Exo group. Although, there were no significant change in the protein level of AKT2 among groups (P>0.05), in case of pAKT2, as the functional form of AKT2, lower protein level in T-Exo group was observed comparing to the control. Moreover, both T-Exo and Exo groups showed increased total apoptosis than the control group (P≤0.05).
Conclusion: It seems that both miR-149-3p and exosomes have the potential to be more studied and applied in novel cancer therapeutic approaches.
Keywords: Breast Cancer, Cell Proliferation, Cell Cycle, Exosome, MiR-149-3p

Ps-21: Evaluation of Biocompatibility and Osteoinductivity Properties of A Plant-Based Cellulose Scaffold: *In Vitro* and *In Vivo* Analysis

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Objective: Tissue engineering (TE) offers promise for treating bone defects in cases where self-healing is not possible. However, some TE-scaffolds lack sufficient biocompatibility essential for cell attachment, proliferation, tissue integration, and minimizing adverse reactions. Natural cellulose scaffolds show a great potential in addressing these challenges. *In vitro* analysis assesses the biocompatibility of cellulose scaffolds on human cells, but *in vitro* findings may not always directly translate to *in vivo* biocompatibility. Therefore, further preclinical testing is necessary to assess their safety and effectiveness for TE applications.

Materials and Methods: Towards the goal of developing a functional biocompatible system for bone TE, we assessed the cellular and tissue compatibility of a porous, fibrous scaffold derived from Monocephala leaves in both *in vitro* and *in vivo* settings. Osteoinductivity was evaluated by studying its effects on human mesenchymal stem cells (hMSCs) differentiation.
Results: Results showed biocompatibility in both models, supporting cell attachment, proliferation, mineralization, and infiltration. Histological analysis revealed a temporary foreign body response post-implantation, diminishing over time. The scaffold's properties, including biocompatibility, hydrophilicity, and mechanical strength, promoted new blood vessel formation, encouraging tissue regeneration.
Conclusion: Enhanced expression of bone markers in cultured cells suggested high potentials of these scaffolds to safe repair osteochondral defects without the risk of toxicity or an immunological reaction. These findings contribute to a better understanding of the cellulose scaffold's biocompatibility and osteoinductive properties, enhancing strategies for bone-TE development.
Keywords: Cellulose Scaffolds, Regenerative Medicine, Stem cell Differentiation, Tissue Engineering

Ps-22: Assessment of The Antibacterial and Wound Healing Effects of Magnesium Oxide Nanoparticles in Male Rats

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Objective: The severity and slow healing process of burn wounds make their treatment a significant challenge. This study aims to investigate the potential of magnesium oxide nanoparticles (MgONPs) in improving the healing process of skin wounds *in vivo*.
Materials and Methods: In the present study, a comprehensive assessment was conducted to investigate the antibacterial and cytotoxic activity of MgONPs. Male Wistar rats with deep burns were randomly assigned to different treatment groups, including untreated burns, silver sulfadiazine (1%), and MgONPs. The effects of these treatments on macroscopic and microscopic tissue alterations were evaluated over a period of 21 days.
Results: The study observed the antibacterial activity of MgONPs against gram-positive bacteria Staphylococcus aureus. The groups treated with MgONPs showed a significant increase in wound closure compared to the untreated group ($P<0.05$). However, the group treated with nanoparticles exhibited the highest rate of wound healing. The histologic results confirmed the healing activity of skin wound by MgONPs.
Conclusion: The use of a MgONPs has shown significant improvement in the microscopic appearance of burn wounds. These findings suggest that the inclusion of nanoparticles in the ointment may expedite the healing process.
Keywords: Burn, Magnesium Oxide, Nanoparticles, Skin

Ps-23: Evaluation of Anticancer Effects of Linoleic Acid-Loaded Niosomes on Hepatocellular Carcinoma Cells

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Objective: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, which is the second leading cause of cancer deaths in the world. Due to the ineffectiveness of common treatments, there is a serious need to find more effective and targeted treatments. Niosomes are one of the common types of nanoparticles in modern drug delivery systems, which are receiving attention nowadays. The purpose of this study was to synthesis, characterize the physicochemical properties of niosomes containing linoleic acid (LA) (an essential fatty acid with anticancer effects) and evaluate their effects on HCC cells (Hep 3-B).
Materials and Methods: We synthesized LA-loaded niosomes by thin film hydration method and characterized their size, polydispersity index (PDI) and zeta potential by zeta sizer (DLS (and morphology of niosomes was evaluated by scanning electron microscope (SEM). Also, we evaluated the toxicity effects by MTS assay and migration capacity of treated cells with 150 μM of LA-loaded niosomes, free niosomes, free LA and control group (non-treated cells) by scratch assay.
Results: Niosomes had size of 105.7 nm, PDI of 0.271 and -26.1 mV zeta potential. The SEM results indicated that niosomes had appropriate morphology. Niosomes with entrapment efficacy (EE) 74%, released LA slowly and sustained. The MTS assay results indicated that LA-loaded niosomes were more toxic than free LA to Hep-3-B cells. But they showed no toxic effects on normal cells (HFF). Also, LA-loaded niosomes reduced migration capacity of Hep-3-B cells better than other groups.
Conclusion: The use of niosome as a nanocarrier can be a good way to target and destroy cancer cells since they have toxic effects on HCC cells and could reduce migration rate of them.
Keywords: Hepatocellular Carcinoma, Niosome, Linoleic Acid

Ps-24: Optimizing Cryopreservation of HESC-Derived RPE Cells to Comply with GMP Conditions

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Objective: Cryopreservation is a critical step for Advanced Therapy Medicinal Products (ATMPs) that adhere to Good Manufacturing Practice (GMP) regulations to achieve the highest possible cell recovery rate while preserving the properties and function of the cells. However, a point to consider when manufacturing a drug product under GMP conditions is the use of reagents to simplify clinical delivery. We previously used a combination of compounds to freeze human embryonic stem cells-derived retinal pigment epithelial (hESC-RPE) cells consisting of 10% DMSO, 40% RPE medium, and 50% knockout

serum replacement (KOSR). In this study, we intend to develop a new freezing formulation for hESC-RPE cells that complies with GMP regulations.
Materials and Methods: We tested the use of two concentrations of DMSO (5 and 10%) and replaced KOSR and RPE medium with human serum albumin (HSA) at concentration of 5 and 10%, and BSS PLUS, respectively, in previous freezing media for cryopreservation of hESC-RPE cells. After 1 week, 1, 3 and 6 months, we thawed the hESC-RPE cells and assessed their survival, morphology, attachment, and RPE markers to compare with the frozen cells in the previous group (control group).
Results: The results showed no significant difference between the GMP formulation media and the control group in cell viability and cell attachment. Furthermore, RPE cells showed pigmented and cobblestone morphology 10-15 days after thawing and expressed CRALBP and MITF markers in all groups.
Conclusion: Our research suggests that low concentrations of DMSO in combination with HSA and BSS PLUS are suitable excipients for freezing hESC-RPE cells for therapeutic purposes.
Keywords: Cryopreservation, GMP Conditions, Retinal Pigment Epithelial Cells

Ps-25: Investigating The Effect of Toll-Like Receptor 4 Inhibition on The Osteogenic Differentiation of Mesenchymal Stem Cells Under Inflammatory Conditions

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Objective: Rheumatoid arthritis (RA) is an autoimmune disease that affects the synovial lining, leading to joint damage and the destruction of cartilage and bone tissue. Research has shown that blocking the activation of Toll-like receptor 4 (TLR4) pathway, can prevent the progression of inflammatory diseases like RA. TAK-242 is a small molecule that specifically inhibits the TLR4 pathway. On the other hand, according to studies, the TLR4 pathway plays a role in osteogenesis. The present study investigated how inhibiting the TLR4 pathway affects the osteogenic differentiation of bone marrow mesenchymal stem cells (BM-MSCs) under inflammatory conditions.
Materials and Methods: BM-MSCs were isolated from rats, cultured, and characterized to confirm their properties. The toxicity of TAK-242 was evaluated using MTS assay. To induce inflammatory conditions, BM-MSCs were treated with 1 µg/ml of LPS. The expression of inflammatory genes and cytokines in BM-MSCs was done by real-time polymerase chain reaction (PCR) and ELISA methods after 24 or 48 hours. Evaluations were done between the control, LPS, and LPS+TAK-242 groups. Finally, real-time PCR and Alizarin red staining were performed to evaluate the effect of TAK-242 on osteogenic differentiation of BM-MSCs on day 7.
Results: The MTS assay indicated that 100 nM is the optimal dose of TAK-242. Real-time PCR and ELISA results demonstrated that TAK-242 decreased the expression of inflammatory genes TNFα and IL-6 in BM-MSCs compared to the control group. Alizarin red staining and osteogenic genes expression showed that TAK-242 did not inhibit the osteogenic differentiation of BM-MSCs under inflammatory conditions.
Conclusion: TAK-242, by inhibiting the TLR4 pathway, not only inhibits inflammation but also can play a role in bone repair. As a result, TAK-242 can be used as a therapeutic approach for RA disease. However, further studies are required for verification.
Keywords: Mesenchymal Stem Cells, Osteogenic Differentiation, Rheumatoid Arthritis, Toll-Like Receptor 4, TAK-242

Ps-27: Therapeutic Potential of Hair Follicular Stem Cells Conditioned Medium in Vascular Dementia: Insights into Cellular and Molecular Mechanisms

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Objective: Vascular dementia (VaD) represents a prevalent form of dementia with significant clinical implications. This study aimed to delve into the cellular and molecular mechanisms underlying the effects of hair follicular stem cells conditioned medium (HFS-CM) in VaD.
Materials and Methods: Forty-eight rats were divided into four groups: control, sham operation, VaD + vehicle, and VaD + HFS-CM. CM was administered intraperitoneally on days 4, 14, and 24 post-2VO induction. Cognitive performance was assessed using open-field (OP), passive-avoidance, and Morris-water maze tests. Field-potential recording evaluated basal synaptic transmission (BST) and long-term potentiation (LTP). Hippocampal tissue was dissected for real-time polymerase chain reaction (PCR) analysis of *β1-catenin*, *IGF-1*, *TGF-β*, *GSK-3β*, *PSD-95*, and *NR2B* gene expression.
Results: VaD rats exhibited impaired performance in behavioral tests, along with reduced BST and LTP induction. Gene expression analysis revealed decreased levels of *β1-catenin*, *IGF-1*, *PSD-95*, and *TGF-β*, alongside increased *NR2B* and *GSK-3β* expression. HFS-CM treatment restored *PSD-95* and *GSK-3β* expression, improved fear memory and spatial learning, and enhanced grooming behavior. However, memory retrieval and exploratory behavior remained unaffected. While BST recovered with HFS-CM administration, LTP induction remained impaired.
Conclusion: This study highlights the therapeutic potential of HFS-CM in VaD, primarily through its role in restoring BST and improving gene expression profiles, ultimately leading to the amelioration of fear memory deficits.
Keywords: Conditioned Medium, Long-term Potentiation, Memory, Synaptic Transmission, Vascular Dementia

Ps-28: A Three-Dimensional Alginate and Alginate Methyl Cellulose Lung Cancer Culture: Characterized Model for Infection and Drug Testing Study

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Objective: In last decade, researchers have been tried to develop different three-dimensional (3D) cell cultures system, these systems are valuable due to potential of mimicking *in vivo* structure and cell-cell communication along with different applications such as drug discovery, tissue engineering, compound screening, vaccine test and personalized medicine to model different diseases. Limitation in access to proper model during pandemic showed requirement of effective and feasible model as immense help to researcher.
Materials and Methods: Therefore, we developed a 3D culture setting of A549 lung cancer cells (human alveolar basal epithelial cells) using alginate beads and alginate MC tested its effectiveness in different conditions. The viability and proliferation of cells were assessed using Alamar blue, the size and morphology was checked by ImajeJ, SEM microscopy and Immunofluorescent assessment in different days (0, 3, 5, 7, 10 in compare with scaffold alone and 2D culture).
Results: Cell viability for A549 resulted in considerable increase in day 5 for Alg group in compare with Alg-MC. in addition, in both group day 5 showed highest size but Alg group had a higher diameter than Alg-MC. SEM result also indicated increased porosity for Alg rather than Alg-MC. Characterization of spheroids by Immunofluorescence showed distinct assembly of Factin filament around the aggregated cells.
Conclusion: In summary, both biomaterials are appropriate to get accurate 3D model suitable for drug testing and viral infection.
Keywords: Alginate, Diseases Modeling, Lung Cancer, Methyl Cellulose, 3D Culture

Ps-29: Do Inhibitors of Apoptosis Proteins Play Role during Differentiation of Human Embryonic Stem Cells into Cardiomyocytes?

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Objective: Caspases are known for their critical roles in inflammation and apoptosis. Increasing evidence, however, has revealed diverse functions for caspases such as promoting differentiation and development of variety of cell types. In the case of differentiation into cardiomyocytes, the non-apoptotic function of caspases, activated through mitochondrial pathway, has been well-documented, indicating a highly precise regulatory mechanism. Inhibitors of apoptosis proteins (IAPs) are the most popular caspases inhibitors which have been less studied in differentiation.
Materials and Methods: To explore whether IAPs, control caspases activity at non-lethal level, the smac-mimetic Brinapant was used in order to antagonize multiple IAPs including XIAP, cIAP-1, and -2 during cardiomyocyte differentiation from human embryonic stem cells (hESCs). Real-time RT-qPCR and western blotting were performed to analyze IAPs expression level. The activation pattern of caspase-9 and -3 was also investigated by western blotting and caspase-like activity assay in differentiating and apoptotic cells.
Results: Brinapant-treated hESCs were differentiated into beating cardiomyocytes with an efficiency comparable to that of untreated cells. This suggested that IAPs may not be the main regulators of caspases during differentiation or expressed at an insufficient level in our study. On the other hand, the results showed functional cleaved caspase-9 in hESCs with a reducing trend at early stages of differentiation, without activation cleavage of cellular caspase-3.
Conclusion: Taken together, our findings suggested that caspase-9-cleavage of caspase-3 may be inhibited by factors besides IAPs, ensuring protection of pluripotent and differentiating cells from apoptosis in the presence of active caspase-9. Further study is expected to clarify the caspase regulators during cardiac differentiation, elucidating the molecular mechanisms involved, with diagnostic and therapeutic approaches.
Keywords: Caspases, Cardiogenesis, Cell Differentiation, Human Embryonic Stem Cells (hESCs), Inhibitors of Apoptosis Proteins (IAPs)

Ps-30: Nicotine Exposure Alters Expression of Stemness-Related Genes in Human Colorectal Cancer Cell Line

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Objective: Epidemiological studies have suggested that cigaret smoking is associated with increased colorectal cancer (CRC) risk. Nicotine is most likely related to the risk of cigaret smoking and exerts many of its effects on cancer cells through the α7-subtype of nAChR (α7nAChR). The development of CRC originated from cancer stem cells, which comprise a minor population of CRC cells. However, the mechanisms by which nicotine influences the stemness properties of cancer cells are not fully understood.
Materials and Methods: To examine the influence of nicotine exposure on cancer stem cell-related gene expression, the present study applied real-time RT-PCR to investigate changes in cancer stem cell-related gene expression in SW-480 CRC cells exposed to nicotine. SW-480 cells were inoculated in 6-well plates to determine the effects of treatments with 1 μM and 10 μM concentrations of nicotine on the expression level of α7nAChR and cancer stem cell markers, including Nanog, Oct-4, Sox-2, CD 44, CD 133, and aldehyde dehydrogenase (ALDH), by quantitative real-time polymerase chain reaction (PCR).
Results: The findings showed that nicotine increases the expression of both α7nAChR and cancer stem cell marker mRNA expression. Transfection with α7nAChR-siRNA prevented the observed effects of nicotine, indicating that these effects of nicotine are α7nAChR dependent.
Conclusion: Nicotine has recently been found to enhance the stemness properties of cancer cells. Nicotine alters Nanog, Oct-4, Sox-2, CD 44, CD 133, and ALDH expression in the SW-480 cell line and exerts its effects through the α7nAChR dependent axis. Studying the exact molecular mechanism of nicotine signaling can be useful both in diagnosing and treating nicotine-

related cancers and in identifying novel potential therapeutic benefits.
Keywords: Cancer, Nicotine, Nicotinic Receptors, Stemness, SW-480

Ps-31: Investigating The Role of Thermosensitive Hydrogel for The Sustained Delivery of Exosomes Extracted from Menstrual Blood Mesenchymal Stem Cells on Triple-Negative Breast Cancer Cells

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Objective: Due to the heterogeneity of triple-negative breast cancer (TNBC), conventional treatments respond poorly, and there is no targeted treatment for this cancer. Menstrual blood stem cell exosomes (Exo-Mens) have been shown to be potent inhibitors of tumor-induced angiogenesis in prostate and breast cancer models. This study was conducted with the aim of investigating the effects of hydrogel-containing Exo-Mens on breast cancer cells.
Materials and Methods: Optimization and characterization of the hydrogel was conducted, then MDA-MB-231 and human umbilical vein endothelial cells (HUVEC) were examined in two groups of hydrogels (control) and hydrogel with Exo-Mens. Initially, the IC_{50} of Exo-Mens was determined by the MTT assay, and the degradability and release pattern of the studied groups were measured. Western blot (VEGF, HIF-1α) and qRT-polymerase chain reaction (PCR) (VEGF, HIF-1α, and KDR) were performed for the HUVEC cell line, and scratch tests, colony formation, apoptosis, and migration for the MDA-MB-231 cell line, and invasion were performed for both cell lines.
Results: The angiogenic activity of Exo-Mens within the hydrogel, revealed a significant shift in the expression of pro-angiogenic genes and related proteins including HIF-1α, VEGFA, and KDR, in the treated HUVECs. The treatment group of hydrogel was also able to reduce migration and invasion in both cell lines compared with the control group. In addition, reduction of colony formation and an increase in apoptosis were observed, especially in cancer cells.
Conclusion: The developed hydrogel system containing Exo-Mens was able to prevent both the proliferation and invasion of tumor cells as well as the formation of angiogenic cells.
Keywords: Anti-Angiogenesis, Exosome, Hydrogel, Menstrual Stem Cells, TNBC

Ps-32: The KDM6A Upregulation Reduced The Cancerous Phenotype in The Gastric Adenocarcinoma Cell Line

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Objective: Gastric cancer is a significant global health concern. About 90% of gastric cancer cases are gastric adenocarcinoma, which ranks fifth in the world in terms of the prevalence of the disease and the third most common cause of death. One of the most common changes in the development of cancer is epigenetic changes. Methylation and demethylation of histones are among these changes. KDM6A is one histone demethylase that mutates in gastric cancer and is role-critical in the process of carcinogenesis. This gene has demethylase activity and removes the trimethyl group from histone 3 lysine 27 and causes transcription and expression. In this study, we will examine the effect of KDM6A overexpressing on the induction of apoptosis rate and reduction of proliferation, viability, and migration of AGS cells so that this molecule can be used as a therapeutic target in the field of molecular targeted therapies.
Materials and Methods: A Lentiviral vector was used to induce KDM6A force expression in AGS cells. Then, the effect of KDM6A force expression on the cancerous phenotype of cells was assessed by assessing their apoptosis, wound healing, colony formation assay, MTS assay, and gene expression in transduced AGS cells.
Results: Force expression of KDM6A induces apoptosis rate and decreases the viability, migration rate, colony formation capacity, and altered specific gene expression in the transduced AGS cell compared to the control group.
Conclusion: Force expression of KDM6A reduces cancerous phenotype which has significant advantages for the diagnosis and treatment of patients and could be hoped for targeted therapy for gastric adenocarcinoma treatment.
Keywords: Cancerous Phenotype, Gastric Adenocarcinoma, KDM6A, Lentivirus Vector, Molecular Targeted Therapy

Ps-33: KDM6A Over-Expression Attenuated Epithelial-Mesenchymal Transition and Induced Mesenchymal-Epithelial Transition in Hepatocellular Carcinoma Cells

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Objective: About 90% of liver cancer is hepatocellular carcinoma and fourth most common cancer and the third leading cause of cancer-related death in the world. Despite the common treatments after surgery, due to the remaining microscopic metastasis residues, the possibility of cancer recurrence is high. Therefore,

molecular-targeted treatments are very important. During the initiation of liver cancer, the progression of epithelial to mesenchyme transition (EMT) is observed. These changes result in increased invasion and metastasis capacity of cancer cells. In liver malignancies, one of the mutations that in the histone modification happens is the down-regulation of KDM6A. KDM6A is a histone demethylase and a key molecule in regulating cell proliferation, development, and division. Mutations of KDM6A are indicated in cancer types. This research investigates the changes in the EMT-MET (Mesenchyme to epithelial transition) process of hepatocytes after up-regulation of the KDM6A in the hepatocellular carcinoma cells

Materials and Methods: A Lentiviral vector was used to induce KDM6A overexpression in Huh-7 human hepatic cells. We evaluated the effect of KDM6A overexpression on the EMT-MET process, migration rate, wound healing, and change morphology.

Results: KDM6A upregulation reduced the migration rate, in transduced Huh-7 cells. Moreover, the expression pattern of EMT-MET process-related markers in HCC cells was changed.

Conclusion: Our study indicated that the up-regulation of KDM6A could be used as a potential non-invasive therapeutic strategy for preventing cancer metastasis and recurrence inhibition and inducing the MET process in HCC cells. It is expected that this research proposes a molecular therapeutic target, and opens innovative ways to create suitable treatments for hepatocellular carcinoma.

Keywords: Epithelial-Mesenchymal Transition, Hepatocellular Carcinoma, KDM6A, Mesenchyme to Epithelial Transition, Migration Rate

Ps-34: Innovative Liver Cell-Based Platforms; A New Insight in Drug Screening and Toxicology

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Objective: Hepatocytes plays a crucial role in drug screening due to their essential liver functions and detoxification capabilities. Current research focuses on hepatocyte toxicity, using hepatocytes sourced from normal livers, stem cell-derived hepatocytes, and various cancer cell lines. Challenges in working with these cells include limited accessibility, rapid dedifferentiation and loss function. Previous studies have explored pathways related to liver development and liver cancer, identifying key genes such as *HNF4α* a crucial transcription factor in hepatogenesis. Also, *HNF4α* is vital for preserving the phenotype and normal function of hepatocytes. Multiple studies have reported a reduction in the expression of *HNF4α* in liver cancer.

Materials and Methods: In this study a novel approach was employed to increase the expression of the key gene *HNF4α* in Huh7 cancer cells using SINEUP technology in a 3D culture. Manipulated Huh-7 were co-culture with HUVEC and MSC cells, and microparticle-derived sheep liver ECM for mimicking realistic micro environment. The micro-tissue comprised of upregulated HNF4α-Huh7 cells was designated as the experimental group, while the micro-tissue consisting of Huh-7 cells served as the control group. Comparative analyses were implemented on morphological characteristics, mRNA and protein expression, and functional test between the control and experimental microtissue groups.

Results: Significant differences were observed in the expression of metabolic genes at the mRNA, protein levels, and functional characteristics following the upregulation of the *HNF4α* gene in experimental microtissues compare to control microtissues.

Conclusion: By upregulating the expression of the *HNF4α* in Huh7 cells, this study demonstrated that the characterization and function of these cells become more similar to normal hepatocytes. The findings suggest the potential of developing 3D platforms based on engineered cancerous cells to address challenges in drug screening and toxicology in a more cost-effective manner.

Keywords: Drug Screening, Hepatocyte, HNF4α, Innovative Cell-Based Platforms

Ps-35: Tapping into The Potential of Cancer Stem Cell Gene Editing For Gene Therapy

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Objective: Glioblastoma is an aggressive brain cancer with a poor prognosis, and cancer stem cells (CSCs) are believed to play a crucial role in its development and treatment resistance. Recent advances in gene editing technologies, particularly CRISPR/Cas9, have created novel opportunities for targeting cancer stem cells in glioblastoma. The utilization of CRISPR/Cas9 has been observed in targeting crucial signaling pathways and genes responsible for maintaining glioblastoma cancer stem cells. Studies have shown that targeting the Hedgehog pathway, crucial for cancer stem cell self-renewal, can effectively inhibit their proliferation and improve treatment outcomes. Additionally, CRISPR/Cas9 has been employed to disrupt the expression of genes associated with cancer stem cell stemness, such as SOX2, Nanog, and Oct4. Downregulation of these genes has been shown to reduce the tumorigenic potential of glioblastoma cancer stem cells and sensitize them to conventional therapies. Researchers have also used CRISPR/Cas9 to target epigenetic regulators that maintain the cancer stem cell state, such as the histone demethylase JMJD3. Inhibition of JMJD3 has been proven to impair the self-renewal and differentiation capabilities of glioblastoma cancer stem cells.

Materials and Methods: In our research, we conducted a comprehensive review of data and research in this field, utilizing bioinformatics resources and in silico work, to inform our study. This approach enabled us to gather an extensive understanding of the subject and build a strong foundation for our research.

Results: Based on the GSE107560 dataset, miR-21 appears to be the most promising target for glioblastoma stem cell gene editing for GBM gene therapy. miR-21 was identified as one of the most significantly deregulated miRNAs in glioblastoma stem cells compared to differentiated tumor cells. Targeting miR-21 has been demonstrated to inhibit glioblastoma growth, and the TCGA data analysis also associated high miR-21 expression with poorer patient survival outcomes.

Conclusion: Overall, the utilization of CRISPR/Cas9 in glioblastoma cancer stem cell research offers valuable insights into the molecular mechanisms underlying cancer stem cell maintenance and has exposed new avenues for targeted therapies. As this field continues to evolve, the integration of CRISPR/Cas9-based strategies with other emerging technologies will further enrich our comprehension of glioblastoma cancer stem cells and lead to more effective treatment strategies.
Keywords: Cancer Stem Cell, CRISPR/Cas9, Gene Editing, Glioblastoma, Targeted Therapies

Ps-36: Effects of Conditioned Medium of Human Adipose-Derived Mesenchymal Stem Cells on Apoptosis of Endometriosis Cells

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Objective: Although the pathogentic mechanisms related with endometriosis are not clearly understood, apoptosis seems to play a critical role . This research is to detect if Conditioned Medium (CM) obtained from human adipose tissue-derived mesenchymal stem (AD-MSCs) can promote apoptosis in Menstrual blood-derived stem cells (MenSCs) in women with endometriosis.
Materials and Methods: About 2 mL of menstrual blood collected from infertile women. MenSCs were cultured up to passage 3 and cell surface markers were analyzed by flow cytometry. To study the effect of CM on apoptosis of endometriosis cells, Annexin V assay followed by flow cytometry analysis was used.To further analyze the effect of CM on apoptosis and survival of endometriosis cells, mRNA expression of *BAX*, *Bcl-2* and survivin was studied using real-time polymerase chain reaction (PCR).
Results: Apoptosis analysis by flow cytometry showed that late apoptosis and early apoptosis significantly increased in comparision with control group.The real-time PCR findings demonstrated that mRNA expression of *BAX* significantly increased as compared with untreated. in this study no significant differences were detected in mRNA expression of *Bcl-2* and *Survivin* between the groups.
C**onclusion:** The research demonstrate that CM could enhance apoptosis in endometriosis. Furthermore, the findings indicate that MenSCs may have a pivotal role in the pathogenesis of endometriosis and further validate the theory of endometriosis formation through retrograde menstrual blood flow.
Keywords: Apoptosis, Conditioned Medium, Endometriosis, Mesenchymal Stem Cells (MSCs), Menstrual Blood

Ps-37: The Effect of Conditioned Medium Obtained from Human Adipose-Derived Mesenchymal Stem Cells on The Restoration of Spermatogenetic Genes in A Rat Model of Azoospermia Induced by Busulfan

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Objective: For men with non-obstructive azoospermia (NOA), assisted reproductive techniques (ART) are often the only viable option to have a biological child. This study aims to investigate whether Conditioned Medium (CM) from Human adipose tissue-derived mesenchymal stem cells (AD-MSCs) can help to restore the expression of spermatogenic genes in a busulfan-induced azoospermic rat model.
Materials and Methods: To investigate the impact of CM intratestis injection on spermatogenesis recovery in a rat model of NOA , AD-MSCs were isolated and cultured. The CM was collected on the 3rd passage. The rats (30 male Wistar rats, aged 8-12 weeks) were divided into 4 groups: control group (no treatment), NOA group (busulfan-induced rats), azoospermia sham group (busulfan + Phosphate-buffered saline), and experimental group (busulfan + ADSC-CM). The NOA model was induced by intraperitoneal busulfan injections with a 21-day interval. After 35 days, the test group received ADSC-CM. Gene expression was analyzed using real-time polymerase chain reaction (PCR).
Results: The analysis indicated a significant increase($P \leq 0.5$) in the levels of DAZL, Miwi and VASA expression within the ADSC-CM group as compared to the control group, whereas a significant decrease was observed in the NOA and Sham groups.
Conclusion: This study suggests that the use of CM obtained from AD-MSCs could potentially assist in the recovery of spermatogenesis in busulfan-induced infertile rats.
Keywords: Adipose Mesenchymal Stem Cells, Conditioned Medium, Non-Obstructive Azoospermia

Ps-38: Human Three-Dimensional Acellular Amniotic Membrane Containing Adipose Tissue-Derived Mesenchymal Stem Cells; How Deep Is The Penetration of Allogenic Stem Cells into The Scaffold?

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Objective: Simulation of *in vivo* conditions for mesenchymal stem cells in a laboratory environment and placing them on a biological ECM can not only improve the structural characteristics of stem cells but also significantly increase their secretory potential and function.
Materials and Methods: In this study, mesenchymal stem cells were extracted from adipose tissue, cultured until passage 3, and confirmed by flow cytometry and differentiation potential. In the next step, the amniotic membrane separated from placenta was decellularized using mechanical and chemical methods. According to the convergent scattering pattern, activated allogeneic mesenchymal stem cells (7×10^5 cells/each scaffold) were propagated for 16±3 hours at 37°C, 5% CO_2 and 90% humidity. The structural, mechanical, biological, and physicochemical health of 3D scaffold was confirmed using SEM, MTT, FTIR and acridine orange staining methods. Safety and efficiency of 3D scaffolds were investigated in the healing process of diabetic wounds in C57 mice.
Results: We designed a three-dimensional biological scaffold composed of decellularized amniotic membrane with a diameter of 20-25 µm, which contains active allogeneic mesenchymal stem cells derived from adipose tissue with a diameter of 1.8-2.8 µm. Allogeneic mesenchymal stem cells not only settled and proliferated on the surface layer of acellular amniotic membrane but also permeated into deeper layers and formed a multilayered

structure. SEM analysis demonstrated that the allogeneic mesenchymal stem cells penetrated into the acellular amniotic membrane from a depth of 5µm to 17µm. The structural, mechanical, biological, and physicochemical health of scaffolds was confirmed using SEM, FTIR, MTT, and acridine orange staining methods. There were no allergic and immunological reactions in treated diabetic mice after treatment.
Conclusion: The 3D amniotic membrane scaffold can have wide therapeutic applications, not only in plastic surgery, tissue engineering, and regenerative medicine, but also in treatment of gynecological and reproductive system diseases, cardiovascular abnormalities, etc.
Keywords: Amniotic Membrane, Mesenchymal Stem Cells, 3D Biological Scaffold

Ps-39: Effects of Kartogenin-Enriched MSC Extracellular Vesicles with Hyaluronic Acid on Chondrogenesis of MSCs Derived from Arthritic Rats

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Objective: Rheumatoid Arthritis (RA), is an autoimmune disease that damages the synovial membrane, cartilage, and bone. Despite advances in cell therapy, RA treatment still faces significant challenges. However, extracellular vesicles (EVs) derived from MSCs show promise as future clinical alternatives. These EVs serve as nanocarriers for miRNAs, and small molecules including kartogenin (KGN), which notably promotes chondrogenesis. The primary challenge lies in targeted drug delivery. This study aims to enhance EV-KGN by applying a hyaluronic acid (HA) coating, potentially leading to more effective drug delivery and improved chondrogenesis *in vitro*.
Materials and Methods: Bone marrow-derived mesenchymal stem cells (Bm-MSCs) were isolated from an arthritis rat model. These cells were differentiated into osteogenic, adipogenic, and chondrogenic lineages. Extracellular vesicles (EVs) from BM-MSCs, obtained through ultracentrifugation, were characterized. KGN (kartogenin) was encapsulated into EVs, and a modified hyaluronic acid (HA) coating was applied. The study evaluated the chondrogenic potential of experimental groups, including EV, KGN, EV-KGN, chondrogenic media, and HA-EV-KGN, using toluidine blue staining and qPCR to measure gene expression related to chondrogenesis.
Results: The identity of Bm-MSCs was confirmed based on their spindle-shaped morphology and differentiation into skeletal lineages. EV-MSCs were characterized by their size (70 to 150 nm) and the presence of the EV marker CD63. Histological results showed cartilage cell matrix formation in all experimental groups. As qPCR, all experimental groups had significantly higher expression of chondrogenic genes (COL II, SOX9, and COL X) compared to control MSCs. Notably, the HA-EV-KGN group exhibited significantly higher expression of these genes compared to other experimental groups (**P<0.01).
Conclusion: The HA-EV-KGN group showed significantly increased delivery of hyaluronic acid (HA)-enriched extracellular vesicles (EVs) targeting chondrogenesis compared to other groups. Importantly, using HA-EVs led to reduced drug consumption. Overall, HA-EV-KGN holds promise as an effective system for targeted drug delivery and therapeutic applications.
Keywords: Extracellular Vesicles, Chondrogenesis, Hyaluronic Acid, Mesenchymal Stem Cells, Kartogenin

Ps-40: Reversing Hepatic Stellate Cell Activation to Attenuate Liver Fibrogenesis by Placenta Derived Biomaterials

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Objective: Liver diseases result in two million deaths annually and pose a significant global health challenge. Anti-fibrotic therapies focus on hepatic stellate cells as the primary fibrogenic cells to alleviate liver fibrosis, a condition that develops following liver diseases in an inflammatory and oxidative environment. Recent progress in biomaterials has introduced promising strategies in regenerative medicine. The decellularized extracellular matrix (ECM) from the placenta has been identified for its antioxidative, anti-inflammatory, and regenerative properties.
Materials and Methods: LX2 cells were stimulated with TGFb for 24 hours and then treated with placental biomaterials for 24 hours. Cell proliferation was determined by BrdU assay. Intracellular ROS levels were evaluated using 2',7'-dichlorofluorescin diacetate (DCFH-DA) staining and mRNA expression of oxidative stress related genes was shown. Protein expression and mRNA expression of ECM components were evaluated. Cell migration was evaluated using trans-well assay and mRNA expression of EMT-related genes was determined.
Results: Placental biomaterials attenuate hepatic stellate cell activation through regulating the cell proliferation, ROS accumulation, ECM expression, and cell migration.
Conclusion: Biomaterials derived from the placenta show potential as a promising treatment for reducing fibrogenic factors associated with liver fibrosis. These innovative placental biomaterials offer a hopeful avenue for addressing the underlying mechanisms contributing to liver fibrosis and may pave the way for more effective therapeutic interventions in this challenging condition.
Keywords: Biomaterials, Hepatic Stellate Cell, Liver Fibrosis, Regenerative Medicine

Ps-41: Designing of Single Guide RNA for Melanogenesis Assay in Zebrafish

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Objective: Many human diseases are deeply intertwined with genetics, underscoring the critical importance of understanding this relationship in the context of therapeutic interventions. Among the array of genetic manipulation technologies, CRISPR-Cas9 stands out for its unparalleled precision in gene editing. Widely embraced for its ability to generate animal and cell models, CRISPR-Cas9 holds significant promise for advancing

genetic studies. Zebrafish, with their transparent embryos and rapid developmental pace, emerge as particularly valuable models for exploring the genetic underpinnings of human diseases. Notably, the *SLC24A5* gene is recognized as a key player in melanogenesis, with mutations often associated with various forms of albinism. In this study, our aim is to design and clone a single guide RNA (sgRNA) targeting the *Slc24a5* gene in zebrafish.
Materials and Methods: Our methodology hinges on precise molecular biology techniques. Initially, we retrieve the *Slc24a5* gene sequence from public databases, such as NCBI, followed by rigorous bioinformatic analysis to design sgRNAs using specialized tools like CHOP CHOP. The designed sgRNA constructs are then seamlessly integrated into the DR274 vector through enzymatic digestion and ligation using T4 enzyme at 37°C. The resulting constructs are introduced into competent E. coli cells for amplification, followed by cultivation in LB Broth medium and long-term preservation at -70°C.
Results: Validation of sgRNA integration into the vector was achieved through a series of meticulous experimental procedures, including colony polymerase chain reaction (PCR) and digestion assays. Furthermore, Sanger sequencing served as a robust quality control measure, ensuring the fidelity of the genetic constructs and guarding against unintended mutations. Three colonies were selected following successful confirmation procedures.
Conclusion: In the DR274 plasmid, sgRNA oligos are cloned under the control of the SP6 promoter, facilitating *in vitro* transcription of sgRNA. The resulting product holds potential for the knockout of the *Slc24a5* gene in zebrafish, thus offering a valuable model for studies related to albinism.
Keywords: CRISPR, Zebrafish, Genetic Manipulation, Melanogenesis

Ps-42: Three-Dimensional NanoFibers Play The Key Role in Regeneration of Neural Stem Cells

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Objective: To begin, spinal diseases, particularly spinal cord injuries (SCI), are severe conditions with no current cure, leading to significant burdens on patients due to permanent neurological impairments. Stem cell-based therapies, specifically neural stem cells (NSCs), have emerged as promising options for repairing injured spinal cords. Neural stem cells are multipotent stem cells capable of differentiating into neuronal and neuroglial cells, making them ideal candidates for regenerating damaged spinal cords. Controlling the fate of neural stem cells is crucial for effective spinal cord regeneration. Neural stem cells possess therapeutic potentials such as modulating inflammatory environments, supporting axonal growth, and differentiating into neural cells, all essential for spinal cord injuries repair. Nanofibers play a pivotal role in regulating the fate of stem cells for spinal cord regeneration. Electrospun fibers, with their ability to provide physical guidance cues and support axonal regeneration, have shown promise in neural tissue engineering applications, including spinal cord injury research. The latest innovation in this field involves three-dimensional nanofibers, which offer a solution to the limitations of conventional three-dimensional scaffolds by providing precise structures that match the spinal cord's architecture. These three-dimensional nanofibers can stimulate and guide axon regeneration, promoting functional recovery in spinal cord injuries.
Materials and Methods: In the course of our investigation, we undertook a thorough examination of data and studies within this particular domain. This methodology facilitated the acquisition of a profound comprehension of the topic and the establishment of a robust basis for our investigation, thereby guaranteeing that our discoveries are well-supported and rooted in current scientific understanding.
Results: By combining neural stem cells with three-dimensional biomimetic scaffolds, a significant advancements in promoting axonal regeneration and functional recovery in spinal cord injuries have been achieved, showcasing the potential of nanofibers in enhancing stem cell-based therapies for spinal cord regeneration.
Conclusion: In conclusion, the use of nanofibers, especially three-dimensional nanofibers, represents a cutting-edge approach in the field of spinal cord regeneration, offering new insights and possibilities for controlling the fate of neural stem cells and ultimately improving outcomes for patients with spinal cord injuries.
Keywords: Nanofiber, Neural Stem Cells, Regenerative

Ps-43: Anti-Inflammatory Effects of TAK-242-Enriched MSC Extracellular Vesicles with Hyaluronic Acid on Mesenchymal Stem Cells Derived from Arthritic Rats

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Objective: Rheumatoid arthritis (RA) is a chronic autoimmune disease that leads to bone and cartilage destruction. Inhibiting toll-like receptor (TLR) activity can halt inflammation. TAK242, a TLR4 inhibitor, has been used in RA and sepsis. On the other hand, MSCs- extracellular vesicles (EVs) are effective drug carriers. Recently, the surface modification of EV with hyaluronic acid (HA) is a promising targeted drug delivery method. This study investigates the effect of HA-modified EV-TAK242 on inflammation reduction.
Materials and Methods: MSCs were isolated from arthritic rat bone marrow and differentiated into osteogenic and adipogenic lineages. Optimal TAK-242 dosage was determined via the MTS test. EV-MSCs were obtained from the Royan Institute's ATMP center and characterized using DLS, western blot, and scanning electron microscopy (SEM). TAK-242 was introduced into EVs through electroporation. The EV surfaces were modified with hyaluronic acid. Inflammation amplified into rMSCs using LPS. After 48 hours, the anti- inflammatory effects of EV, TAK-242, EV-TAK242, and HA-EV-TAK-242 were examined on rMSCs. Subsequently, the expression of cytokines TGF-ß, IL-10, TNF-α, IL-6, and NK-KB were evaluated through qPCR, ELISA, and immunocytochemistry (ICC) techniques.
Results: Spindle-shaped morphology and rMSC differentiation into skeletal lineages confirm their mesenchymal characteristics. The identity of EVs was validated through the presence of

the CD63 marker and an EV size range of 70-150 nm. Post-treatment, the expression of anti-inflammatory genes TGF-β and IL-10 significantly increased across all groups compared to the control after 48 hours (**P<0.01). However, there was no significant difference in the expression of these genes between the TAK242 and EV groups (**P<0.001, *P<0.01). The ICC analysis showed the NF-κB expression decreased in rMSCs after 48 hours. ELISA assessment showed TNFα and IL-6 cytokines were decreased in all groups in comparison to the control group, while it was not statistically significant.
Conclusion: This study shows that HA-EV-TAK242 can reduce inflammation via labeling drugs for targeted release.
Keywords: Anti- Inflammatory Properties, Extracellular Vesicles, Hyaluronic Acid, Mesenchymal Stem Cells, TAK-242

Ps-44: Histopathological Evaluations of The Colon in Induced Ulcerative Colitis: The Role of The Supernatant of Mesenchymal Stem Cells Containing 17-Beta Estradiol

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Objective: The role of estrogen in the accelerating regeneration of the intestinal mucosa and the presence of estrogen receptors on the surface of mesenchymal stem cells (stimulator of proliferation and differentiation of MSC) led us to investigate the role of the supernatant of mesenchymal stem cells containing 17-beta estradiol in the histopathological changes of the colon of rats suffering from ulcerative colitis (UC).
Materials and Methods: Following the induction of ulcerative colitis in all groups [colitis (C), mesenchymal (MSC) and MSC + estradiol (MSC + EST)] except the control, in 2 groups MSC and MSC + EST respectively: 200 µl supernatant of $2x10^6$ mesenchymal stem cells and 200 µl supernatant of $2x10^6$ mesenchymal stem cells treated with 17-bED were injected 3 times during 10 days (IP). After the experimental period, Tissue specimens were collected and following the tissue passage and section, the slides were stained by H&E for histopathological evaluations.
Results: The results obtained from the colon tissue of the C group showed the presence of many inflammatory cells in the mucous layer (the space between the crypts), many eosinophilic cells in the sub-epithelial, collagenous colitis in the mucosa, severe vascular congestion in the submucosa and as well as the loss of normal structure of the epithelial cells. In the mucosal layer of MSC groups and especially MSC + EST, inflammatory cells were seen scattered along with a decrease in eosinophil cells and collagenous colitis. Mild to moderate vascular congestion and loss of epithelial cells and crypts were reported in some areas.
Conclusion: The present study showed the improvement of histopathological symptoms caused by the induction of UC following the use of mesenchymal stem cell supernatant, especially in MSC + EST group.
Keywords: Histopathology, Mesenchymal Stem Cells, 17-Beta Estradiol

Ps-45: Evaluation The Levels of Inflammatory Cytokines TNF-α, IL-1 and IL-6 in Acetic Acid Induced Ulcerative Colitis in Rats Treated with Condition Medium of Mesenchymal Cells Treated with 17-Beta Estradiol

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Objective: Ulcerative colitis (UC) is a form of inflammatory bowel disease with increased levels of inflammatory cytokines. The present study aims to investigate the ameliorating effects of condition medium of mesenchymal cells (CMm) treated with 17-beta estradiol (17-bED) on the level of inflammatory cytokines in an animal model of UC.
Materials and Methods: In this study, 40 male Wistar rats (150-200 g) were randomly placed into 4 groups; control, colitis (C), mesenchyme (MSC) and MSC+estradiol (MSC+EST). MSCs obtained from the bone marrow of male Wistar rats were cultured in the presence of 100 nM 17-bED for 24 hours, and UC was induced using 4% acetic acid in all groups except the control group. In the MSC and MSC+EST groups, after UC induction, respectively: 200 µl CMm obtained from $2x10^6$ cells and 200 µl CMm obtained from $2×10^6$ cells treated with 17-bED were injected intraperitoneally 3 times during 10 days. At the end, the rats were sacrificed and the mRNA expression of cytokines, including TNF-α, IL-1 and IL-6 in homogenous colon tissue samples was performed by RT-PCR method.
Results: The results showed that the highest and lowest levels of mRNA expression of cytokines were respectively in colitis and control groups, which had significant differences with each other and with other groups (P<0.05). Also, the obtained results indicate that the cell groups (MSC and MSC+EST) significantly reduce the levels of TNF-α, Il-1 and Il-6 compared to the Colitis group and even the MSC+EST group shows a significant decrease compared to the MSC group (P<0.05).
Conclusion: The present study showed the effect of CMm and especially CMm containing 17-beta estradiol in reducing the levels of inflammatory factors.
Keywords: Condition Medium, Inflammatory Cytokine, Ulcerative Colitis,17-Beta Estradiol,

Ps-46: Ameliorating Role of Condition Medium of Mesenchymal Cells Treated With 17-Beta Estradiol on The Oxidative Factors in An Experimental Model of Ulcerative Colitis

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Objective: Application of the condition medium obtained from the culture of mesenchymal stem cells accelerates the regeneration process of damaged tissue by reducing the levels of oxidants. The present study aimed to the role of condition medium of mesenchymal cells treated with 17-beta estradiol (17-BED) on the levels of myeloperoxidase (MPO) and nitric oxide (NO) in acetic acid induced ulcerative colitis (UC) in male rats.
Materials and Methods: MSCs obtained from the bone marrow of male Wistar rats were cultured in the presence of 100 nM 17-bED for 24 hours, and UC was induced using 4% acetic acid in colitis(C), mesenchyme (MSC) and MSC+estradiol (MSC+EST) groups. After UC induction, in the MSC and MSC+EST groups, respectively: 200 µl CMm obtained from $2×10^6$ cells and 200 µl CMm obtained from 2x106 cells treated with 17-bED were injected intraperitoneally (3 times during 10 days). After 10 days, the rats were sacrificed and the levels of MPO and NO in homogenous colon tissue samples was per-

formed.
Results: The results of this study showed a significant increase in MPO and NO in the C group compared to other groups ($P<0.05$) that MSC and MSC+EST groups were able to create a significant decrease ($P<0.05$). Also, the level of NO in the MSC+EST group was significantly reduced compared to the MSC group ($P<0.05$).
Conclusion: This study showed that CMm and CMm containing 17-beta estradiol decreased MPO and NO by ameliorating the levels of oxidants.
Keywords: Myeloperoxidase, Nitric Oxide, 17-Beta Estradiol, Ulcerative Colitis

Ps-47: Gene Expression Evaluation to Investigate Novel Hub Gene in Cardiomyocyte Differentiation

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Objective: Heart dysfunction is one of the most problems in our country. To solve that we have many traditional approaches, such as heart transplantation; however, this therapeutic way has some challenges.
Materials and Methods: So, the cellular therapy used alternative approaches in this way. The problem in this novel wat lacks of molecular information about the cardiomyocyte differentiation. To this end, we used bulk-RNA-seq data to gain differential expression genes (DEGs) by GEO2R software. In addition, to find protein-protein interaction network authors utilize STRING database via Cytoscape software. besides these programs, we used the CentiScape package to find hub genes. In the current study, we used GSE18514 data to indicate 4624 DEGs. To understand more about the protein interaction PPIs network constructed
Results: The ACTB node reported as main hub gene in cardiomyocyte differentiation from stem cells for the first time in this study.
Conclusion: The results of this study could be used to produce cardiomyocyte tissue as part of heart muscle, which increases the chance of cell therapy approaches in future.
Keywords: Bioinformatics, Cardiomyocyte, Cell Therapy, Differentiation, Heart Failure

Ps-48: Using Transgenic Zebrafish Model to Evaluation of Hyperglycemia Effects

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Objective: Diabetes mellitus (DM) is characterized by sustained elevation of systemic glucose levels. Three major complications of DM, collectively termed triopathy, comprise diabetic neuropathy, nephropathy, and retinopathy. Hyperglycemia, a key diagnostic parameter in both types of diabetes, significantly contributes to these complications, notably impairing sensory and/or motor functions affecting 50 percent of patients with this metabolic disorder. While a cohort of diabetic animal models has been established to investigate diabetes-related nerve disorders, the emergence of the zebrafish (Danio rerio) as a potent new model organism in central nervous system (CNS) disease modeling offers distinct advantages in this area. This research, conducted at the Kawakami zebrafish lab at the National Institute of Genetics (NIG, Japan), aimed to assess the impact of hyperglycemia on the nervous system and pancreas, utilizing two transgenic zebrafish lines with green fluorescent protein (GFP) expression in the entire nervous system [SAGFF(LF)19B] and pancreatic cells (gSA2AGFF323A).
Materials and Methods: Hyperglycemia was induced in transgenic larvae at 5 days post-fertilization by exposure to a 120 mM glucose solution for 5 days. Subsequently, the GFP area of transgenic larvae was quantified using Fiji software and statistically analyzed using Prism software in both treatment and control groups.
Results: The results revealed a significant decrease in the GFP area in treated larvae compared to the control group. For the SAGFF(LF)19B line (n= 15, ***P=0.0002) and the gSA2AGFF323A line (n= 30, **P =0.0053), this decrease was statistically significant.
Conclusion: Prior studies have highlighted that hyperglycemia in zebrafish larvae leads to motor axon defasciculation, disruptions in perineurial glial sheath formation, reduced myelination of motor axons, and mislocalization of sensory neurons. This study further demonstrates that a 5-day exposure of larvae to a 120 mM glucose solution results in damage to the nervous system and impairs pancreas development.
Keywords: Diabetes Mellitus, Diabetic Neuropathy, Hyperglycemia, Transgenic Zebrafish,

Ps-49: Rapid Synthesis of Silver Nanoparticles Using Henna Extract for Induced Apoptosis on Retinoblastoma Cell Line

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Objective: This study aims to rapidly synthesize silver nanoparticles (AgNPs) using Henna extract to trigger apoptosis in retinoblastoma cells, a common intraocular cancer in children. Green synthesis methods are utilized for their reproducibility, biocompatibility, and lack of toxicity, with Henna extract selected for its medicinal properties.
Materials and Methods: AgNPs are synthesized with Henna leaf extract and a 0.01 molar silver nitrate solution. Physicochemical properties of the AgNPs, including size, surface charge, and morphology, are characterized using techniques such as spectrophotometry, energy-dispersive X-ray spectroscopy (EDS), dynamic light scattering (DLS), and field emission scanning electron microscopy (FESEM). Toxicity tests are performed on retinoblastoma (Y79) and retinal pigment epithelia (ARPE19) cells to evaluate cellular viability, apoptosis and mitochondrial function with flowcytometry used to assess apoptotic and necrotic cell percentages.

Results: Successful synthesis of AgNPs is confirmed by a peak at 400 nm in spectrophotometric analysis. DLS indicates an average size of 103 nanometers with a surface charge of -43.5 mV. EDS verifies 50% silver content in the nanoparticles, while FESEM images show spherical nanoparticles. Toxicity tests on the Y79 cancer cell line demonstrate significantly higher toxicity at 100 mg/ml compared to Henna extract or silver solution alone, with no impact on healthy ARPE19 cells. Staining and apoptosis assays reveal cytotoxic effects on cancer cells, resulting in around 40% cell death versus <10% in healthy cells. The AgNPs induce mitochondrial dysfunction and nuclear abnormalities in cancer cells, with 15% undergoing necrosis and 23% experiencing late-stage apoptosis per flow cytometry analysis.
Conclusion: Green-synthesized AgNPs exhibit stabilized, eco-friendly properties with improved anticancer effects. Cytotoxicity assessments on the Y79 cell line suggest the potential of AgNPs as an effective cancer treatment delivery method. Further research in this area could lead to enhanced strategies for tackling difficult tumors.
Keywords: Green Synthesis of Nanoparticles, Retinoblastoma, Henna Plant, Silver Nanoparticles, Y79 Cell Line

Ps-50: Enhancing Dental Pulp Stem Cell Potential: Chick Embryo Extract Preconditioning For Neurotherapeutic Applictions

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Objective: Dental pulp stem cells (DPSCs) are promising candidates for cell-based therapies in neurodegenerative diseases, exhibiting potential for neural lineage differentiation and promoting tissue regeneration through paracrine effects. However, challenges such as limited *ex vivo* expansion and inefficient differentiation into neural lineages hinder their clinical application. In the present study, we aimed to explore the impact of chick embryo extract (CEE) preconditioning on the properties of DPSCs during *in vitro* cultivation, aiming to enhance their suitability for neurotherapeutic applications.
Materials and Methods: DPSCS were isolated from the pulp region of rat teeth and cultured in media supplemented with either 10% fetal bovine serum (FBS) alone or combined with 10% CEE. The study assessed cell multipotency, morphological changes, and gene expression associated with paracrine effects across both experimental conditions.
Results: Our results showed that CEE supplementation can preserve the rate of DPSCS multipotency. The preconditioned stem cells acquire neural-like morphology with longer neurites. Furthermore, CEE preconditioning upregulates the expression of brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and vascular endothelial growth factor (VEGF).
Conclusion: These outcomes indicate that CEE preconditioning could equip DPSCs with beneficial attributes during *in vitro* culture, potentially improving their efficacy in treating neurodegenerative disorders. This approach represents a significant advancement towards leveraging the full therapeutic potential of dental pulp stem cells in neurotherapeutic applications.
Keywords: Chick Embryo Extract, Dental Pulp Stem Cells, Neurotrophic Factors, Preconditioning

Ps-51: Neuroprotective Effect of Hair Follicle Stem Cell-Derived Conditioned Medium in An Aschemia Model of Astrocytes

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Objective: Hair follicle stem cells (HFSCs) have emerged as promising candidates for cell-based therapy in stroke preclinical studies. The therapeutic potential of HFSCs towards neuroprotection is essentially based on their paracrine effects, referred as secretome. Secretome contains various biologically active molecules including growth factors, chemokines, and exosomes. The paracrine effect of HFSCs is suggested as a key mechanism contributing to the protection and restoration of brain cells after ischemic injury. Astrocytes play pivotal roles during brain ischemia, and understanding their role in HFSC-mediated protection against ischemic injury is crucial. This study investigates HFSC-derived secretome in an astrocyte ischemia model to assess its protective effects and underlying mechanisms for potential stroke therapy.
Materials and Methods: This study utilized an *in vitro* model of ischemic stroke using primary astrocyte cultures exposed to 24 hours oxygen-glucose deprivation (OGD) conditions. Subsequently, OGD-induced astrocytes were treated with HFSC conditioned medium (HFSC-CM) for 48 hours to create an environment rich in paracrine factors. The neuroprotective effect of HFSC-CM on injured astrocytes was evaluated using the MTT assay, apoptosis flow cytometry, and qRT-PCR.
Results: The HFSC-CM mitigated cell death and apoptosis in OGD-induced astrocytes. Additionally, the HFSC-CM reduced the mRNA expression levels of pro-inflammatory cytokines IL-6, IL-1β, and TNF-α compared to the injured cells. Furthermore, it upregulated the mRNA levels of neurotrophic factors BDNF, and VEGF after OGD in astrocytes.
Conclusion: These findings suggest that the reparative effects of the secretome are associated with astrocyte neuroprotection, anti-inflammatory properties, and anti-apoptotic effects. The neuroprotective effect of HFSC secretome may be associated with the upregulation of neurotrophic and angiogenic factors. The restored astrocytes create a conducive environment for repair, thereby expediting the recovery of impaired brain function. This study provided preclinical evidence for further use of HFSC secretome in stroke therapy to improve the treatment outcome of patients who suffered from ischemic stroke.
Keywords: Astrocyte, Hair-Follicle Stem Cell, Ischemic Stroke Model, Neuroprotection, Oxygen-Glucose Deprivation

Ps-52: Novel Approached for Identification Prognostic Biomarker for Osteosarcoma base on Data-Mining and scRNA-Seq

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Objective: Osteosarcoma is considered one of the most severe types of bone cancer, with a 5-year survival rate as low as 40% in younger age groups. The lack of validated methods for prognosing this disease leads to individuals suffering and dying due to insufficient biomarkers. Therefore, identifying the most effective and patient-specific biomarkers associated with prognosis could significantly enhance their quality of life and overall health.
Materials and Methods: For this goal, an extensive literature search was carried out in the Scopus database (https://www.scopus.com/home.uri) to pinpoint relevant prior studies centered on discovering osteosarcoma biomarkers using bioinformatics workflows. Initially, 832 pertinent articles were identified through this process. Subsequent refinement and analysis reduced this figure to 99 articles that satisfied the inclusion criteria for our study. The search queries and filtering criteria primarily focused on articles containing keywords such as “osteosarcoma”, “biomarker”, and “prognosis”. Through data mining, all potential markers mentioned in each article were extracted and organized into terms pertinent to osteosarcoma. Finally, a list was compiled featuring related terms (38 terms) and genes. For future analysis and identification of the most effective prognostic markers for osteosarcoma, a single-cell RNA sequencing (scRNA-seq) dataset encompassing six osteosarcoma patients was retrieved from the NCBI Gene Expression Omnibus (GEO) database. During this process, all data underwent analysis using the Seurat package within R Studio (R version 4.3.3, https://www.r-project.org/). Cells exhibiting expressions below 300 or above 7000 were excluded, as were those with a mitochondrial percentage exceeding 15%. Subsequently, variable features were identified in all normalized samples using the "FindVariableFeature" function to pinpoint the top 2000 genes and the initial 30 principal components (PCAs) for cluster identification with a resolution of 0.5. Integration of all samples and the removal of batch effects were achieved utilizing the "rPCA" integration pipeline within the Seurat package, followed by cluster annotation. Ultimately, our investigation focused on establishing the relationship between each marker's expression in our integrated samples to discover an optimal biomarker panel associated with the osteosarcoma disease term through the utilization of scRNA-seq and data-mining techniques. Remarkably, we identified a significant relationship between specific biomarkers, such as “DCN” and “CD74” with the prognosis of osteosarcoma.
Results: Results were merged into material and methods.
Conclusion: Based on our findings, we propose DCN and CD74 as the best biomarkers for assessing osteosarcoma prognosis. However, *in vitro* validation assessments are needed for future results and to propose the best biomarker panel for the prognosis of osteosarcoma.
Keywords: Osteosarcoma, Biomarker, scRNA-seq, R Studio, Prognosis

Ps-53: Multi-Omics Approach Identifies ACADM as a Biomarker for Head and Neck Cancer

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Objective: Head and neck squamous cell carcinoma (HNSCC) ranks as the seventh most prevalent cancer globally and is increasingly associated with poor lifestyle choices. Despite advancements in therapeutic strategies, the 5-year survival rate for early-stage (I–II) HNSCC patients ranges from 70–90%, whereas it declines to approximately 40% for those with advanced stages (III–IV). This disparity highlights the heterogeneity inherent in HNSCC. The identification of reliable diagnostic and prognostic biomarkers could markedly enhance life expectancy and overall patient health.
Materials and Methods: In this context, analysis of RNA expression data was conducted to evaluate differentially expressed genes (DEGs). A binomial deep learning algorithm was employed to ascertain genes with notable variability in expression. The study also included comprehensive evaluations through protein-protein interaction (PPI) networks, the construction of Receiver Operating Characteristic (ROC) curves, and single-cell RNA sequencing (scRNA-seq) assessments.
Results: The findings indicate a strong association of ACADM with the carcinogenic processes in HNSCC, underscoring its potential as a significant biomarker. These insights pave the way for improved diagnostic precision and therapeutic outcomes, ultimately enhancing patient management and survival rates in HNSCC. Additionally, integrating ACADM expression patterns with clinical data may offer novel insights into the stratification of patient risk groups, enabling more personalized treatment approaches.
Conclusion: Lastly, ongoing research into the modulation of ACADM activity could lead to the development of targeted therapies that specifically exploit metabolic vulnerabilities in HNSCC, offering a promising avenue for enhancing treatment efficacy and reducing recurrence rates.
Keywords: Biomarker, Deep Learning, HNSCC, Prognosis, scRNA-seq,

Ps-54: Resveratrol-loaded PLA-PEG nanoparticles trigger apoptosis in 4T1 Breast Cancer Cells

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Objective: Researchers continue to grapple with the challenge of effectively treating cancer while minimizing the adverse effects associated with current therapies. In light of this, there is a growing need to investigate the potential of natural sources to provide anticancer antioxidants as an alternative to chemo

drugs. Resveratrol is a natural polyphenol found in grape skins, plums, peanuts, and bilberries. It has various therapeutic effects, including modulation of cell-signaling pathways, antioxidant properties, anti-aging attributes, anti-inflammatory effects, anticancer properties, and neuroprotective qualities. Polymeric nanoparticles are highly effective as nanocarriers due to their outstanding properties, including biocompatibility, biodegradability, non-toxicity, non-immunogenicity, and the ability to control drug delivery. The present study aimed to evaluate the effects of Resveratrol-loaded PLA-PEG nanoparticles [polylactic acid–poly (ethylene glycol)] on the cell death mechanism by increasing the expression of pro-inflammatory genes.

Materials and Methods: 4T1 cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) and supplemented with 10% fetal bovine serum and 100 ug/ml penicillin/streptomycin. Cell lines were kept in a CO^2 incubator at 5% CO^2 and 37° C temperatures. 4T1 cells were treated with Resveratrol-loaded PLA-PEG nanoparticles (0, 100 µM) for 1, 12, and 24 hours. Cells were trypsinized and frozen to perform quantitative RT-PCR experiments

Results: Real-time polymerase chain reaction (PCR) analysis showed that Resveratrol-loaded PLA-PEG nanoparticles significantly increased the transcriptional levels of IL-1β and TNF-α after 24 hours. However, no significant changes were observed in the expression of IL-1B and TNF-a mRNA levels after treatment with Resveratrol-loaded PLA-PEG nanoparticles for 12 hours.

Conclusion: Finally, our study found that the Resveratrol-loaded PLA-PEG nanoparticles caused apoptosis in the breast cancer cell line, effectively eliminating 4T1 cells. This indicates that PLA–PEG polymeric nanoparticles can be used as a reliable method to release resveratrol and control the growth of breast cancer cells.

Keywords: Cancer, PLA-PEG Nanoparticles, Resveratrol, 4T1

Ps-55: Cytotoxic Effect of Gold Nanoparticles-Conjugated Thymoquinone in Human MDA-MB-231 Breast Cancer Cells

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Objective: Breast cancer is the most prevalent form of cancer in women worldwide. Detecting it in its early stages and identifying potential drugs to combat multi-drug resistant metastatic breast cancers offers the potential for improved treatment options and extended life expectancy. Following the selection of the phytochemicals, attention is then directed towards the delivery systems that possess inert properties and are capable of effectively transporting drugs to specific cancer sites. Extensive research has been conducted by scientists on various delivery systems, including nanoparticles such as silver and gold, as well as liposomes. These investigations have been carried out both in controlled *in vitro* and within living organisms, to enhance therapeutic efficacy against different types of cancers, as well as metastatic and drug-resistant tumors. Among the nanoparticles, gold nanoparticles (AuNPs) have been widely utilized as drug delivery systems due to their ease of synthesis, large surface area, ability to be easily functionalized, and high biocompatibility. This new formulation shows promise in effectively delivering hydrophobic chemotherapeutic to tumors for cancer treatment.

Materials and Methods: The study aimed to assess the anti-metastatic effects of thymoquinone in combination with gold nanoparticles (AuNP-thymoquinone) in *in vitro* models (MDA-MB 231).

Results: The combination treatments of AuNP-thymoquinone were highly effective in the apoptosis of breast cancer cells (MDA MB 231) *in vitro*. In-depth studies on gene expression showed that these anti-cancer effects were linked to the suppression of *VEGF* and *HOXB9* genes, while the overexpression of the apoptotic *Caspase-3* gene was observed.

Conclusion: The findings suggest that administering gold-conjugated thymoquinone formulations could potentially impact the effectiveness of chemotherapy. This research is strongly backed by data obtained from cell-based experiments and molecular investigations.

Keywords: Breast Cancer Cell Lines (MDA-MB 231), Gold Nanoparticles, Thymoquinone

Ps-56: Effects of Mesenchymal Stem Cell Transplantation on Major Adverse Cardiovascular Events and Cardiac Function Indices in Patients with Chronic Heart Failure: A Systematic Review and Meta Analysis of Randomized Controlled Trials

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Objective: Trials on efficacy of mesenchymal stem cells (MSCs) transplantation in heart failure (HF) have been controversial. This study was conducted to investigate whether MSCs transplantation after HF could improve clinical outcomes and myocardial indices.

Materials and Methods: Using systematic approach, electronic databases were searched for randomized controlled trials (RCTs) until July 2023. Outcomes of interest included clinical outcomes and myocardial function indices. Using random-effects method, relative risk (RR) , mean difference (MD) and corresponding 95% confidence intervals (CI) were pooled.

Results: In Seventeen RCTs with 1684 patients (927 intervention, 757 control) the mortality RR was 0.78 (0.62; 0.99) in the MSC group compared to controls. Rehospitalization only decreased significantly in autologous MSC group [RR= 0.67 (0.49; 0.90)]. LVEF significantly increased among those receiving MSC [MD= 3.38 (1.89; 4.87)]. LVESV [MD= -9.14 (-13.25; -5.03)], LVEDV [MD= -8.34 (-13.41; -3.27)], and scar size [standardized MD= -0.32 (-0.60; -0.05)] significantly decreased. NYHA class [MD= -0.19 (-0.34; -0.06)], and MLHFQ [MD= -11.55 (-16.77; -6.33)] significantly decreased and 6-minute walk test significantly improved [MD = 36.86 (11.22; 62.50)] in the MSC group. Heart failure cause did not influence outcomes. However, trials with the autologous source of cells, doses less than 100 million cells, and intracoronary injection performed significantly better in some outcomes.

Conclusion: Transplantation of MSCs for HF patients may reduce all-cause mortality and improve clinical condition. Moreover, this treatment would improve left ventricular function indi-

ces and reduce scar size.
Keywords: Cardiomyopathy, Heart Failure, Meta-Analysis, Mesenchymal Stem Cells, Randomized Controlled Trials

Ps-57: Effectiveness of Stem Cell Transplantation Therapy Using Mesenchymal Stem Cells Versus Bone Marrow-Derived Mononuclear Cells in Heart Failure Patients: A Meta-analysis of Randomized Controlled Trials

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Objective: Mesenchymal stem cells (MSCs) and bone marrow-derived mononuclear cells (BM-MNCs) transplantation have emerged as heart failure therapies. However, clear comparison between them has never been established. This meta-analysis aimed to compare the cardiovascular outcomes of MSCs and BM-MNCs therapies.
Materials and Methods: Pertinent studies in online databases were systematically searched to find eligible randomized trials on heart failure patients receiving either MSCs or BMMNCs. Clinical outcomes and echocardiographic indices were included. Random-effects approach was used to combine the relative risk (RR) or mean difference (MD) along their respective 95% confidence intervals (CI).
Results: A total of 32 studies with 2487 patients (1356 in intervention, 1131 in control group) were included. Stem cell therapy did not yield reduced risk of MACE [RR 0.83 (0.64; 1.06)] but could decrease the risk of all-cause death by 21% [RR 0.79 (0.64; 0.97)] and marginally decrease rehospitalization by 24% [RR 0.76 (0.59; 1.00)] compared with placebo with no between-subgroup differences (P>0.05). Transplantation of stem cells resulted in a significant increase in LVEF [MD 3.00 (1.80; 4.20)] and LVESV [MD -8.02 (-13.24; -2.80)] but not LVEDV [MD -4.11 (-10.35; 2.12)] with no difference between stem cells (P>0.05).
Conclusion: Stem cell therapy with BMMNCs and MSCs demonstrated efficacy in improving LVEF and LVESV and also decrease rehospitalization and all-cause death although no change in MACE was found. Both types of stem cells are efficient in patients with heart failure with no significant difference between cells.
Keywords: Bone Marrow-Derived Mononuclear Cells, Mesenchymal Stem Cells, Heart Failure, Randomized Controlled Trials, Meta-Analysis

Ps-58: Cell Spheroids Derived from Human Keratinocytes and Fibroblasts as Biological Models

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Objective: Current skin models *in vitro*, including skin equivalents, skin-on-chip and organoids, reproduce the architecture and cellular composition of native skin well. However, their fabrication methods are time-consuming and laborious. This has led to the search for new approaches to human skin modeling. One such approach involves the use of cell spheroids. In this study, we aimed to investigate the effect of formation conditions on the structure and properties of keratinocyte and fibroblast multicellular spheroids in order to determine optimal co-culture conditions for achieving a histotypic structure.
Materials and Methods: Four experimental groups of spheroids were formed: two groups of monospheroids composed of HaCaT keratinocytes or 977hTERT fibroblasts, and two groups of heterospheroids derived from co-cultures of these cells. Spheroids were analyzed using immunocytochemistry (ICC), quantitative real-time polymerase chain reaction (qRT-PCR), western blotting, biomechanical tests, phase-contrast and confocal microscopy.
Results: Diameter measurements have shown that fibroblast monospheroids increased in size, while the diameters of other groups gradually decreased. Reactivation experiments have demonstrated that heterospheroids spread out on a substrate more actively than monospheroids. Biomechanical tests revealed differences in stiffness between groups of mono- and heterospheroids. Localization of protein markers (keratin 19, ZO-1, vimentin, collagen I) and epidermal differentiation analysis showed that heterospheroids formed by layering keratinocytes on a fibroblast core have a more precise epidermal-dermal architecture and more differentiated keratinocytes compared to heterospheroids obtained by mixing two cell types.
Conclusion: Heterospheroids formed by layering can be used as an *in vitro* skin models, while heterospheroids obtained by mixing are better suited for studying cell interactions within the spheroid. This work was supported by the Russian Science Foundation (23-25-00503).
Keywords: Skin Models, Spheroids, Co-Culture

Ps-59: Novel identification of two THBS1 and RUNX2 hub genes in neuron differentiation

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Objective: Spinal cords Injuries are the most important reason of movement disorders. Based on the slow rate of proliferation of nerve cells, this disorder still untreatable. In this perusal, the authors attempted to understand more about the molecular behaviors via high-throughput array techniques such as microarrays.
Materials and Methods: In addition, our evaluation has indicated 3008 differential expressed genes (DEGs) from GSE27334 via GEO2R software, which demonstrated differential molecular alteration during neural differentiation from P19 stem cells through Retinoic Acid (RA). In order to understand protein behavior, we used STRING database from Cytoscape software to construct the protein-protein interaction (PPIs) network. Which showed 3209 nodes and 28266 edges. Moreover, to find the genes that have most interaction from PPIs, we utilized CentiScape plug-in that reported 30 hub genes within higher than or equal 138 interactions.
Results: Among that two THBS1 and RUNX2 reported for the first time in the current study as novel hub genes, which could be important during the neural differentiation for the novel thera-

peutic approaches.
Conclusion: Among that two THBS1 and RUNX2 reported for the first time in the current study as novel hub genes, which could be important during the neural differentiation for the novel therapeutic approaches.
Keywords: Hub Gene, Cell Therapy, Bioinformatics, Neural Differentiation, RNA-seq

Ps-60: Optimizing the Chimera Formation in Chicken Embryos Using Human Pluripotent Stem Cells

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Objective: The formation of chimeras is an important technique for evaluating the developmental potential of human pluripotent stem cells (hPSCs). Our previous research indicated that the chicken embryo is a suitable host due to its developmental similarity to the human embryo at the primitive streak stage. However, we encountered challenges associated with the lengthy process of transferring the fertilized egg to the surrogate eggshell, leading to increased embryo mortality. To address this issue, we suggest a new approach to enhance human-chicken chimera formation.
Materials and Methods: We injected primed Royan H6 cells at a rate of 3000-5000 cells in 3 microliters into HH4 stage embryos. Instead of using the previous method, where a surrogate eggshell was used to inject the cells from the wide top, fertilized eggs were placed on their side for at least 1 hour and injected the cells directly into that side. After 7 days, we examined the morphology and survival of the embryos, as well as the presence of human cells using the STEM121 antibody.
Results: After comparing the old and new methods, we found that increased efficiency in chimera formation reduced fetal mortality during cell injection into the primitive streak after 7 days and decreased the number of consumed chick embryos. Although we observed abnormalities in the morphology of some chick chimera, staining with STEM121 antibody revealed the presence of hPSCs.
Conclusion: Our approach enhances precision, simplifies handling, and speeds up chimera generation. Furthermore, we validated the reproducibility of chimera formation in chick embryos using hPSCs.
Keywords: Chick Embryo, Interspecies Chimera, Human Pluripotent Stem Cell, ,

Ps-61: Effect of Calligonum Plane Eetract on Colonization Olonization and Reactive Oxygen Species Production in Sheep Spermatogonial Stem Cells

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Objective: The balance between self-renewal and differentiation of spermatogonial stem cells (SSCs) in the testicles of adult species causes the creation of regular cycles,the most important results of which are spermatogenesis and the possibility of potential fertility
Materials and Methods: The testes of immature Afshari lambs were first washed with 70% tincture of iodide.Then mechanical digestion was performed by strile scissors.in two stages of enzymatic digestion the collagenase IV, hyaluronidase II and trypsin enzymes were used.The samples assigned into four groups. i. The control ii. H_2O_2, iii. Calligonum iv. Calligonum+H_2O_2. Flow cytometry was used to measure the amount of reactive oxygen species(ROS)inside the cell.The mRNA genes expression of BAX, BCL2 in all groups were measured.An inverted microscope equipped was used to evaluate the number of colonies on days 5,14,and 21of culture
Results: The number of spermatogonia colonies in the control and Calligonum groups was higher than the other two groups ($P\leq0.05$) on the 5thand 14thdays of culture.The highest and lowest number of spermatogonial colonies were observed in Calligonum and H_2O_2 groups($P\leq0.05$),respectively on the 21st day.The lowest and highest percentage of cells containing ROS, BAX expression,and BAX/BCL2 ratio were observed in Calligonum and H_2O_2 groups ($P\leq0.05$), the highest amount of BCL2 expression was in Calligonum group ($P\leq0.05$)
Conclusion: The use of Calligonum in the culture improves the colonization rate in sheep spermatogonial stem cells and controls the amount of ROS production rate in these cells.The expression of BCL2 and BAX genes,as inhibitory and compelling factors of apoptosis,also increases and decreases in the presence of Calligonum
Keywords: Spermatogonia, Calligonum, Colonization, ROS, Sheep

Ps-62: Janus-Like Collagen Membrane: A New Platform in The Urethra Restoration

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Objective: Collagen-based materials, especially membranes, are widely used in the biomedical field due to their mechanical properties and suture retention ability, which makes them useful for surgical procedures, particularly urethroplasty. There are two main approaches to produce collagen-based membranes: decellularization and reconstruction. We have developed an innovative modification of the collagen electrophoretic deposition method to produce robustly no-defect membranes that can be industrially scaled. This study aims to show its applicability to restore the urethra using a rabbit as a model.
Materials and Methods: Bovine Achilles tendons obtained fresh frozen from local farms and were used as a source of type I collagen. The tendons were treated with NaCl, homogenized, hydrolyzed, and purified. The resulting suspension was precipitated electrophoretically in an electrochemical cell separated by a semi-permeable barrier. The obtained membranes were dried, crosslinked, and mechanically perforated. Soviet Chinchilla rabbits (n=15) were chosen as an animal model. The experiments were carried out in accordance with international regulations.

Results: The high membrane's biocompatibility was proven by the Live/Dead assay using 3T3 cells and MSCs. *In vivo* experiments showed the complete epithelialization by the urothelium at day 45 with implant's resorption and replacement by own tissues. α-SMA staining revealed the absence of fibrosis. Urethrocystography showed no signs of stenosis by day 180. In contrast, the control group had the marked stenosis without the mucosal regeneration.
Conclusion: The developed approach allows creating implants applicable in urethroplasty and possessing appropriate mechanical properties. The membranes' application was efficient and ensured high epithelization level without the stricture formation.
Keywords: Tissue Engineering, Urethra, Collagen, Urethroplasty, Mechanics

Ps-63: Modulation of The Inflammatory Response of Macrophages by Mesenchymal Stromal Cells-Derived Exosomes and Matrix-Bound Nanovesicles

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Objective: The ability to control the process of inflammation for chronic inflammation, for example, osteoarthritis, is one of the most important challenge in the field of regenerative medicine. There is a need for therapies that are able to modulate the immune response the inflammatory process without having systemic side effects. Extracellular vesicles [matrix-bound nanovesicles (MBV) and exosomes] have shown the ability to modulate the inflammation process by changing the polarization of macrophages in the direction of the anti-inflammatory phenotype.
Materials and Methods: The study used EVs derived from the secretions and ECM of human umbilical cord mesenchymal stromal cells (U-MSCs). Both types of EVs were added to macrophages together with polarization inducers in the M1 (IFN-γ+LPS) and M2 (IL-4) phenotypes. The effects of EVs on the macrophage polarization were evaluated using ELISA and qRT-polymerase chain reaction (PCR). Production of reactive oxygen species (ROS) production in macrophages was evaluated by luminol-dependent chemiluminescence (CL).
Results: ELISA and qRT-PCR showed that МИV and exosomes reduced the production of inflammatory cytokine TNF-α and IL-6 in M1 macrophages compared with control cells receiving only IFN-γ+LPS. QRT-PCR showed that MBV increased the expression level of CD-206 of the M2 polarization marker to a greater extent than exosomes. In addition, in the MBV group there was a more pronounced decrease in the expression of the NF-kB subunit responsible for inflammatory activation of macrophages. Incubation of macrophages with EVs caused a decrease in ROS production by M1 macrophages compared with control cells, which was confirmed by the CL method.
Conclusion: MBV and exosomes of the U-MSCs inhibit the pro-inflammatory activity of M1 macrophages, enhance M2 anti-inflammatory polarization of macrophages and the radical-generating activity of macrophages, which shows their potential use as therapy for diseases caused by chronic inflammation. This research was funded by grant from Russian Science Foundation (# 23-25-00497, rscf.ru/en/project/23-25-00497/).
Keywords: Extracellular Vesicles, Matrix-Bound Nanovesicles, Macrophage Polarisation, ,

Ps-64: Biofabrication of Urethral Equivalent From Spheroids of The Buccal Epithelium and Mesenchymal Stromal Cells

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Objective: Currently, diverse scaffolds with immobilized cells formed with 3D bioprinting are widely used in regenerative medicine. 3D cellular spheroids serve as building blocks for the biofabrication, owing to the cell-cell, cells-matrix interconnections, as well as nutrient and oxygen gradients. These spheroids help preserve cell viability during bioprinting by reducing stress and mimicking microenvironments. Primary cell cultures sourced from human tissues present a promising resource due to the potential for personalized medicine. Extended genitourinary stricture stands out as a prevalent affliction causing considerable discomfort in affected individuals. Innovative methods using tissue equivalents can be effective treatments alongside traditional urethroplasty techniques. In urethral biofabrication, integrating multilayered epithelium and stroma mimics human tissue complexity. The main goal was complex characterization of spheroids from buccal epithelium and MSCs and formation of bilayered bioequivalent from epithelial and mesenchymal cells by 3D-bioprinting.
Materials and Methods: Buccal epithelium and multipotent mesenchymal stromal cells (MSCs) were isolated from explants of human oral cavity tissues, characterised using flow cytometry. Spheroids were formed using 3D petri dish agarose plates (MicroTissue, USA). Bioequivalents were created using a 3D CELLINK BioX bioprinter (CELLINK, Sweden) based on fibrin hydrogel.
Results: Comprehensive characterization of the spheroid's cytoskeleton, extracellular matrix components morphology, mechanical properties, and expression profiles of key markers, was performed. Cell viability in spheroids was assessed, along with their ability to reorganize on plastic surfaces and in a fibrin-based hydrogel. Distinct phenotypes of spheroids, representing epithelial and mesenchymal lineages, were successfully generated. Buccal epithelium-derived spheroids exhibited a mixed marker expression profile, indicative of both epithelial and mesenchymal cell characteristics. MSC-derived spheroids predominantly displayed mesenchymal features and demonstrated faster compaction and reactivation abilities. Bilayer bioequivalents were constructed using epithelial and mesenchymal cells in conjunction with fibrin-based bioinks. During maturation, epithelial spheroids form a cell layer on the upper surface, while mesenchymal spheroids create a dense layer at the base.

Conclusion: The study was supported by the Russian Science Foundation (№ 23-15-00481).
Keywords: Spheroids, 3D-Bioprinting, Biofabrication, Buccal Epithelium, MSCs

Ps-65: The Effect of Conditioned Medium from Bone marrow-derived Mesenchymal Stem Cells on The Prefrontal Cortex in Type 2 Diabetic Rats

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Objective: Diabetes leads to the damage of prefrontal cortex neurons and causes behavioral disorders. It has been suggested that conditioned medium (CM) derived from bone marrow mesenchymal stem cells (BM-MSCs) can improve the complications caused by diabetes. This study examines the effect of CM from BM-MSCs on the prefrontal cortex of type 2 diabetes model rats.
Materials and Methods: 40 Male Wistar rats (8 wk, 200-250 gr), were randomly divided into following groups: control, diabetes, diabetes+Dulbecco's Modified Eagle Medium (DMEM), diabetes+CM, and pretreatment. To induce diabetes, 120 mg/kg nicotinamide and 15 minutes later 65 mg/kg STZ (streptozotocin) were injected intraperitoneally. One week later, 250 µl of CM (or DMEM) was injected intravenously to group 3 and 4. Pretreatment group received CM two weeks before diabetic induction and also for three weeks afterwards. Two months after the first CM injection, the rats were examined by Elevated Plus Maze behavioral test to investigate anxiety-like behavior. Histopathological examination was done by Nissl staining and pyknotic neurons were counted. The expression of Bcl-2 and Bax genes were evaluated by quantitative real-time polymerase chain reaction. The biochemical tests including total antioxidant capacity, and malondialdehyde were assessed. Finally, data was analyzed by SPSS software and ANOVA test.
Results: The present study showed that the induction of diabetes in rats increased the number of pyknotic neurons ($P<0.0001$), Bax mRNA expression ($P<0.05$) and malondialdehyde level ($P<0.05$) in diabetic rats. The CM administration decreased the number of pyknotic neurons ($P<0.001$) and increased the expression of Bcl2 and total antioxidant capacity ($P<0.01$). Also, the CM injection decreases anxiety-like behavior in rats ($P<0.001$).
Conclusion: These findings suggest that CM produced from BM-MSCs may be valuable for therapeutic approaches in diabetes and reduce its negative effects.
Keywords: Bone Marrow Mesenchymal Stem Cells, Conditioned Medium, Prefrontal Cortex, Streptozotocin, Apoptosis

Ps-66: Viral Fusion Peptide Stimulated The Interaction of Engineered Exosomes with Breast Cancer Stem Cells

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Objective: Currently, numerous researches have been concentrated to increase the therapeutic effects of extracellular-vesicles, especially exosomes for the alleviation of several diseases. It has been indicated that systemically injected exosomes can distribute easily in all biofluids, resulting in the increase of off-target effects. Thus, attempt should be concentrated to increase the uptake rate of exosomes by the target cells. In this research, engineered exosomes were produced using viral fusion peptide, using covalent interaction
Materials and Methods: MDA-MB-231 breast cancer stem cell exosomes were isolated using consequential centrifugation step and decorated with respiratory syncytial virus (RSV) fusion peptide using the click chemistry. The engineered exosomes were assessed using DLS analysis in terms of hydrodynamic diameter. To assess the reciprocal interaction of engineered exosomes with endothelial cells, surface plasma resonance (SPR) biosensing method was performed. MDA-MB-231 cells were cultured on gold-coated SPR chip to yield single cell layer and exposed to naïve and engineered exosomes at flow rate of 15 µl/minute for 5 minutes.
Results: Data indicated the increase of exosome diameter,135 vs. 315 nm, after the addition of viral peptide coincided with the elevation of zeta potential values from -10 to -1 mV. SPR analysis confirmed the increase of RU values between the engineered exosomes compared to the naïve exosomes. These data indicate the increase of physical contact between the circulating exosomes with the of single MDA-MB-231 monolayer.
Conclusion: Decoration of exosomes with viral fusion peptide can be used for the increase of therapeutic efficiency and delivery efficiency of exosomes to the target cells.
Keywords: Breast Cancer Stem Cells, Exosomes, Viral Fusion Peptide, Physical Interaction, On-Target Efficiency

Ps-67: Evaluation Of Anti-Aging Effects Of Human Placenta Extract On Senescent Cell Model

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Objective: One key strategy for preventing skin aging involves safeguarding fibroblast cells from oxidative stress. Extensive research has already explored the beneficial impact of human placenta extract (HPE) on wound healing, including re-epithelization, synthesis of extracellular matrix (ECM) proteins particularly collagen and elastin, cell proliferation, and migration. In this experimental study, the focus was on assessing HPE's antioxidant properties in countering hydrogen peroxide (H_2O_2)-induced damage model to human dermal fibroblasts (HDFs).
Materials and Methods: To create the aging model, HDFs were exposed to varying concentrations of hydrogen peroxide (H_2O_2)

for a 2-hour duration. The optimal dose was determined using the MTS test. Furthermore, we assessed the expression levels of aging-related genes, including P53 and P21, to validate the cell senescent model. HDFs were initially treated with an optimal dose of HPE for 24 hours, followed by exposure to H_2O_2. After a total treatment duration of 26 hours (24 hours of HPE treatment and 2 hours of H_2O_2 exposure), we assessed the expression levels of aging-related genes and compared them to those in the established model.
Results: In the presence of oxidative stress, HPE demonstrated a significant increase in cellular viability. Moreover, it not only enhanced cell proliferation but also mitigated apoptotic induction. HPE effectively reduced the expression of senescence marker genes.
Conclusion: The findings suggest that HPE holds promise as a natural-based cosmeceutical agent for delaying and managing skin aging.
Keywords: Human Dermal Fibroblast Cells, Human Placenta Extract, H_2O_2, Anti-Aging

Ps-68: Re-purposing Clove Oil in Ovarian Cancer Treatment: A Multi-Dimensional Approach Involving Bulk RNA-Seq and scRNA-Seq

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Objective: Ovarian cancer (OC) is currently the fifth leading cause of cancer-related deaths among women, with approximately 140,000 fatalities globally per year. To improve the prognosis of OC patients, novel therapeutic approaches are essential . Cancer stem cells (CSCs) are integral to ovarian cancer's entire development process, including initiation, metastatic progression, therapeutic resistance, and disease recurrence. Thus, targeted therapy, particularly against ovarian cancer stem cells (OCSCs), is expected to be more effective and less toxic, potentially improving patient survival and reducing tumor relapse.
Materials and Methods: Our initial analysis utilized GSE13237 dataset, from NCBI gene expression omnibus (GEO), which includes data from 11 pairs of primary and metastatic Ovarian Cancer tumors. We employed RNA-seq pipeline,"DESeq2" package, setting a threshold of $p < 0.05$ and $|logFC| > 1$ to identify highly variable genes. This yielded 2134 significant genes. Subsequently, using the "clusterProfile" package and David online enrichment tool for KEGG, we repurposed "clove oil"—a biotech-type, reliever of neuropathic pain used for toothache and mild pain—as a potential agent targeting *COL1A1* gene. This gene plays a significant role in metastasis and stem cell regulatory pathways. To ensure accuracy, we further analyzed scRNA-seq datasets GSE184880 and GSE158937, comprising 8 samples (5 healthy and 3 high-grade serous metastatic OC) with "Seurat" package in R studio.
Results: The results significantly enriched the *COL1A1* gene in the cancer cell cluster, providing clear evidence of its association with OCSCs. We have found out that there is a significant relationship between "*COL1A1*" expression and "Clove oil".
Conclusion: By understanding the genetic underpinnings and potential therapeutic targets like the COL1A1 gene, we can pave the way for more effective and personalized treatments for ovarian cancer, offering hope for improved outcomes.
Keywords: Ovarian Cancer, Cancer Stem Cells, Clove Oil, Collagen Type I Alpha 1 (COL1A1)

Ps-69: The Potential of Oncolytic Virus as A Therapeutic Strategy for Glioblastoma Treatment (2D and 3D Model)

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Objective: This study aimed to evaluate the cytotoxic effects of the oncolytic virus Onco-VV-TT on glioblastoma (GB) cell lines and its impact on glioblastoma stem cell (GSC) properties. Specifically, the study examined the virus's ability to inhibit cell migration and colonization, and analyzed the temporal dynamics of virus-induced cell death and its penetration into spheroids. The findings aim to highlight the potential of Onco-VV-TT as a therapeutic agent for GB treatment.
Materials and Methods: Human (U251 and U87) and rat (C6) GB cell lines were treated with various multiplicities of infection (MOI) of Onco-VV-TT for 24, 48, and 72 hours to assess cell viability and proliferation. Colony formation and wound healing assays were performed to evaluate the inhibition of cell migration. Flow cytometry (FACS) was used to determine the type of cell death induced by Onco-VV-TT. Spheroids were infected with the virus, and their viability and virus penetration were assessed over 72 hours using confocal microscopy. The ability of spheroids to reattach post-virus infection was also evaluated.
Results: Onco-VV-TT significantly reduced cell viability and proliferation of GB cell lines in a dose-dependent manner. At an MOI of 1, the virus effectively decreased colony formation in U251, U87, and C6 cell lines, with the most pronounced effect observed in U251 cells. Wound healing and colony formation assays confirmed the inhibition of cell migration and colony formation following Onco-VV-TT infection.
Conclusion: Onco-VV-TT demonstrated significant cytotoxic effects on GB cell lines, inhibiting GSC properties, cell migration, and colonization. The virus induced a temporal progression of cell death and effectively penetrated spheroids, resulting in substantial cell death. These findings suggest that Onco-VV-TT holds promise as a therapeutic agent for GB treatment.

Keywords: Oncolytic Virus, Glioblastoma, Cancer Stem Cell, Immunoviro Therapy

Ps-70: Astrocyte Exosomes; Novel Therapeutic Source in Central Nervous System

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Objective: Among different glial cell within the central nervous system, astrocytes are valid cell component involved in maintaining brain homeostasis and neurogenesis. It has been thought that extracellular vesicles (EVs), especially exosomes, can regulate inter astrocyte-neuron communication in a paracrine manner. In this study, we performed protocol for the isolation and enrichment of mouse fetus astrocyte exosomes for possible application in biomedical fields.
Materials and Methods: Cerebral cortexes of fetal mouse were sampled and cut into small pieces without enzymatic solution. The cell suspensions were transferred into culture flasks and allowed astrocyte population attached the bottom surface while gentle agitating. The supernatants containing non-astrocyte cells were discarded. Upon 80-90% confluence, astrocytes were subjected to starvation in FBS-free culture medium to collect the exosomes. After consequential centrifugation steps, the isolated exosomes were characterized and immunophenotyped using DLS, SEM, and western blotting.
Results: Wester blotting revealed the existence of tetraspanins such as CD63, CD81, and TSG101 in exosome pellets. SEM images confirmed heterogenous round-shape particles. DLS analysis indicated hydrodynamic diameters of ~336 nm with and zeta potential of -32 mV, indicating typical exosome physicochemical properties.
Conclusion: Taken together, primary astrocyte exosomes can be isolated and pooled from astrocytes for different regenerative purposes.
Keywords: Mouse, Cerebral Cortex, Astrocytes, Exosomes, Biomedical Application

Ps-71: The Effect of Biocompatibility Properties of Electrospun Polycaprolactone-Gelatin Scaffold Containing Layered Double Hydroxide Nanoparticels on L929 Mouse Fibroblast Cells

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Objective: Nanofibrous scaffolds provide cells with an environment similar to the body's extracellular matrix, which is effective on cell morphology, adhesion, proliferation and function. The present study aims to investigate the biocompatibility properties of polycaprolactone and gelatin electrospun scaffold containing LDH nanoparticles on L929 mouse fibroblast cells.
Materials and Methods: In this research, LDH nanoparticles were synthesized by co-precipitation method. Using the electrospinning method, a scaffold made of polycaprolactone and gelatin with optimal nanoclay 1% by weight was made. The synthesized nanoparticles were evaluated by X-ray diffraction (XRD) and SEM. 20 thousand L929 fibroblast cells were planted on the scaffolds for 3 to 5 days. Then they were placed in alcohols of different percentages to dehydrate the scaffolds. After drying the scaffolds, a scanning electron microscope (SEM) was used to characterize and examine the stabilized cells. MTT was used to evaluate the toxicity of the scaffolds. Cell evaluations were evaluated by examining adhesion, viability, and proliferation of L929 cells
Results: By adding LDH, the fiber diameter decreased. The biocompatibility of the PCL-GEL-LDH scaffold was investigated after the culture of L929 mouse fibroblast cells with the MTT test, and the results showed that the survival rate of the cells cultured on it increased compared to PCL-GEL. Also, SEM images showed better interaction and proliferation of L929 cells on PCL-Gel-LDH than PCL-GEL.
Conclusion: Adding LDH to PCL-GEL nanofibers has a positive effect on cell adhesion and viability.
Keywords: Polycaprolactone-Gelatin, Double Layered Hydroxide Nanoparticles, Mouse Fibroblast Cells

Ps-72: The Effect of Wound Dressing Containing Polycaprolactone-Gelatin-Layered Double Hydroxide Carrying The Drug On Wound Healing In Guinea Pigs

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Objective: Nanofibrous scaffolds containing layered double hydroxide nanoparticles are very important in drug delivery for healing skin wounds. The purpose of this study is to investigate the effect of PCL-GEL-LDH wound dressing containing the drug 4-methyl-7-hydroxycoumarin on wound healing in guinea pig skin tissue.
Materials and Methods: In this research, a scaffold made of polycaprolactone and gelatin with 1% by weight optimized nanoclay containing coumarin drug was made using electrospinning method. The synthesized nanofibers were evaluated by X-ray diffraction (XRD) and SEM. There male guinea pigs were used to make the animal model. Three square wounds with dimensions of 2 x 2 cm were created in the skin of the back surface of each guinea pig. In each guinea pig, the wounds were divided into 3 groups: wounds without receiving any treatment, wounds with polymer, and wounds with drug-carrying polymer-nanoparticles. The wound surface and wound healing rate in the studied groups were checked on days 7 and 14 after treatment using H&E staining and Image J and SPSS software.
Results: In examining the wound surface on the 7th and 14th day, the smallest and largest wound surface was observed in the polymer-nanoparticle-coumarin, polymer-nanoparticle, and untreated wound groups, respectively. In examining the percentage of wound healing on days 7 and 14, the highest percentage of healing was observed in the polymer-nanoparticle-coumarin

group, polymer-nanoparticle, and untreated wound, respectively, and there was a significant difference between these 3 groups on day 14.
Conclusion: The wound dressing containing polymer-nanoparticles carrying the drug coumarin had a good compatibility with the skin wound in guinea pigs and had a better effect on accelerating the healing of skin wounds in guinea pigs than the polymer without LDH carrying the drug.
Keywords: Polymer-Nanoparticle Wound Dressing, Coumarin Drug, Guinea Pig Skin Wound

Ps-73: Gabapentin Reduced Osteo-Differentiation of Bone Marrow Mesenchymal Stem Cells Via Down-Regulation of Related Genes

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Objective: Gabapentin (GP) as an analog of GABA, is the most commonly used medicine to treat neurological pain. Studies revealed continuous application of GP causes bone mass density reduction in patients. The present study aimed to investigate its effect on the osteogenic differentiation of bone marrow mesenchymal stem (BMSCs) which are responsible for producing osteoblasts.
Materials and Methods: Rat BMSCs were extracted and treated with different concentrations of GP for 21 days, and then 10-8 and 10-4 M were selected for further study in osteogenic media. Matrix production was investigated using alizarin red quantitative analysis and calcium level estimation. In addition, the activity of alkaline phosphatase (ALP) and expression of osteo-related genes including BMP 2 and 7, RUNX2, osteocalcin, Alp, and collagen 1 were determined.
Results: Statistical analysis showed that the matrix production by differentiated BMSCs reduced significantly ($P<0.05$) in a dose-dependent manner after treatment with GP. In addition, the activity of ALP reduced significantly and the expression of all the investigated genes also reduced significantly ($P<0.05$) in the presence of GP.
Conclusion: GP reduces the osteo-differentiation of BMSCs by affecting related genes which finally causes the reduction of bone matrix production.
Keywords: Gabapentin, BMSCs, Osteogenic Differentiation, Gene Expression,

Ps-74: Resveratrol-Coated Chitosan Mats Promote Angio-Genesis for Enhanced Wound Healing In Animal Model

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Objective: Growing incidences of chronic wounds recommend the development of optimal therapeutic wound dressings. Electrospun nanofibers have been considered to show potential wound healing properties when accompanied with other wound dressing materials. This study aimed to explore the potential role of Chitosan (CS) nanofibrous mats coated with resveratrol (RS) as an antioxidant and pro-angiogenic agent in rat model of skin wound healing.
Materials and Methods: Electrospun chitosan/polyethylene oxide (PEO) nanofibers were prepared using electrospinning technology and coated by 0.05 and 0.1 mg.ml resveratrol named as (CS/RS 0.05) and (CS/RS 0.1), respectively. The scaffolds were characterized physio-chemically such as *in vitro* release study, TGA, FTIR spectroscopy analysis, biodegradability and human dermal fibroblasts seeding assay. The scaffold subsequently used *in vivo* as a skin substitutes on a rat skin wound model.
Results: *In vitro* tests revealed that all scaffolds promoted cell adhesion and proliferation. However, more cell viability was observed in CS/RS 0.1 scaffold. The biocompatibility of the scaffolds was validated by MTT assay, and the results did not show any toxic effects on human dermal fibroblasts. It was observed that RS coated scaffolds had the ability to release RS in a controlled manner. In *in vivo* tests CS/RS 0.1 scaffold had the greatest impact on the healing process by improving the neodermis formation and modulated inflammation in wound granulation tissue. Histological analysis revealed enhanced VEGF expression, epithelialization and increased depth of wound granulation tissue.
Conclusion: The RS coated CS/PEO nanofibrous scaffold accelerates wound healing and may be useful as a dressing for cell transfer and clinical skin regeneration
Keywords: Chitosan, Resveratrol, Electrospinning, Angiogenesis, Wound Healing

Ps-75: Investigating The Immunogenic Properties of Artificial Insulin Producing Cells

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Objective: Immune rejection presents a substantial challenge in diabetes treatment by stem cell transplantation. The current work investigated the immunological features of insulin-producing cells (IPCs) generated from adipose-derived mesenchymal stem cells (ADSCs) both *in vitro* and *in vivo*.
Materials and Methods: The research was carried out at Ahvaz Jundishapur University of Medical Sciences in 2023. ADSCs were derived from rat adipose tissues and differentiated into IPCs. The control group consisted of undifferentiated ADSCs. The amount of secreted insulin was measured using ELISA. The *in vitro* expression of MHC-I and MHC-II, CD40, and CD80 by IPCs was assessed using Western blot analysis. The *in vivo* study was performed on ten male diabetic rats. The experimental group received 107 IPCs in the peritoneal cavity. The control group received 10 7 Un-differentiated ADSC. After 4 hours, the expression of CD3a and CD45 by immune cells collected from the peritoneal cavity was measured using Flow cytometry.
Results: The differentiated cells secreted much higher amounts of insulin than the control group. ($P=0.004$). IPCs exhibited higher expression of MHC-I and MHC-II, CD40, and CD80.

(P= 0.02, P= 0.008, P= 0.07, P= 0.02 respectively). The experimental group showed higher levels of CD3a and CD45 expression than the control group. (P= 0.07, P= 0.04 respectively).
Conclusion: Functional IPCs generated by ADSC differentiation exhibited immunogenic activity both *in vitro* and *in vivo*. As a result, immune-modulating strategies are required for the effective transplantation of the differentiated IPCs generated in our study.
Keywords: Adipose-Derived Mesenchymal Stem Cell, Insulin, Cell Differentiation, Immunogenicity

Ps-76: Gabapentin Effects Osteo-Differentiation of Bone Marrow Mesenchymal Stem Cells Due to The Induction of Oxidative Stress

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Objective: Gabapentin (GP) is an anticonvulsant medicine prescribed to treat neurological complications. Studies showed that GP causes osteoporosis in patients following continuous consumption. We investigated the oxidative stress induced by GP on bone marrow mesenchymal stem cells (BMSCs) differentiated to osteoblasts *in vitro*.
Materials and Methods: Rat BMSCs were treated with different concentrations of BP and then concentrations of 10-8 and 10-4 M were selected for further analysis. Osteogenic differentiation, cell viability, nuclear and cytoplasm morphology, total protein concentration, malondialdehyde (MDA) level, total antioxidant capacity (TAC), and antioxidant enzymes (CAT and SOD) activity was determined. In addition, the expression of oxidative stress-related genes (NFkB and Nrf2) was estimated using real-time polymerase chain reaction (PCR).
Results: Alizarin red staining showed osteogenic differentiation of BMSCs was reduced significantly following treatment with selected dose of GP. In addition, GP reduced the cell viability and total protein concentration and decreased the nuclear diameter and cytoplasm area of the treated cells significantly ($P<0.05$) compared to the control one. It was shown that GP caused elevation of MDA and reduced the TCA as well as the activity of the antioxidant enzymes. It was revealed that the expression of NFkB increased and Nrf2 decreased after treatment with the selected dose of GP.
Conclusion: GP reduces cell viability and changes the morphology of osteo-differentiation of BMSCs via induction of oxidative stress.
Keywords: Gabapentin, Morphology, Oxidative Stress, Gene Expression,

Ps-77: Anti-Tumor Effect of Lapatinib In Combination with Standard Care In Patient Derived Bladder Cancer Organoid (PDO)

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Objective: Bladder Cancer (BLCa) inter-patient heterogeneity is the primary cause of treatment failure, suggesting that patients could benefit from a more personalized treatment approach. Organoid as an aggregation of adult stem cell, progenitor of cells or induced pluripotent stem cell can recapitulate organ function in miniature. Patient derived organoids have been used as a preclinical model to predict or personalize treatment.
Materials and Methods: In our study, we stablish PDO culture from different stage of BLC of 30 patients. Patient-derived organoid characterized for ability of grow, sub culturing capability, long term proliferation potential, size, viability and also expression of specific bladder organoid markers by RT-PCR. The organoid forming efficiency was evaluated by confocal microscopy for CK20, GATA3a, CK20, UPK III and proliferation marker (KI67). Then the generated model was used for early stage of drug screening in different concentration of Lapatinib in compare with standard care.
Results: Our drug screening pipeline is implemented using PDOs, testing standard-of-care and FDA-approved compounds for other tumors. We successfully establish and culture organoids from NMIBC and MIBC that recapitulate the PTs' key aspects. Integrative analysis of drug response profiles with matched PDO analysis is used to determine enrichment thresholds for candidate markers of therapy response and resistance. Finally, the result indicate synergic effect of lapatinib in combination with cisplatin and gemcitabine and in compare with cisplatin and gemcitabine (or either alone) on the well stablished preclinical model of bladder tumor organoids.
Conclusion: In summary, with this study, we have generated a unique model of BLCa organoids which retain cancer heterogeneity and can be employed in drug-sensitive screens. Moreover, we showed the dose dependent behavior of Lapatinib in combination with standard care and the specific concentration with synergic effect significantly inhibit the proliferation and growth tumor organoids.
Keywords: Bladder Tumor, Organoid, Drug Screening, Disease Modeling, Lapatinib

Ps-78: Development of an Optimized Protocol for Generating Knockout Cancer Cell Lines Using The CRISPR/Cas9 System, with Emphasis on Transient Transfection

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Objective: The clustered regularly interspaced short palindromic repeats (CRISPR) system, a bacterial immunity mechanism against phage and other invading factors, has instigated a profound revolution in eukaryotic genome editing over the last dec-

ade. This system empowers researchers to conduct intricate genetic modifications such as gene knockout, knock-in, activation, repression, and single-base editing. Compared to conventional gene editing methods, CRISPR/Cas9 offers distinct advantages: affordability, exceptional efficiency, remarkable accuracy, and unparalleled user-friendliness.
Materials and Methods: Here, we develop an optimized approach for generating knockout cancer cell lines using a plasmid vector and nonviral delivery method. In this paper, we present a detailed nine-step instruction set encompassing various precise procedures. This includes employing a lipid nanoparticle-based delivery strategy, leveraging robust bioinformatics tools, conducting indispensable assays, isolating monoclonal cells via limiting dilution, and validating biallelic gene knockout through sequencing analysis.
Results: These steps collectively enable the creation of knockout cell lines within ten weeks. We successfully created several new generations of colorectal cancer cell lines with monoallelic and biallelic epithelial cell adhesion molecule (EpCAM) gene knockout by using this method.
Conclusion: Consequently, by employing our method, which contains stepwise instructions and comprehensive troubleshooting recommendations, researchers with limited laboratory equipment and minimal funding, especially for researchers undertaking CRISPR projects for the first time, can produce knockout cell lines efficiently to significantly enhance their investigations in cancer research, unravel disease mechanisms, and pave the way for potential therapeutic interventions.
Keywords: CRISPR, Gene Knockout, Cancer Cell Lines, Transient Transfection, Monoclonal Cell Population

Ps-79: Scalable Generation of Functional Retinal Pigmented Epithelial Cells from Human Embryonic Stem Cells in Stirred Suspension Bioreactor Technology

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Objective: Human embryonic stem cells possess the capability to differentiate into a variety of cell types, including retinal pigmented epithelium cells (RPEs), showing potential for addressing conditions such as age-related macular degeneration. The progress in utilizing human pluripotent stem cells in animal models of retinal failure highlights the necessity for practical protocols to produce functional RPE cells at a larger scale.
Materials and Methods: In this experimental investigation, we introduce a technique for growing functional RPE cells from human embryonic stem cells in a scalable stirred-suspension bioreactor. The differentiation process entailed the utilization of Noggin, retinoic acid, and Shh to prompt neural ectoderm and RPE specification. The characterization of the RPEs was conducted through Real-time polymerase chain reaction (PCR), immunofluorescent staining, flow cytometry, and a phagocytosis assay.
Results: Over a span of 60 days, hESCs effectively transitioned into RPE cells. The isolated pigmented cells exhibited a hexagonal morphology reminiscent of mature native RPEs. By day 56, the differentiated cells displayed elevated expression levels of crucial RPE markers like Mitf, RPE65, Bestrophin, and ZO-1, with RPE65 expression surpassing 70% in suspension culture.
Conclusion: A comparison with adherent conditions, where the expression stood at 40%, indicates a notable improvement in RPE cell culturing efficiency in suspension culture. This scalable enrichment approach establishes a fresh framework for producing functional RPEs, providing a plentiful cell source for applications such as drug screening, disease modeling, and potential cell replacement therapies for individuals affected by retinal degenerative diseases.
Keywords: Retinal Pigmented Epithelium Cells, Scale-Up Differentiation, Stirred Suspension Bioreactor, Human Embryonic Stem Cells

Ps-80: Turmerone-Loaded Niosomes Inhibit Proliferation and EMT in Hepatocellular Carcinoma *In Vitro* Models

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Objective: Hepatocellular carcinoma (HCC) is the most frequent primary liver malignancy and the fourth most lethal cancer. Current HCC therapies have different challenges, including tumor recurrence, drug resistance, and low bioavailability of the drugs. Recently, nanodrug delivery systems such as non-ionic surfactant-based nanocarriers (also known as niosomes) have attracted much attention in cancer treatment due to their appropriate encapsulation, stability, bioavailability, and release properties.
Materials and Methods: In this study, turmerone-loaded niosmes (tur-nio) were synthesized using the thin film hydration method. Niosome characteristics such as size, polydispersity index (PDI), and Zeta potential were assessed with dynamic light scattering (DLS). In addition, the storage stability of niosomes was examined at 4 °C and 25 °C using DLS. The anti-cancer effects of tur-nio were investigated through performing proliferation, apoptosis, cell cycle, and scratch assays on Huh7 and Hep3B cells. Additionally, the effect of tur-nio on the expression of genes related to epithelial-mesenchymal transition (EMT), and apoptosis examined.
Results: DLS analysis indicated that niosomes were completely stable at 4 °C for two months. Tur-nio significantly inhibited proliferation of Huh7 and Hep3B cells. Flowcytometry analysis indicated that tur-nio treatment efficiently induced apoptosis and cell cycle arrest in Huh7 and Hep3B cells. Furthermore, the scratch assay showed that in tur-nio treated cells, migration capacity was significantly inhibited. EMT related genes were down regulated as well. The expression level of N-cadherin and Vimentin was significantly decreased in tur-nio treated cells in comparison with the control group. In addition, tur-nio treatment increased the expression level of E-cadherin. Gene analysis assays indicated that viability of Tur-nio cells were significantly decreased.

Conclusion: Conclusion: This study showed that turmerone-loaded niosomes have anti-cancer properties and could be used as a promising device for HCC treatment. In addition, these results suggest that niosomes could be a novel candidate for drug delivery in HCC.
Keywords: Niosome, Cancer, HCC, Nanotechnology,

Ps-81: Advanced Tissue Clearing Technique for High-Resolution Visualization of Ocular Structures: A Comparative Study Using FACT Protocol

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Objective: It is essential to have high-resolution visualization of the physiological ocular structure and cellular interactions to better understand and treat eye diseases. In this study, we aim to achieve this by utilizing advanced tissue clearing techniques, specifically employing the Freeof-Acrylamide Clearing Tissue (FACT) method.
Materials and Methods: In this study, we established the FACT protocol on eye tissues using C57BL/6 mice and transgenic zebra fish. We examined five groups: 1) Intact, 2) Treated with 10% H_2O_2 (hydrogen peroxide) for 3 hours at 55°C, 3) Treated with 2% SDS (Sodium dodecyl sulfate) for 48 hours at 37°C, 4) Treated with 2% SDS for 72 hours at 37°C, and 5) Treated with 4% SDS for 48 hours at 37°C. We conducted retinal morphometry through cryostat sectioning and DAPI staining, followed by image analysis using ImageJ. Transgenic zebra fish were used to assess the impact of FACT on GFP.
Results: Comparison between the 2% and 4% SDS-treated groups revealed a significant decrease in the outer nuclear layer length in the 2% group (p value <0.0001). However, no significant morphological changes were observed in the retinal layers treated with 4% SDS for 48 hours at 37°C compared to the H_2O_2-treated and Intact samples. Notably, the FACT protocol did not alter the retinal layers in zebra fish, and fluorescence remained preserved.
Conclusion: In conclusion, the FACT protocol demonstrated varying effects of SDS treatments on ocular tissue morphology, with the 4% SDS group showing minimal alterations. The preservation of fluorescence in zebra fish retina post-clearing underscores the potential of FACT in maintaining cellular integrity for enhanced visualization of ocular structures.
Keywords: Tissue Clearing, Ocular Morphology, Sodium Dodecyl Sulfate, Setinal Integrity, Fluorescence Preservation

Ps-82: HCC Cells Decreased Hepatic Characteristics After Exposing to Fibrotic Liver ECM-Derived Microparticles in 3D Liver Micro-Tissues

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Objective: Hepatocellular carcinoma (HCC), the third most fatal cancer worldwide, accounts for 164.9% increase in their mortality by the end of 2045 in Iran. HCC mostly occurs in the background of liver fibrosis and cirrhosis. Fibrosis is characterized by excessive accumulation, and remodeling of the extracellular matrix (ECM) which is followed by the development of hepatic nodular regenerative hyperplasia in liver cirrhosis. The incidence of hyperplasia is associated with the altered microenvironment in inflammation-driven liver fibrosis, and the substantial changes in ECM might contribute to this regenerative nodule. This study aims to identify whether alteration in biochemical compounds of fibrotic ECM can regulate the hepatic maturation in HCC cells in biomimetic platform.
Materials and Methods: Initially, to produce ECM, rat liver tissues from both control (normal) and carbon tetrachloride (CCl4)-induced liver fibrosis were decellularized using sodium dodecyl sulfate (SDS) and Triton X-100. The extracellular matrix (ECM) obtained from the decellularized tissues was processed into microparticles through the water-in-oil emulsion technique. These microparticles were then integrated into three-dimensional liver biomimetic micro-tissues (MTs) composed of Huh-7 cells, human umbilical vein endothelial cells (HUVECs), and LX-2 cells. The liver microtissues were cultured ex-vivo for 14 days and their gene expression profile, and protein secretions were analyzed.
Results: The results indicated that the fibrotic matrix from CCl4-treated rat livers significantly decreased the expression of hepatic maturation genes *HNF4A*, *ALB*, and *CYP3A4* in the MTs. Moreover, MTs incorporating the fibrotic matrix showed a marked increase in the secretion of alpha-fetoprotein.
Conclusion: Our findings suggest a regulatory role for the fibrotic matrix in accelerating dedifferentiation, which could potentially facilitate the progression of malignancy in the liver.
Keywords: Hepatocellular Carcinoma, Liver Fibrosis, Extracellular Matrix, Liver Micro-Tissue, Hepatic Differentiation

Ps-83: The Study of The Modulation of Collagen Alignment By Stem Cells in Mechanical Properties of Wound Healing

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Objective: Recently, the use of random flaps has become widespread in the field of plastic surgery. With the clinical application of random flaps in plastic surgery, skin defects can be improved in terms of aesthetics and functionality. With the development of regenerative medicine, stem cell therapy is a promising way to increase regeneration and accelerate tissue healing in chronic wounds.
Materials and Methods: Twenty male albino Wistar rats with an average weight of 250-300 g were used in this research. Also, five rats of the same breed weighing 30 to 40 g were used to extract bone marrow cells. The animals were divided into two healthy groups of without therapeutic intervention and with therapeutic intervention of stem cell injection so that there were

ten animals in each group. In both groups, day zero was the day of surgery and all the 20 animals underwent surgery. A random skin flap measuring 3 x 8 cm was created in the back area of the animals and the results were checked on the 14th day in the transitional area.
Results: BMMSCs injection increased the survival level and decreased the necrotic wound level, but this improvement was not statistically significant. Also, the injection of BMMSCs improved the biomechanical properties of wound healing, the increase of which was statistically significant except for energy absorption, but in terms of the synthesis, content, and arrangement of collagen fibrils, the injection of BMMSCs was ineffective. In the untreated group, there was more collagen and more organized arrangement in collagen fibrils.
Conclusion: Local injection of BMMSCs did not significantly increase the survival level of random skin flap, but improved the wound healing process. Also, the injection of BMMSCs did not increase collagen content and improve the arrangement of collagen fibrils, but significantly improved the biomechanical properties of the wound.
Keywords: Random Skin Flap, Bone Marrow-Derived Mesenchymal Stem Cells, Collagen, Wound Healing, Biomechanical Properties of Skin

Ps-84: Stem-Cell-Based Cancer Therapy with The Aid of "HYPOXIA"; The Common Feature of Mesenchymal Stem Cell Niche and Tumor Microenvironment

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Objective: Uncontrolled tumor cell division and proliferation cause a rapid increase in cell number and oxygen consumption, leading to a hypoxic condition in the tumor microenvironment (TME) of the most developing tumors (Pathologic Hypoxia). Similarly, the adipose tissue-derived mesenchymal stem cells (hASCs) reside in a healthy hypoxic niche (Physiologic Hypoxia). Studies have shown mesenchymal stem cells (MSCs) are inherently prone to migrate toward solid or developing tumors. Therefore, hypoxia, as a common feature of hASCs niche and TME, and tumor tropism could be used in cancer therapy that would introduce hypoxia-regulated-gene-engineered hASCs as a potential carrier to transfer and release the anti-tumor agents, specifically at the TME.
Materials and Methods: We investigated the effect of TME-mimicked hypoxia on a therapeutic transgene expression level in hASCs. For this purpose, a hypoxia-regulated therapeutic gene was constructed which contained the hypoxia-responsive elements (HRE) adjacent to the full cytomegalovirus (CMV) promoter, and cytosine deaminase::uracil phosphoribosyltransferase fusion gene (CD: UPRT), separated with an IRES2 from the enhanced green fluorescent protein gene (EGFP). Following the transfection of this recombinant construct into hASCs via lipofectamine, the cells were incubated in normoxia (21% O_2) and hypoxia (2-5% O_2) conditions. The expression level of the transgene was evaluated using fluorescence microscopy and flow cytometry.
Results: Although the transgene expression in hASCs was transient and had a significant toxicity effect on the cell population, the microscopic evaluation shows the transient expression of transgene (12 hours post-transfection) was significantly increased under hypoxic conditions while the low expression in normoxia.
Conclusion: This study indicates that the HRE results in higher transgene expression under hypoxia in hASCs. This result is optimistic for using engineered hASCs as a carrier for the antitumor therapeutic genes to exert their potential therapeutic effects after migration to the hypoxic TME.
Keywords: Mesenchymal Stem Cells, Cancer Therapy, Hypoxia

Ps-85: Associations between Endothelial Cell Biomarkers and GVHD

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Objective: Hematopoietic stem cell transplantation (HSCT) is a treatment for various hematologic disorders, but it can also lead to serious complications, such as graft-versus-host disease (GVHD). Elevated levels of coagulation factors indicate endothelial dysfunction, which can contribute to GVHD.
Materials and Methods: In this study, investigate the presence of enhanced endothelial dysfunction in patients who develop acute GvHD following allogeneic hematopoietic cell transplantation (allo-HCT). *In vitro* experiments were conducted where endothelial cells (ECs) were exposed to serum samples obtained from patients with aGvHD (n = 31) and without GvHD (NoGvHD, n = 13). plasma levels of various factors such as von willebrand factor VWF, TNF receptor 1 (TNFR1), soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular adhesion molecule 1 (sICAM-1), and plasminogen activator inhibitor-1 (PAI-1) were measured. In the Immunofluorescence assay, ECs were cultured and treated with specific monoclonal antibodies against ICAM-1 and VCAM-1 and a polyclonal antibody against VWF. Finally, using a fluorescent microscope for capturing images. Furthermore, plasma levels of VWF, TNFR1, sICAM-1, PAI-1, and sVCAM-1 were quantified in individual patients using ELISA, and the results were statistically analyzed using Student's t test for paired data.
Results: VWF, sVCAM-1, PAI-1, ICAM-1 and TNFR1 exhibited significant increases in patients with GvHD.
Conclusion: Our findings suggest that ECs damage might be linked to GVHD. The relationship between endothelial cell activation and GvHD has been a topic of interest in recent research and have the potential to make a significant impact in the medical field and ultimately benefit those suffering from this challenging condition. Understanding how endothelial cell activation plays a role in the pathophysiology of GvHD could improve our knowledge of this condition. By focus on this relationship, further research may lead to improved diagnostic and treatment approaches for patients.
Keywords: Graft-Versus-Host Disease, Von Willebrand Factor, Endothelial Cell, Hematopoietic Stem Cell Transplantation

Ps-86: The Effect of Human Placenta Extract on Bone Differentiation of Mesenchymal Stem Cells

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Objective: Stem cell and molecular medicine
Materials and Methods: Mesenchymal stem cells with the ability to self-renewal and differentiate into various tissues were extracted and cultured from the umbilical cord. Then they were oriented towards ossification using a differentiation solution. Their bone differentiation was measured through alizarin red dye and this dye was also used to measure calcium density in the process of ossification. Human placenta extract was prepared in laboratory conditions and its protein content was checked. Differentiated bone cells were treated with human placenta extract, and the MTT test was performed on them to detect the absence of toxicity and cell viability, it was found that not only the placenta extract is not toxic, but also it caused the treatment of mesenchymal stem cells. Also, the differentiated cells that were treated with different concentrations of the extract were evaluated in terms of the amount of alkaline phosphatase and the amount of calcium, which are markers of ossification. Calcium deposits gradually increased with the effect of placenta extract. Then it was time to examine the osteocalcin gene, which is one of the genes at the end of the pathway. This gene plays a role in ossification differentiation, which was investigated by the real-time polymerase chain reaction (PCR) method, which in the dilution of 1 ml of placenta extract increased the osseous differentiation of stem cells compared to the control group, and the significant difference in the graph fully illustrates this issue
Results: Differentiated bone cells were treated with human placenta extract, and the MTT test was performed on them to detect the absence of toxicity and cell viability, it was found that not only the placenta extract is not toxic, but also it caused the treatment of mesenchymal stem cells. Also, the differentiated cells that were treated with different concentrations of the extract were evaluated in terms of the amount of alkaline phosphatase and the amount of calcium, which are markers of ossification. Calcium deposits gradually increased with the effect of placenta extract. Then it was time to examine the osteocalcin gene, which is one of the genes at the end of the pathway. This gene plays a role in ossification differentiation, which was investigated by the Real-time PCR method, which in the dilution of 1 ml of placenta extract increased the osseous differentiation of stem cells compared to the control group, and the significant difference in the graph fully illustrates this issue
Conclusion: So it can be concluded that despite the high ability of stem cells in treating diseases, placenta extract also increases the differentiation to ossification due to its many properties and the effect of treatment on stem cells so that the effect of these stem cells on treatment Diseases especially of the bone type will increase.
Keywords: Placenta Extract, Mesenchymal Stem Cells, Distinction, Umbilical Cord, Bone

Ps-87: Role of Autophagy in Homotypic Mitochondrial Transfer via Tunneling Nanotubes between Mesenchymal Stem Cells

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Objective: Here, the possible role of autophagy was investigated in mitochondrial donation via tunneling nanotubes (TNTs) between the human mesenchymal stem cell (MSCs) *in vitro*.
Materials and Methods: MSCs were treated with 15 μM Metformin (Met) and/or 3 μM 3-methyladenine (3-MA) for 48 hours. The generation of TNTs was monitored using bright-field and SEM images. The possible effects of autophagy modulation were assessed on mitochondrial membrane integrity. The crosstalk of autophagy signaling pathway was investigated with Wnt signaling pathway via polymerase chain reaction (PCR) array analysis. Using gas chromatography, the changes in fatty acid profile was also studies in different groups.
Results: The induction of autophagy via Met increased the TNT formation (number↑ and length↑) compared to 3-MA-treated group. Along with these changes, protein levels of Rab8 and p-FAK were diminished in 3-MA-treated MSCs with suppression of TNT formation. The stimulation/inhibition of autophagy can modulate the expression of different genes associated with Wnt signaling transduction pathway. Data revealed the increase of unsaturated fatty acids with the induction of autophagy compared to control and 3-MA-treated MSCs.
Conclusion: Autophagy modulation can influence TNT formation in MSCs coincided with homotypic transfer of intracellular compartments.
Keywords: Mesenchymal Stem Cells, Autophagy, Tunneling Nanotubes, Juxtacrine Interaction, Homotypic Transfer

Ps-88: Mitochondria-Incorporated Electroconductive Alginate-Based Hydrogel Promoted Myocardial Regeneration Via Vascularization In A Rat Model Of Myocardial Infarction

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Objective: Here, the angiogenesis properties of mesenchymal stem cell (MSC) mitochondria were investigated in a rat model of myocardial infarction
Materials and Methods: Mesenchymal stem cells were pretreated with mitochondria-induction compounds metformin (Met; 50 μM), and dichloroacetic acid (DCA; 40 μM). After the

isolation of mitochondria, the isolated particles were characterized and incorporated (~2 × 107 particles) inside the alginate (3%)-gelatin (1%) hydrogel containing 1 μM pyrrole in the presence of FeCl3. The physicochemical properties of the final composite were monitored using several assays such as FTIR, swelling, biodegradation, porosity tests, and SEM. Mitochondria-loaded hydrogels (~2 × 107 particles/100 μl) were injected into the border zone of infarcted myocardium induced by LAD ligation. After two weeks, rats were euthanized and mitochondrial uptake and angiogenesis status were monitored using histological examination.
Results: Data showed appropriate physicochemical (swelling rate, biodegradation, porosity) and cytocompatibility properties of alginate-gelatin pyrrole hydrogel evaluated by SEM, FTIR, and MTT assays. Flow cytometry analysis indicated high-rate purity and functionality of isolated mitochondria. Masson's Trichrome staining and ECG analysis indicated appropriate myocardial infarction in the control rats. Immunohistochemistry analysis showed the stimulation of angiogenesis in the infarcted area (vWF+ capillaries↑, and α-SMA+ arterioles↑) in rats that received mitochondria-loaded hydrogel compared to mitochondria and hydrogel groups ($P<0.05$). Data showed a significant increase in anterior wall thickness in the mitochondria-loaded hydrogel group ($P<0.05$).
Conclusion: Mitochondria-loaded hydrogels are suitable delivery platforms for the induction of angiogenesis and reduction of fibrosis in ischemic myocardium.
Keywords: Mesenchymal Stem Cells, Mitochondria, Hydrogel, Angiogenesis, Infarction

Ps-89: Human Placental Biomaterials Ameliorate Cancerous Phenotype in Hepatocellular Carcinome

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Objective: Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and is responsible for the fourth cause of cancer-related deaths worldwide. It is predicted that by 2025, more than 1 million individuals will be influenced by HCC yearly; therefore, due to the insufficient methods of treatment, it represents a major health-care challenge globally. Recently, a new field in regenerative medicine led to the formation of Advanced Therapy Medicinal Products (ATMPs). Tissue-engineered medicinal products are a category of ATMPs. Placenta is a good source of extracellular matrix (ECM) and different biomaterials which are known for their antioxidative and anti-inflammatory properties. Hence, the objective of this study is to utilize the decellularized ECM of the human placenta in order to investigate the cancerous phenotype in HCC cells.
Materials and Methods: Hep-3B cells were treated with placental biomaterials. In order to find the preferable dosage, cell viability was evaluated using MTS after 24, and 48 hours treatment. Epithelial-mesenchymal transition (EMT) was assessed utilizing the mRNA expression of EMT markers. Cell migration was performed using wound-healing assay.
Results: Placental biomaterials ameliorated the cancerous phenotype in Hep-3B cell line. A reduction in cell viability was noticed after the treatment with placental biomaterials. Furthermore, we demonstrated an increase in CDH1, a decrease in CDH2 expression and switch cadherin in Hep-3B cell line.
Conclusion: Placental-derived biomaterials has shown a promising potential in attenuating cancerous phenotype such as cell viability, migration, and EMT process. It is hoped that with further studies placental-derived biomaterials can be used in amelioration and apoptosis of cancerous cells or might be a good candidate for combination therapies in HCC.
Keywords: Hepatocellular Carcinoma, Human Placental Biomaterials, Cell Migration, Epithelial-Mesenchymal Transition

Ps-90: Evaluation of The Effect of Saffron (Crocus Sativus L.) On The Viability Bone Marrow-Derived Mesenchymal Stem Cells of Rat (rBM-MSC)

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Objective: Medicinal plant extracts possess tremendous potential as agents for stem cell and regenerative therapy in the realm of public health, owing to their easy availability, cost-effectiveness, and least side effects. Saffron has been utilized in traditional medicine for an extensive period of time. Moreover, according to reports, saffron is utilized in the treatment of various diseases including cardiovascular disorders and cancer. Mesenchymal stem cells (MSCs) have exhibited remarkable immunomodulatory and antioxidant properties, thereby offering potential benefits in the treatment of various diseases. In the current study, we have evaluated the effects of saffron on the viability of MSCs, in order to clarify on the potential therapeutic effects of this compound in the context of stem cell-based therapies.
Materials and Methods: Bone marrow cells from rat's femurs and tibias were isolated and subsequently characterized by flow cytometry. The cytotoxicity of saffron on rBM- MSCs was accomplished through the utilization of the MTT assay. Following successful adherence of the cells to the culture plates, they were incubated for 24 hours and 37°C, during which they were treated with varying concentrations of saffron (3.25 to 400 μM).
Results: Flow cytometry analysis demonstrated the presence of positive expression of CD90 and CD44 in rBM-MSCs at passage 3, while the expression of CD34 and CD45 was found to be negative. Furthermore, our findings revealed that saffron at low concentrations, exhibited remarkable effects on the viability and growth promotion of rBM-MSCs. However, it should be noted that higher concentration of saffron were observed to exert cytotoxic effects.
Conclusion: Based on our research, it has been determined that saffron has the potential to augment the survival rate of MSCs and may be considered as supplementary therapeutic approach individuals undergoing MSC transplantation.
Keywords: Saffron, Bone Marrow-Derived Mesenchymal Stem Cells, Cytotoxicity, Cell Therapy

Ps-91: Musashi-2, A Knock-Out Target for Controlling The Malignancy of Melanoma Cells

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Objective: Musashi-2 (MSI2) is a novel oncoprotein that promotes cancer cell growth and invasion. Studies have shown that the expression of MSI2 increases in many types of cancers, such as cervical, kidney, papillary, and lung cancers. Melanoma is the most deadly form of skin cancer, often appearing as a dark spot on the skin. Metastasis occurs when melanoma cells spread through the skin lymph nodes, leading to skin lesions between the primary tumor site and the lymph region. The aim of this study is to investigate the potential oncogenic role of the MSI2 gene in melanoma cancer as a prognostic and/or therapeutic target. To achieve this, the MSI2 gene was knocked out in a melanoma cell line using the CRISPR/Cas9 technique to examine its impact on growth, proliferation, and invasiveness compared to unmodified cancer cells in the control group.
Materials and Methods: Initially, the expression level of *MSI2* was measured in two melanoma cell lines, A2058 and A375, using RT-qPCR. The cell line with the highest *MSI2* expression was chosen for further investigation. Two sgRNAs were selected to target the *MSI2* oncogene using bioinformatics and were then cloned into the pX459 plasmid. The melanoma cells were transfected with the Cas9 vector, and puromycin-resistant clones were isolated. Subsequently, the selected clones were analyzed to identify InDels in the genomic sequence of the *MSI2* gene through genomic polymerase chain reaction (PCR) and sequencing.
Results: The RT-qPCR results revealed a significantly higher expression level of *MSI2* in both melanoma cancer cell lines compared to healthy cells, with A2058 exhibiting the highest *MSI2* expression. Consequently, A2058 cells were selected for further investigation. On the other hand, the accuracy of the cloned sgRNA sequences was validated through PCR, enzymatic digestion, and sequence analysis. Following the transfection of A2058 cells with pX459 using Lipofectamine 2000 and the isolation of resistant clones, genomic DNA samples were extracted for genomic PCR.
Conclusion: Our findings confirmed biallelic deletions in the *MSI2* gene sequence between the two sgRNAs in the tested clones, resulting in a notable decrease in the proliferation rate of the cancer cells.
Keywords: Musashi-2, CRISPR/Cas9, Melanoma

Ps-92: Formulation, Physicochemical and Biological Characterization of Etoposide-Loaded Microemulsion

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Objective: Etoposide (ETO), an anticancer chemotherapeutic agent, is used in treatment of many types of malignancies. However, its low solubility restricts its use in clinical practice where in today's medicine it is tried to apply nano encapsulation to overcome its medical limitation. As an alternative, Microemulsion (ME) system as a nanoparticle drug delivery has been developed to improve ETO solubility and increase its delivery to malignant tissues. Therefore, in this study a newly developed ME system containing ETO was used to evaluate the physicochemical and its growth inhibition property against the MDA-MB-231 cancer cell line.
Materials and Methods: The ME drug delivery system has been prepared by phase titration method. Particle size and zeta potential were evaluated using dynamic light scattering (DLS), and drug release rate from the system was investigated *in vitro*. Safety of the DDS and its gradient was checked using bone marrow mesenchymal stem cells (BMSCs) and red blood cells (RBC). MDA-MB-231 cell line was used to check the DDS+ DXR and CXR only.
Results: The particle size was reported to be approximately 5.43 nm and its poly dispersity index (PDI) was 0.163. It was observed that the system and its components are safe. In addition, the drug loading rate was 92% and the releasing percentage of the drug after 24 hours through mouse skin and dialysis membrane was obtained as 49 and 62 %, respectively. The half maximal inhibition concentration (IC_{50}) of the drug for 48 and 72 hours of treatment was calculated to be 33.26 and 31.49 µg/ml respectively.
Conclusion: Newly developed ME shown to have high capacity to carry ETO its acceptable releasing rate which makes it a suitable carrier for this chemotherapeutic agent.
Keywords: Etoposide, Microemulsion, Breast Cancer, Drug Delivery

Ps-93: GATA1's Regulatory Network In Embryonic Stem-Like Cells: Bridging Spermatogonial Stem Cells To Pluripotency

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Objective: Embryonic stem-like cells (ES-like cells) offer promising potential in regenerative medicine since they can differentiate into any cell type, providing therapeutic potential for various disorders and injuries. Generating these cells from spermatogonial stem cells (SSCs) *in vitro* has gained attention as it involves a non-controversial, ethical source of pluripotent cells, bypassing the moral issues associated with embryonic stem cells. This method supports personalized medicine by using SSCs from an individual's tissue, reducing immune rejection risks and advancing reproductive health. The *GATA1* gene balances self-renewal and differentiation in embryonic stem cells by modulating gene expression networks. In pluripotent stem cells, *GATA1* is involved in the JAK/STAT, MAPK/ERK, and BMP signaling pathways.
Materials and Methods: We employed Fluidigm qPCR and immunocytochemistry staining to assess *GATA1* gene expression and protein levels in both cell types. Furthermore, we examined the expression of the Oct4 gene, a well-validated marker known to be up-regulated in pluripotent stem cells, to provide a comparative baseline for our data. We conducted protein-protein interaction (PPI) network analysis to enhance our study to iden-

tify proteins connected to the *GATA1* gene.
Results: Our analysis revealed significant fold changes in gene expression levels between SSCs and ES-like cells. Based on our data, the GATA1 gene is up-regulated in ES-like cells derived from SSCs. Immunocytochemistry analysis demonstrated presence of GATA1 protein within ES-like pluripotent stem cells. The PPI network analysis indicated a close relationship between *GATA1* and several key genes, including *TAL1*, *TCF3*, *RUNX1*, *LMO2*, and *LDB2*. We applied enrichment analysis based on gene ontology (GO) molecular functions. GO analysis highlighted the involvement of the GATA1 PPI network in transcription co-regulator binding, transcription co-regulator activity, and DNA binding.
Conclusion: The *GATA1* gene is assumed to exert a remarkable effect on the differentiation and function of ES-like cells derived from SSCs. We demonstrated that *GATA1* is significantly upregulated in ES-like cells and interacts with key genes involved in pluripotency and differentiation. These findings have promising implications for regenerative medicine, presenting a non-controversial, patient-specific source of pluripotent cells for disease treatment and fertility restoration.
Keywords: GATA1, Stem Cell, Regenerative Medicine, Embryonic Stem-Like Cells, Spermatogonial Stem Cells

Ps-94: A New Bioequivalent Based on Extracellular Vesicles and Spheroids for Wound Healing

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Objective: Non-healing wounds are common complications in patients with diabetes and venous insufficiency, leading to significant financial costs and a decreased quality of life. A combined bioink, consisting of hydrogel, cell spheroids, and extracellular vesicles, is being considered as a promising solution. In this system, the hydrogel provides support, while the spheroids form the basis of the construct. Our study focused on two types of extracellular vesicles - exosomes and matrix-bound vesicles (MBV) from mesenchymal stem cells as a potential agents for bioequivalent maturation.
Materials and Methods: For our study, we chose a hydrogel based on fibrin and gelatin due to its well-studied nature and optimal properties for bioprinting. We developed a protocol for creating a combined bioink and demonstrated that fluorescently labeled exosomes are absorbed by cells without negatively affecting the viability of spheroids in the hydrogel.
Results: We then optimized the standard protocol for MBV isolation. Through proteomic comparison of exosomes and MBVs, we found that exosomes have a richer proteomic profile than MBVs and contain more proteins responsible for construct maturation, particularly vascularization. *In vitro* experiments indicated that exosomes contributed to the formation of a more branched and longer network of tubules from MSC spheroids encapsulated in hydrogel, indirectly suggesting construct maturation. Based on *in vivo* experiments, we demonstrated that pure hydrogel, as well as hydrogel with MSC spheroids, when subcutaneously implanted in mice, did not trigger an immune response and integrated well into surrounding tissues.
Conclusion: This study represents the initial step towards creating combined constructs for treating non-healing wounds and generally open new approaches to the generation of combined bioequivalents. The study was financially supported by the Russian Science Foundation № 22-75-10120.
Keywords: Bioink, Hydrogel system, Extracellular Vesicles, Non-Healing Wounds,

Ps-95: A study of The Interesting Relationship between Differential Gene Expressions in Ovarian and Testicular Cancer

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Objective: Ovarian cancer is one of the most fatal gynecologic malignancies worldwide, with an estimated incidence of 239,000 cases and 152,000 deaths each year. Ovarian cancer and testicular cancer are distinct types of cancer with unique genetic landscapes and differential gene expression patterns.
Materials and Methods: We analyzed data from GO datasets (GSE172159, GSE17586, GSE15220, and GSE12630). Differentially expressed genes were studied in the limma package in the R language and TAC software. Enrichr carried out gene ontology, gene set enrichment, and genomic pathway analysis. Gephi, Cytoscape, and STRING databases were employed to build the network of protein-protein interactions.
Results: Ovarian cancer is characterized by genetic mutations in genes such as *BRCA1/2*, TP53, and *PIK3CA*, which drive cell proliferation, survival, and metastasis. In contrast, testicular cancer often involves mutations in genes like *KIT*, *KRAS*, and abnormalities in chromosomal structure, impacting cell growth and differentiation. Despite these differences, both cancers exhibit dysregulation of common pathways, including PI3K/AKT/mTOR signaling and DNA repair mechanisms, contributing to tumor development. Differential gene expression analyses reveal specific genes that are upregulated or downregulated in each cancer type, influencing cell cycle control, DNA repair, and stem cell-like properties.
Conclusion: Understanding these genetic differences is crucial for developing targeted therapies and identifying potential biomarkers for improved diagnosis and treatment outcomes in ovarian and testicular cancers.
Keywords: Ovarian Cancer, Testicular Cancer, Gene Expression, Signaling Pathway, Biomarkers

Ps-96: Safety of Autologous Exosomes Injection in Infertile Patients

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Objective: Some new researches have focused on mesenchymal stem cells (MSCs) as an option to ovarian regeneration. MSCs exert their effect by secreting exosomes in a paracrine manner. Evidence has shown that exosomes secreted from MSCs have

the same effect as MSCs. Given the role of exosomes in the improvement of ovarian function in animal models, this study investigated the safety and feasibility of autologous exosomes derived from menstrual blood MSCs injection into the ovaries of diminished ovarian reserve (DOR) patients.
Materials and Methods: In the first step, autologous MSCs were isolated from the menstrual blood of patients. Then, from the culture medium of the cultured cells, exosomes were isolated (using chromatography technique) and characterized (using flow cytometry, transmission electron microscopy, and dynamic light scattering). We tested the feasibility of intraovarian injection of autologous exosomes in the follicular phase in 10 DOR patients. The patients were aged 25-42 years without chronic malignancies. To assess the safety, some clinical symptoms such as fever, pain, infection, bleeding and allergic reactions were monitored for three months after the injection.
Results: The isolated exosomes had cup-shaped morphology. Their size was between 30-150 nm. Also, the expression of exosomal markers (CD9, CD63, and CD81) was positive for these vesicles. After the injection, the patients were discharged from hospital without pain, nausea, bleeding or fever. However, two patients experienced pain in the injection area. This pain lasted for 24 hours. Then it decreased and disappeared completely after 48 hours. In addition, there were no suspicious symptoms such as swelling, fluid collection, infection, or fever on physical examination or ultrasound during follow-up.
Conclusion: Due to the absence of serious side effects after injection, autologous exosomes derived from menstrual blood MSCs can be considered as a promising candidate for ovarian disorders after more extensive studies on efficacy.
Keywords: Diminished Ovarian Reserve, Exosomes, Mesenchymal Stem Cells, Regeneration Medicine, Clinical Applications

Ps-97: Different Artificial Antigen Presenting Cells in T Cell and Natural Killer Cell Expansion and Activation

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Objective: Artificial antigen-presenting cells (aAPCs) represent a promising technological advancement in immunotherapy, particularly for the expansion and activation of T cells and natural killer (NK) cells. These synthetic constructs mimic natural antigen-presenting cells (APCs) by presenting specific antigens in conjunction with necessary co-stimulatory signals and cytokines to immune cells, thereby facilitating their proliferation and functional maturation. Cytokines play a crucial role in immune cells. The development of aAPCs involves the integration of various biomaterials, bioengineering techniques, and cytokines to create platforms that can precisely control the presentation of antigens and co-stimulatory molecules. Recent studies have demonstrated the efficacy of aAPCs in expanding specific T cell and NK cell populations *ex vivo*, which can then be reintroduced into patients to target cancers, viral infections, and autoimmune disorders. Incorporating cytokines such as interleukin-2 (IL-2), interleukin-15 (IL-15), and interleukin-21 (IL-21) into aAPCs has been shown to enhance the proliferation and cytotoxic function of T cells and NK cells.
Materials and Methods: Various cell types can serve as the backbone for artificial antigen-presenting cells (aAPCs) each offering unique advantages for specific applications. K562 Cell Line, CHO (Chinese Hamster Ovary) Cells, HEK293 Cells, Jurkat T Cells, and Fibroblast Cell Lines are some backbones that have been used more. Each of these cell types has distinct characteristics that can be leveraged depending on the specific requirements of the immunotherapy being developed. We have chosen K562 and characterized the cell line by STR test and flow cytometry using specific antibodies.
Results: The report of the STR test demonstrated that our cell line(K562) has 16 markers matched with the STR profiling of the ATCC website. One marker had a different number of repetitions so flow cytometry data could cover this issue. The specific markers of APC had high expression on the K562 cell surface.
Conclusion: Choosing the best backbone to be aAPC is one of the challenges that has an important role in presenting antigens based on their use.
Keywords: Artificial Antigen-Presenting Cells, T Cell, NK Cell, Cytokines, K562 Cell Line

Ps-98: Investigating Foreign Body Response of Taurine Modified Transplantation Device for Type 1 Diabetes Cell Therapy

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Objective: Encapsulation devices for insulin-producing cells represent an advanced approach to treatment of type 1 diabetes (T1D). One of the major challenges in this approach foreign body response (FBR) of transplanted device. The FBR is the reaction of host immune cells to any implanted biomaterials and devices followed by transplantation preventing the diffusion of oxygen and nutrients in the device, leading to cell death. Despite attempts for reducing biomaterials fibrosis, development of cell therapy devices with low FBR remains challenge.
Materials and Methods: In this research, we design and fabricate a 3D-printed biocompatible device made of polylactic acid (PLA) and implanted subcutaneously on the dorsal side of mice. Surface modification of the device with taurine was performed to reduce FBR. Taurine, an anti-fouling and anti-inflammatory molecules, has been coated on the device surface in two methods including: physical coating, and covalent conjugation. Surface modification was confirmed by SEM-EDX, contact angle and colorimetry. *In vitro* anti-fouling properties were examined by measuring the amount of absorbed protein and macrophage adhesion on the surfaces. The gene expression of inflammatory markers was also investigated. Finally, the devices were implanted in mice for four weeks and retrieved for histological analysis.
Results: The SEM-EDX analysis revealed the distribution of taurine on the device surfaces, and the amount of the taurine coating was quantified using a colorimetric assay. The contact angle measurements of the modified surfaces indicated increased

hydrophilicity compared to the control group. Moreover, taurine effectively reduced protein fouling of the human serum albumin. The number of attached macrophages on taurine coating was significantly less than in the control group.
Conclusion: Our finding suggests that surface modification of devices with taurine coating can significantly reduce FBR *in vitro* model. This device showed a therapeutic potential approach for treatment of T1D.
Keywords: Type 1 Diabetes, Encapsulation, Foreign Body Response, Fibrosis, Taurine

Ps-99: Expression of miR-17-5p, SMAD6 and SMAD4 on *In Vitro* Cardiogenic Differentiation of Human Endometrium-Derived Stem Cells

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Objective: Myocardial infarction (MI) is the most common cause of heart failure and mortality worldwide. Due to the limited regeneration capacity of myocardium tissue, stem cell therapy is considered as a promising strategy to restore cardiac function. In this regard, molecular mechanisms involved in cardiogenic differentiation of MSCs, in particular the regulation mechanisms mediated by miRNAs and BMP–Smad signaling components remains elusive. In this study, we investigated the expression level of has-miR-17-5p, Smad4 and Smad6 Proteins in cardiac cells derived from human endometrium-derived mesenchymal stem cells (hEMSCs).
Materials and Methods: hEMSCs were primed with a cardiac-inducing medium containing 5-azacytidine and bFGF for a period of 24days. Smad6 and Smad4 proteins level, as well as the expression level of miR-17-5p were evaluated by immunofluorescence staining and qRT-PCR, respectively every 6 days.
Results: Immunofluorescence staining of Smad proteins indicated that upon cardiogenic differentiation of hEMSCs, the amount of Smad4 was significantly upgraded, while the expression levels of miR-17-5p and Smad6 during the induction period showed a completely oscillating differentiation. The Pearson correlation coefficients analysis revealed that there is a negative correlation between the Smad-6 with miR-17-5p.
Conclusion: Our results indicate that miRNAs-17-5p is involved in the cardiac differentiation of hEMSCs potentially by regulation of Smad6 levels through inhibitory effect. Although, more mechanistic experiments are required to confirmation of this idea.
Keywords: Endometrium-Derived Mesenchymal Stem Cells, Cardiomyocyte, Smad4, Smad6, Hsa-mir-17-5p

Ps-100: Predicting The Probability of Effectiveness of Terpenoid In The Treatment of Type 2 Diabetes Using in Silico Methods

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Objective: Type 2 diabetes is caused by the destruction of pancreatic β cells or insulin resistance in fat cells, liver, muscle, etc. Studies have shown that tumor necrosis factor (TNF-α) and Interleukin 6 (IL-6) among other cytokines, are two effective factors in the development of diabetes. so we are looking for them in this research by using in silico methods among terpenoids (plant compounds that have been seen to be useful in controlling diabetes) more favorable terpenoids be introduced and presented for further research.
Materials and Methods: Based on this, 1100 terpenoid structures along with all absorption, distribution, metabolism, excretion (ADME) properties were obtained from the website http://pscdb.appsbio.utalca.cl/ and after passing the screening process using the ADME properties and drug-likeness feature. Some of the ADME predictions included gastrointestinal absorption by BOILED-Egg method, interactions with Cytochrome P450 (CYP450) enzyme and being P-glycoprotein (P-gp) substrate. Drug-likeness is determined by Lipinski's "Rule of 5". These compounds were docked with TNF-α, IL-6 using Auto Dock Vina software .
Results: Using the screening methods, we reached 274 compounds that 4 compounds had good binding energy with both cytokines. These 4 compounds are Androstenedione, Testosterone, Limonin, Strigol, whose binding energy to TNF-α and IL-6 were -7.4 and -7.1, -7.1 and -6.9, -7 and -6.5, -7 and -6.4 respectively.
Conclusion: It seems that these 4 compounds (Androstenedione, Testosterone, Limonin, Strigol) that have been screened from 1100 terpenoids can moderate the effects of diabetes by simultaneously inhibiting TNF-α and IL-6 with their favorable binding energy.
Keywords: Type 2 Diabetes, Terpenoid, TNF-α, IL-6, In Silico

Ps-101: The Comparing Atudy among Various Human Mesenchymal Stromal Cell-Extra Vesicles on Mouse Retinal Ganglion Cells Injury

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Objective: Extracellular vehicles (EVs) derived from various mesenchymal stromal cells (MSCs) show significant potential for protecting retinal ganglion cells (RGCs). This study aims to identify the most effective EVs from the optimal MSC source for nerve regeneration.
Materials and Methods: EVs from various sources, including clonal bone marrow (cBM), dental pulp (DP), and trabecular meshwork (TM) MSCs isolated using different centrifugation methods, were tested in a mouse model with optic nerve crush injury. Following treatment, functional assessments, immunostaining, retrograde tracing of RGCs, and western blot analysis were used to evaluate recovery and identify superior EVs.
Results: These findings suggest that all four types of administered MSC-EVs improved the optomotor response. However, EVs derived from TM exhibited a lower survival rate for Brn3a+ RGCs. Due to the compliance with GMP standards of clonal MSC-EVs, we proceeded with these EVs. When comparing the cBM EVs to the control group, retrograde tracing of RGCs and visual behavior tests indicated that the results were more in line with those of an intact group. The 20K cBM EVs were found to upregulate the p-AKT/AKT and p-PI3K/PI3K ratios in the retina and optic nerve, which are known antiapoptotic indicators. In addition, vehicle was shown to quickly increase procaspase and caspase levels in the control group, whereas these apoptotic markers were reduced in retinas treated with EVs. Lastly, a comparative analysis of specific candidate proteins in the 20K and 110K cBM EVs revealed a higher concentration of TGFβ, a finding that was substantiated by western blot analysis of the retinal tissue.
Conclusion: Increased levels of AKT and PI3K through cBM EV 20K may be responsible for the degenerating RGCs protection afforded by multi-trophic factor within EVs. This discovery represents a significant advancement towards potentially employing cBM EVs in the healing of injured optic nerves.
Keywords: Optic Nerve Injury, Mesenchymal Stromal Cell, Extracellular Vesicle, Retinal Ganglion Cells, PI3 Kinase

Ps-102: Neural Differentiation of Mesenchymal Stem Cells Derived From Adipose Tissue On A Scaffold Prepared With A 3D Printer Using Exosomes Extracted From Neural Stem Cells

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Objective: Neural stem cell-derived exosomes (NSC-exosomes) are extracellular vesicles secreted by Neural Stem Cells (NSCs) that play essential roles in intercellular communication within the central nervous system (CNS). These exosomes are small membrane-bound vesicles containing various bioactive molecules such as proteins, lipids, and nucleic acids, which enable them to exert diverse therapeutic effects on neural cells and tissues. NSC-exosomes have been extensively studied for their potential therapeutic applications due to their ability to modulate cellular functions, promote tissue regeneration, and enhance neuroprotection. Several studies have demonstrated that these exosomes can transfer genetic information, including microRNAs and mRNAs, to target cells, leading to changes in gene expression and cellular behavior. One compelling feature of NSC-exosomes is their ability to promote neural cell proliferation and differentiation. These exosomes carry growth factors, transcription factors, and other signaling molecules that can induce the proliferation and differentiation of neural progenitor cells, thereby potentially aiding in the generation of new neurons and glial cells. This regenerative potential makes NSC-exosomes attractive candidates for therapeutic approaches to repair damaged neural tissue in neurodegenerative diseases or after neural injury.
Materials and Methods: First, nerve cell isolation, exosome isolation, scaffold construction, and then cell differentiation and the method of examining the differentiation of exosomes using ultracentrifuge method from neural stem cells extracted from mouse brain and identified by TEM microscope, western blot and DLS technique. Also, adipose stem cells placed on the 3D PAN scaffold were treated with exosome concentration for 14 days. Cell survival on the scaffold was evaluated by MTT and acridine orange ethidium bromide methods. Cell differentiation process was also investigated by immunocytochemistry and Real Time-PCR techniques. Examining the identity of exosomes confirmed the existence of exosomes with an approximate size of 70 nm. The results of cell survival indicate the ability of cells to survive and multiply within 14 days. Also, the expression of MAP2 (protein associated with microtubules) and Nestin (interstitial filament protein) was confirmed using immunocytochemistry method.
Results: Results (DLS diameter 49.79 nm), TEM (lipid bilayer membrane), expression of CD81, CD9 and CD63 markers and total protein concentration (160 µg/ml) identified exosomes. Immunophenotype of adipose mesenchymal stem cells (AdMSCs) was positive for CD73, CD90, CD105 markers and negative for CD34, CD45 markers. Morphological examination showed the transformation of AdMSCs towards neural differentiation from the fifth day under exosome treatment compared to the untreated group. The results of the expression of Nestin, Map2, Tuj-1 and NF markers in these two groups indicated a significant expression of neural differentiation of adipose mesenchymal stem cells located on the scaffold on the fourteenth day after treatment.
Conclusion: These results show that the use of NSC-exosomes can cause neural differentiation of adipose mesenchymal stem cells. Therefore, exosomes can be considered as a repair tool for the regeneration of the lost nerve tissue.
Keywords: Exosome, Adipose Mesenchymal Stem Cell, Neural Differentiation, 3D Printer

Ps-103: Neural Differentiation of Mesenchymal Stem Cells Derived from Adipose Tissue on A Scaffold Prepared with A 3D Printer Using Exosomes Extracted from Neural Stem Cells

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Objective: Neural stem cell-derived exosomes (NSC-exosomes) are extracellular vesicles secreted by neural stem cells (NSCs) that play essential roles in intercellular communication within the Central Nervous System (CNS). NSC-exosomes have been extensively studied for their potential therapeutic applications due to their ability to modulate cellular functions, promote tissue regeneration, and enhance neuroprotection. Several studies have demonstrated that these exosomes can transfer genetic information, including microRNAs and mRNAs, to target cells, leading to changes in gene expression and cellular behavior. One compelling feature of NSC-exosomes is their ability to promote neural cell proliferation and differentiation. These exosomes carry growth factors, transcription factors, and other signaling molecules that can induce the proliferation and differentiation of neural progenitor cells, thereby potentially aiding in the generation of new neurons and glial cells. The purpose of this project is to investigate the potential of inducing neural differentiation of these exosomes on adipose-derived mesenchymal stem cells (ADMSCs) in cultured conditions using a scaffold made with a 3D printer.
Materials and Methods: First, nerve cell isolation, exosome isolation, scaffold construction, and cell differentiation were conducted. The exosomes were extracted using the Anacell kit from neural stem cells and identified through TEM microscope, western blot, and DLS technique. Additionally, ADMSCs placed on the 3D PAN scaffold were treated with 100 µg/ml exosome concentration for 14 days. Cell survival on the scaffold was evaluated using MTT and acridine orange ethidium bromide methods. The cell differentiation process was further investigated using immunocytochemistry and Real Time-PCR techniques
Results: The results (DLS diameter 40.19 nm), TEM (lipid bilayer membrane), expression of CD81, CD9, and CD63 markers, and total protein concentration (160 µg/ml) identified exosomes. The immunophenotype of ADMSCs was positive for CD73, CD90, CD105 markers and negative for CD34, CD45 markers. Morphological examination showed the transformation of ADMSCs towards neural differentiation from the fifth day under exosome treatment compared to the untreated group. The results of the expression of Nestin, Map2, Tuj-1, and NF markers in these two groups indicated a significant expression of neural differentiation of adipose mesenchymal stem cells located on the scaffold on the fourteenth day after treatment.
Conclusion: These results demonstrate that the use of NSC-exosomes can induce neural differentiation of adipose mesenchymal stem cells. Therefore, exosomes can be considered a reparative tool for regenerating lost nerve tissue.
Keywords: Neural Differentiation, 3D Printer, Exosomes, Stem Cells

Ps-104: Identification of Potential Biomarkers with Breast Cancer Based on Bioinformatics Analysis

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Objective: Breast cancer is the most common malignancy in women worldwide, despite great advances in the diagnosis and treatment of cancer. Nowadays, with the advances in the bioinformatics analysis of RNAseq, it can identify potential biomarkers for diagnosis, treatment, and evaluation of metastasis and relapse. In this study, we are going to evaluate RNAseq data obtained from TCGA in breast cancer (BRCA) and investigate prognostic and protective genes in survival.
Materials and Methods: GEPIA2 (http://gepia2.cancer-pku.cn/) was used to examine the TCGA BRCA dataset in order to identify all DEGs linked with BRCA among high throughput RNA-Seq data. GEPIA2 is an online program that uses the genotype-tissue expression projects and the TCGA database to evaluate the transcriptional patterns of human malignancies and normal tissues. Genes with adj-P-value < 0.05 were considered significant; after that, significant genes were divided into 4 groups including up/down prognostic or protective genes according to the logFC and HR. After that, a Protein-protein interaction (PPI) network of significant genes associated with BRCA was constructed with STRING at the Cytoscape software.
Results: Of 3556 genes acquired from TCGA-RNAseq for AML, 334 genes are considered significant; of which 127 genes are prognostic and 207 genes are protective. PPI networks were drawn for significant and also hub genes; according to the result, 7 genes are considered as hub genes based on their interaction and degree including CXCL1, SELL, CXCL9, CD3E, CCL5, CXCR3, and CCR5 (degree more than 20). All genes were protective (adj P<0.05, HR <1).
Conclusion: This study identifies hub genes as a promising prognostic, protective, and diagnostic biomarker for breast cancer. Further investigations are warranted to identify the diagnostic potential of these genes.
Keywords: Breast Cancer, TCGA, RNAseq, Bioinformatics, Significant Genes

Ps-105: Understanding Specific Potential Genes for Acute Myeloid Leukemia by Pan-Cancer Analysis

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Objective: Acute myeloid leukemia (AML) is caused by functionally complementary genetic mutations that cause uncontrolled proliferation and maturational arrest of myeloid precursor cells. Nowadays, gene expression profile analysis is a potent research technique that reveals patients' dysregulated genes by integrating data from functional genomics, molecular transcription, and genetics. In this study, we are going to evaluate TCGA datasets in acute myeloid leukemia and investigate genes that are exclusively related to this cancer with pan-cancer analysis.
Materials and Methods: First, GEPIA2 was used to examine the AML dataset to identify all DEGs linked. Genes with a P<0.05 were considered significant; after that, significant genes were divided into 4 groups including up/down prognostic or protective genes according to the logFC and HR. A Protein-protein interaction network of significant genes associated with AML was constructed with STRING at the Cytoscape software, and hub-genes were identified based on their scores and interaction. In the end, we used Pan-cancer analysis with GEPIA2 and UALCAN for hub genes and got to the 4 genes that were exclusive.
Results: Of 7965 genes acquired from TCGA-RNAseq for AML, 843 genes are considered significant (adjusted P<0.05), of which 666 genes are prognostic and 174 genes are protective.

According to the result, 8 genes are significantly related to survival including PSMG1, SLC37A1, FAM207A, GPS2, CSTB, CHAF1B, AKAP9, and ABCG1 (p-value and P<0.05). PPI networks were drawn for significant genes and also hub genes. All of the genes except HSPA5 were prognostic. After pan-cancer analysis, we found NDUFS8, MRPL12, MRPL2, and SOD1 which are expressed significantly less than usual (Compared with other cancers as well as with normal samples).
Conclusion: This study identifies 4 genes that are involved specifically in the occurrence and progression of AML. This information may hold promise as potential biomarkers and therapeutic targets.
Keywords: Acute Myeloid Leukemia, TCGA, RNAseq, Bioinformatics, Pan-Cancer Analysis

Ps-106: Identification of Potential Biomarkers Associated with Ovarian Cancer Using An Integrated Bioinformatics Analysis

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Objective: Ovarian Cancer (OC) is an extremely deadly gynecological cancer. women are often diagnosed at an advanced stage due to the lack of available biomarkers. This study aims to analyze the potential hub-genes associated with the survival of these patients.
Materials and Methods: At first, GEPIA2 was used to examine the TCGA OV dataset in order to identify all DEGs linked with OV among high throughput RNA-Seq data. GEPIA2 is an online program that uses the Genotype-Tissue Expression (GTEx) projects and the TCGA database. Thereafter, significant genes (p-value ≤ 0.05) were divided into 4 groups including up/down prognostic or protective genes according to the logFC and HR. Also, a Protein-Protein Interaction (PPI) network of significant genes associated with OV was constructed with STRING at the Cytoscape software for identifying hub-genes associated with OV cancer. Next, we used TISIDB to evaluate the immune system's interaction with hub-genes which play a major role in cancer onset, development, and treatment. Ultimately, enrichment analysis including Gene Ontology (GO) was used to explore the related biological processes, molecular function, cellular components, and Kegg pathway analysis.
Results: From 7638 genes obtained from TCGA-RNAseq for OV, 341 genes are prognostic and 241 are protective, divided into down and up categories. According to PPI networks, we identified 7 hub-genes based on their degree and interaction, including CXCR4, CXCL10, RPL23, CXCL9, UBD, GZMB, and TNFSF13B. Also, the result of TISIDB showed that most hub-genes have a strong interaction with the immune system except RPL23 and CXCR4 which have less interaction. Ultimately, the enrichment analysis showed these genes have the strongest significance in the inflammatory response, cytolytic granule, CXCR Chemokine Receptor Binding, Cytokine-cytokine receptor interaction, etc.
Conclusion: Our study identified 7 hub-genes that might be involved in the prognostic, diagnosis and survival of OV. Further investigations are warranted to identify the therapeutic potential of these genes.
Keywords: Bioinformatic Analysis, Ovarian Cancer, Biomarker

Ps-107: Mesenchymal Stromal Cell-Extracellular Vesicles-Loaded MiR-29a Induced Apoptosis in HCC Cell Lines

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Objective: In spite of significant advancements in therapeutic modalities for Hepatocellular Carcinoma (HCC), there is still a high annual mortality rate with a rising incidence. There are different treatment strategies that can induce apoptosis in cancer cells. MiRNAs (miRs) are short, 18-24 nucleotide-long molecules belonging to a class of highly conserved Non-Coding RNAs (ncRNAs) that regulate the expression of genes related to main cellular pathways post-transcriptionally. MiR-29a belongs to the miR-29 family and exerts anti-tumor impacts in diverse cancers including HCC. Since myriad biological processes are regulated by miRs, their pharmacological modulation has gained increasing interest as anticancer therapy. However, their effective delivery is hampered due to their negatively charged nature which affects their uptake into the cells. Extracellular Vesicles (EVs) encompass a wide range of vesicles originating from cells, among them endosome-derived exosomes, cell membrane-derived ectosomes, and apoptotic bodies released from dead cells. Mesenchymal stromal cells-derived EVs (MSCs-EVs) are particularly valued in drug delivery system in recent years. In the current study, we aimed to investigate the impact of treatment with miR-29a on the apoptosis in HCC cells using EVs as a natural delivery system given their potential in miRNA delivery both *in vitro* and *in vivo*.
Materials and Methods: Human Wharton's Jelly mesenchymal stromal cell-derived extracellular vesicles (WJ-MSC-EV20K) were lately isolated through centrifugation, characterized by Western blot, scanning electron microscopy, and dynamic light scattering. MiR-29a was subsequently loaded into the EVs and its loading efficiency was evaluated via RT-qPCR. Flow cytometry assessment was then performed on Huh-7 and HepG2 cell lines to evaluate apoptosis.
Results: Findings indicated that MSC-EVs can effectively delivered miR-29a to HCC cells. Furthermore, EV20K-miR-29a treatment significantly induced apoptosis in Huh-7 and HepG2 cells probably through the downregulation of anti-apoptotic gene MCL1.
Conclusion: Given the efficient delivery of miR-29a by EV20K, and the role of miR-29a ability in apoptosis induction in HCC cells, the use of EV20K-miR-29a could be a promising approach for liver cancer treatment.
Keywords: Hepatocellular Carcinoma, Apoptosis, MiR-29a, Extracellular Vesicles

Ps-108: *In Vitro* Differentiation of Adipose Derived Mesenchymal Stem Cells into Insulin-Producing Cells on Decellularized Peritoneal Scaffold

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Objective: Diabetes mellitus (DM) is a set of chronic metabolic diseases caused by insulin insufficiency. DM is highly prevalent, and its incidence is rising globally. At present, there are different treatments for diabetes. Recently, the differentiation of stem cells, especially Adipose-derived stem cells (ADSCs), and using natural scaffolds have demonstrated considerable promise as an alternate technique in the treatment of diabetes patients. Therefore, the aim of this study is to produce insulin-secreting cells from ADSCs using peritoneal scaffolds.
Materials and Methods: For this purpose, ADSCs were extracted from rat fat and differentiated during 2 stages using ITS and nicotine in 14 days. Scaffold formation and cell differentiation were evaluated using Masson's trichrome staining, Diamidino-2-Phenylindole (DAPI) staining, and western blotting.
Results: Histological analysis of Masson's trichrome-stained processed peritoneum samples shows complete decellularization with preservation of extracellular matrix microarchitecture. Further, staining of tissue samples with DAPI demonstrates the complete removal of DNA fragments. Western blot analysis also showed that the differentiation groups with peritoneal scaffold expressed beta cell-specific proteins such as MAFA and PDX1 significantly more than the control group ($P<0.0001$). Also, the 3D group demonstrated MAFA ($P<0.0001$) and PDX1 ($P<0.01$) protein expression significantly more than the 2D group.
Conclusion: These results collectively have evidenced that the decellularized peritoneum is a suitable scaffold for stabilizing artificial pancreatic islands. Peritoneum scaffold is thus capable of producing insulin-secreting cells from ADSC and could be widely applied as one of the differentiation agents.
Keywords: Adipose Tissue Derived Mesenchymal Stem Cells, Diabetes Mellitus, Decellularized Scaffold, Peritoneal Scaffolds, Insulin-Producing Cells

Ps-109: Overcoming Cisplatin Resistance in Ovarian Cancer: A Promising Approach with Quercetin-Loaded Solid Lipid Nanoparticles

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Objective: Ovarian cancer (OCa) is a significant health concern globally, with rising mortality rates despite it being less common in Iran compared to Western countries. The prevalence of epithelial carcinoma, the most common type of OCa, and the development of resistance to platinum-based chemotherapy, such as cisplatin, present major therapeutic challenges. This study aims to investigate the potential of quercetin (QU)-loaded solid lipid nanoparticles (SLN) to overcome cisplatin resistance in OCa cells.
Materials and Methods: Human ovarian cancer cell lines SKOV-3 was exposed to 36 µl/ml of QT-SLN or 0.001 µl/ml cisplatin for 48 hours. To assess the toxicity of QT-SLNs, cell viability and RT-PCR were measured. ovary cancer cell proliferation was investigated through MTT assay. Finally, the effect of treatments on gene expression of XIAP was evaluated by quantitative real-time PCR.
Results: We discovered thate quercetin facilateds cispelatin-induced apoptosis in human ovary (cell line SKOV-3) by inhibiting the anti-apoptotic protein XIAP. Following QT-SLNs treatment, the expression of the XIAP protein significantly decreased, while the XIAP expression showed some change in comparison with free QT-treated cells. when cancer cells are pretreated with nano-quercetin.
Conclusion: The application of QT-SLN in conjunction with cisplatin not only diminishes cell proliferation and augments apoptosis but also effectively curtails the expression of genes associated with drug resistance. These findings suggest that QT-SLN could be a viable strategy to overcome cisplatin resistance, offering a promising therapeutic avenue for inclusion in clinical OCa treatment protocols.
Keywords: Solid Lipid Nanoparticles, Resistance, Cisplatin, Quercetin, Ovarian Cancer

Ps-110: Comparison of The Effects of Exosomes Secreted from Menstrual Blood-Derived Stem Cells and Ginger-Derived Exosomes on Apoptosis in Endometriosis Women

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Objective: The most important theory for endometriosis initiation is the return of blood during menstruation. Menstrual blood-derived stem cells from endometriosis patients (E-MenSCs) have shown different gene expression compared with healthy non-Endometriotic women's cells (NE-MenSCs). Today, exosomes extracted from mesenchymal stem cells and plants have been considered for the treatment of various diseases. In this study, we compared the effect of NE-MenSCs-derived exosomes and exosomes derived from the root of ginger on E-MenSCs.
Materials and Methods: Menstrual blood samples were collected from healthy and endometriosis women. MenSCs were isolated through Ficoll-Paque density gradient centrifugation and cultured till 3rd passage. After a gradual decrease of FBS

concentration in the culture medium, NE-MenSCs-derived exosomes (C-Exo) were isolated and characterized. In addition, plant exosomes (P-Exo) were extracted from the root of ginger. 72 hours after treating E-MenSCs with C-Exo/ P-Exo, cell viability was measured using apoptosis assay, and the expression of genes related to apoptosis was analyzed by Real-Time polymerase chain reaction (PCR).
Results: After treatment, increased apoptosis was observed in both P- and C-Exo groups compared to the untreated group of E-MenSCs. Also, the expression level of BAX/BCL2 in the P-Exo group was significantly decreased compared to the E-MenSCs group. Besides, the apoptosis level was decreased after P-Exo treatment compared to E-MenSCs.
Conclusion: Although the effect of exosomes, especially P-Exo, has been significant in this study, it seems that further studies on the wider range of treatment doses, as well as preclinical and clinical studies, are necessary for providing an effective treatment method for endometriosis.
Keywords: Endometriosis, Exosome, Mesenchymal Stem Cells, Menstrual Blood, Ginger

Ps-111: The Comparative Evaluation of The Effects of Small Molecule C-82 and Naringenin on The Inflammatory Factors of Menstrual Blood Derived-Stem Cells in Endometriosis Patients

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Objective: One of the leading causes of endometriosis - a chronic estrogen-dependent disease associated with pelvic pain and infertility- is the return of menstrual blood flow into the pelvic cavity and the establishment of menstrual blood mesenchymal stem cells (MenSCs) in areas outside the uterine cavity. Menscs from endometriosis patients (E-MenSCs) and healthy women have been shown to vary, particularly in terms of surface markers and gene expression, which may suggest the involvement of these cells in the development, expansion, and maintenance of ectopic lesions. The purpose of this study is to investigate the effect of small molecule C-82 and naringenin as inhibitors of involving pathways on E-MenSCs to modulate their gene expression and functional pattern.
Materials and Methods: Briefly, menstrual blood samples (2-3 ml) were collected from women with endometriosis. E-MenSCs isolated by Ficoll-Paque density-gradient centrifugation were characterized by flow cytometry. Then E-MenSCs cells were treated with small molecule C-82 and naringenin in the third passage, then the cell behavior of the studied groups was investigated using ELISA and Western methods.
Results: In this study, using the ELISA test, we found that naringenin and C-82 treatment can reduce the production level of ROS, IL-6, and IL-8 in the endometriosis cell line, moreover, when we compared the treated groups, showed that combined treatment with C-82 and Naringenin was more effective in reducing all three relevant genes in E-MenSCs. Also, using Western analysis, the expression level of the β-catenin gene and α-SMA protein showed a significant decrease in all treated groups compared to untreated E-MenSCs.
Conclusion: The results obtained from this study clarify the function of C-82 together with naringenin in modulating E-MenSCs. However, it seems that more research is needed to analyze the precise effects of small molecule C-82 and naringenin on endometriosis.
Keywords: Endometriosis, Menstrual Blood Derived-Stem Cells, Small Molecule C-82, Naringenin, Inflammatory Factors

Ps-112: Identification of Three Key microRNAs (miR-302d, miR-371, miR-372) Involved in The Cardiomyocytes' Cell Cycle Arrest

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Objective: Cardiomyocytes' cell cycle arrest is the major underlying mechanism for the limited cardiac regenerative capacity. As a result, myocardium is replaced with fibrotic tissue after ischemic or non-ischemic damage, which reduces the heart's blood pumping efficiency. In order to enhance the regenerative capacity, one solution could be the identification of the molecular basis of cardiomyocytes' cell cycle arrest. In the current study, we aimed to investigate miRNAs related to this cellular process.
Materials and Methods: Initially, we reanalyzed the RNA sequencing (RNA-Seq) data for differentiating human induced pluripotent stem cell-derived cardiomyocytes available in public dataset NCBI (GSE35672) using GEO2R with $-2 \geq LogFC \geq 2$ and adj.$P \leq 0.05$ with focus on miRNA expression profile. The Differentially Expressed miRNAs (DEmiRNAs) were identified and their corresponding signaling pathways were determined using Enrichr. In a further screen, those DEmiRNAs related to proliferation, growth and cell cycle were shortlisted. To verify these candidate DEmiRNAs, human embryonic stem cells (hESC, Royan H6 line) were expanded and differentiated into cardiomyocytes using four small molecules targeting Wnt/α-catenin, TGFα and sonic hedgehog pathways. Cardiomyocytes were harvested at day 10 (D10), D20 and D30 of differentiation and subjected to cell cycle analysis by flow cytometry. The expression of candidate DEmiRNAs were evaluated in hESC and cardiomyocytes at D10, D20 and D30 using real time qRT polymerase chain reaction (PCR).
Results: miR-302d, miR-371 and miR-372 were identified as the candidate DEmiRNAs, where miR-302d and miR-372 was related to Wnt/α-catenin, sonic hedgehog and cancer pathways and miR-371 to Wnt/α-catenin and cancer pathways. The cell cycle analysis showed that while $53.2 \pm 2.5\%$ of hESC were in G1, the percentage was significantly increased in D10, D20 and D30 cardiomyocytes (74, 77.4 and 80%, respectively). Conversely, the percentage of cells in S phase was significantly decreased in D10, D20 and D30 cardiomyocytes compared to hESC (13.1, 8.61 and 5% compared to 23.7%, respectively). miR-302d, miR-371 and miR-372 showed downregulation in hESC-derived cardiomyocytes at D20 and D30.
Conclusion: Thus, the decreased expression of candidate DEmiRNAs by increasing differentiation window, suggests them as potential molecular regulators of cardiomyocytes' cell cycle arrest and possible candidates for cell cycle reentry by overexpression.

Keywords: Human Pluripotent Stem Cell, Cardiac Differentiation, Regulatory Non-Coding RNA, Cell Cycle

Ps-113: Treatment with Cabergoline Reduced The Expression of Immune Checkpoints in Monocyte-Derived Dendritic Cells and Shifted Their Phenotype to Tolerogenic Dendritic Cells

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Objective: Dendritic cells (DCs) are regarded as specialist antigen-presenting cells, several strategies have been used recently to increase the effectiveness of antigen presentation, tolerogenisity and improving immune system against several disorders including autoimmune diseases. Tolerogenic dendritic cells inhibit autologous T-cell responses, making them appealing therapeutic candidates for autoimmune disorders.
Materials and Methods: We investigate the impact of cabergoline on the expression of immune checkpoints in dendritic cells generated from peripheral blood mononuclear cells. Ficoll was used to isolate PBMCs, while magnetic activated cell sorting was used to separate monocytes with high purity. DCs were generated from monocytes after cell were cultured in a special medium for 5 day and Apoptosis assay was done to determine the optimal dosage of cabergoline Using flow cytometry and Annexin v/Propidium iodide labeling. Finally, qRT-PCR assay was done for recognizing the tolerogenic profile of DCs and analyzing the level of immune checkpoint expression in DCs.
Results: The purity of isolated monocytes separated from PBMCs using MACS method was 92.5%. Administering 10 μM of cabergoline to the cells does not result in a significant loss of life. The both the mDC group and the treatment group with 10 μM drug doses, expressed CD11c, HLA-DR, and CD86 markers. Then, the mean fluorescence intensity showed the level of the cabergoline-treated group had a significant increase in CD11c and CD86 expression (p,0.05). While the decrease in HLA-DR expression was not significant in the Cabergoline-mDC group. The expression of the CTLA-4 genes (P≤0.01), B7H7 (P≤0.05) and ICOS-L (P≤0.0001) decreased significantly in Cabergoline-mDCs, and TIM-3 expression level had a significant elevated after cabergoline treatment (P≤0.01). however, PD-L1 and CD39 decrease was not significant.
Conclusion: Our findings demonstrated that cabergoline treatmented DCs could be effective in autoimmune diseases according to their remarkable role in reduction of immune checkpoints expression.
Keywords: Cell Therapy, Autoimmune Disease, Cabergoline, Immune Checkpoint Molecules, Dendritic Cell

Ps-114: Treatment with Cabergoline Reduced The Expression of Oncogenic Immune Checkpoints in Monocyte-Derived Dendritic Cells and Shifted Their Phenotype to Immunogenic Dendritic Cells

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Objective: Dendritic cells (DCs) regarded as specialist antigen-presenting cells. Strategies used recently to increase the effectiveness of antigen presentation, tolerogenic and generate strong immune response against disorders such as autoimmune disorders. Tolerogenic dendritic cells inhibit autologous T-cell responses, making them appealing therapeutic candidates for autoimmune diseases.
Materials and Methods: DCs were generated from monocytes after cell were cultured and Apoptosis assay was done to determine the optimal dosage of cabergoline Using flow cytometry and Annexin v/ iodide labeling. Administering 10 μM of cabergoline to the cells doesn't result in a significant loss of life. m DC group and the treatment group with 10 μM drug doses, expressed CD11c, HLA-DR, and CD86 markers. the mean fluorescence intensity showed the level of the cabergoline-treated group had a significant increase in CD11c and CD86 expression (p,0.05). While the decrease in HLA-DR expression was not significant in the Cabergoline-m DC group. qRT-PCR assay was done for the recognizing the tolerogenic profile of DCs and analyzing the level of immune checkpoint expression in DCs.
Results: Results indicated that the expression of the CTLA-4 genes (P≤0.01), B7H7 (P≤0,05) and ICOS-L (P≤0.0001) decreased significantly in Cabergoline-m DCs, and TIM-3 expression level had a significant elevated after cabergoline treatment (P≤0.01). PD-L1 and CD39 decrease was not significant.
Conclusion: Findings demonstrated that cabergoline treatment DCs could have an impressive effect in autoimmunotherapy according to their remarkable role in immune checkpoints expression.
Keywords: Cell Therapy, Autoimmune Disease, Cabergoline, Immune Checkpoint Molecules, Dendritic Cell

Ps-115: Diagnosis of Drug Resistance in Brain Tumor Patients Using Chemobiogram Kit

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Objective: The chemogram kit is an innovative method used in oncology to optimize the selection of chemotherapy drugs for cancer patients. Oncologists extract a sample from the patient's tumor. After tumor cells are cultured in the laboratory, the cells are exposed to a diverse panel of chemotherapy drugs. By evaluating the response of tumor cells to different drugs, specialists can identify the most effective treatments. This helps in the early detection of drug resistance
Materials and Methods: Initially, common chemotherapy drug doses used in adult human treatment were adapted from Medscape.com and converted to culture medium doses. Tumor cells from cancer patients were cultured in both 2D and 3D culture. Subsequently, tumor cells were exposed to chemotherapy drugs for 24, 48, and 72 hours. To assess cell viability, the MTS Assay was employed, and measurements were obtained using a Micro-

plate reader.
Results: The greatest effect of drugs on the glioblastoma cell line (U251) was for: % Viability 2% Viability 1 Group (24,72H 2D) 100 100 Control 1 69 68 Gemsiban 72H 2 18 20 Cisplatin 24H 3 17 20 Doxorubicin 24H 4 18 17 Bevacizumab 24H 5 % Viability2 % Viability1 Group (72H 3D) 100 100 Control 1 69 68 TMZ 72H 2.
Conclusion: The Chemobiogram Kit is a powerful tool used by oncologists to optimize chemotherapy drug selection for individual cancer patients.
Keywords: Brain Cancer, Glioblastoma, U-251G Cell Line, Drug Resistance, Drug Treatment

Ps-116: Selenium-Containing Polycaprolactone 3D-Printed Scaffold for Osteogenesis

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Objective: Bone injuries are a prevalent issue that affects millions of people worldwide. Selenium is an essential trace element that plays crucial roles in maintaining bone health due to antioxidant and anti-inflammatory properties. Selenium deficiency delays or disrupts bone healing. In the present study, with the approach of producing a simple and efficient implant containing the selenium, PCL scaffolds containing the selenium were produced using a 3D printer and its effect on bone repair was investigated in the *in vitro* conditions.
Materials and Methods: Net and selenium-containing (0.5-10 uM) PCL scaffolds were produced with 3D printer. Rat bone marrow mesenchymal stem cells were cultured on the scaffolds, and the osteo-induction potential of PCL-Sel scaffold was compared with net PCL scaffold during the bone differentiation of BMSCs. The biocompatibility of 3D-printed scaffolds was assayed using MTT assay.
Results: The study's findings revealed that incorporating selenium to PCL enhanced the scaffold's hydrophilicity. In addition, selenium had a gradual release from the scaffolds and Bone differentiation of cells on PCL-selenium scaffolds was higher compared to pure PCL. The MTT test results showed that PCL scaffolds incorporated with 0.5-10 µM selenium had not toxic effect on the BMSCs and did not inhibit their growth.
Conclusion: The results of the current study demonstrated that selenium-incorporated PCL scaffold had improved bone adaptability and osteogenesis capacity because of selenium's favorable bone-formation capabilities and might be used to treat bone defects.
Keywords: Polycaprolactone, 3D-Printed Scaffold, Selenium, Tissue Engineering

Ps-117: Bardoxolone Methyl Increases The Antioxidant Content of Bone Marrow Mesenchymal Stem Cell-Derived Exosomes

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Objective: Bone marrow mesenchymal stem cell (BMSC)-derived exosomes have been recognized as a promising therapeutic modality due to their low immunogenicity, and the ability to penetrate biological barriers. The cargo carried by exosomes is determined by the cells from which they originate. By inducing donor cells with specific small molecules before isolating exosomes, it would be feasible to generate exosomes that carry specific molecules, such as antioxidants. This research aimed to enhance the antioxidant capacity of exosomes by activating the nuclear factor Erythroid 2-Related Factor 2 (*NRF2*) gene in BMSCs.
Materials and Methods: BMSCs were harvested from Wistar rats and treated with Bardoxolone methyl (BaMet) to upregulate the *NRF-2* gene. Real-time polymerase chain reaction (PCR) was performed to determine the expression level of *Nrf2*. Exosomes were isolated by ultracentrifugation. Their identity was confirmed using DLS, SEM, TEM, and Western blot analyses to detect markers specific to exosomes. The antioxidant contents of exosomes were determined using Superoxide Dismutase (SOD) and catalase (CAT) assay kits.
Results: The BaMet-treated BMSC-derived exosomes exhibited spherical morphology with a mean size of 84.79 ± 10.79 nm. Immunoblotting revealed that the exosome tested positive for CD63, CD9, and CD81. BaMet-treated BMSC-derived exosomes exhibited significantly higher levels of SOD (2.890 ± 0.085 vs. 1.070 ± 0.071) and CAT content (0.333 ± 0.016 vs. 0.159 ± 0.008) ($P<0.05$).
Conclusion: Our findings demonstrated that the elevated expression of the *Nrf2* gene in BMSCs stimulated the secretion of exosomes with increased antioxidant content.
Keywords: Exosome, Mesenchymal Stem Cell, Antioxidant, NRF2

Ps-118: Synergistic Effects of Kartogenin and TAK242-Loaded Extracellular Vesicles in Treatment of Rheumatoid Arthritis

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Objective: Extracellular vesicle (EV)-based drug delivery systems show great promise for treating systemic inflammatory diseases like rheumatoid arthritis (RA). Unlike conventional anti-inflammatory agents, which often have non-specific tissue distribution and uncontrolled drug release, EV-based systems provide precise delivery and controlled release of therapy. This study specifically focused on EVs loaded with Kartogenin (KGN) to promote chondrogenesis and TAK-242 to reduce inflammation in a collagen-induced arthritis (CIA) rat model.
Materials and Methods: TAK242 and KGN were loaded into EVs using electroporation and sonication, respectively, and were characterized by SEM, DLS, and zeta potential analysis. For *in vivo* evaluations, animals were divided into five treatment

groups: EV TAK242, KGN, combined TAK242 and KGN, and EV/TAK242-KGN. Results were compared with sham, healthy, and methotrexate control groups. Over a 28-day post-treatment follow-up, physical examinations, imaging, histopathological, and molecular tests were conducted to evaluate treatment response and underlying biological mechanisms.
Results: TAK242 and KGN were efficiently loaded into EVs, with encapsulation efficiencies of 11.15 and 39.85%, respectively, while maintaining the general characteristics of EVs. Physical observations showed significant reductions in paw swelling and arthritis severity, along with increased body weight in the treatment groups. The EV/TAK242-KGN group exhibited the most notable improvements compared to the other groups. Radiographic and histological analyses confirmed the therapeutic effects of EV/TAK242-KGN in CIA rats. Additionally, there was a decrease in serum levels of the pro-inflammatory cytokines IL-6, IL-17, and TNF-α, and an increase in the anti-inflammatory cytokine IL-10 across all treatment groups compared to the sham group, with the most significant changes observed in the EV/TAK242-KGN group.
Conclusion: EV-based drug delivery systems, especially EVs loaded with KGN and TAK-242, offer a promising therapeutic strategy for RA treatment and could apply to targeted therapies for systemic conditions.
Keywords: Rheumatoid Arthritis, Extracellular Vesicles, Drug Delivery, TAK242, Kartogenin

Ps-119: Design and Fabrication of Conductive Hydrogel Based on Polyaniline/Sodium Alginate for Bone Tissue Engineering

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Objective: Bone tissue engineering aims to mimic the natural extracellular matrix (ECM) environment to promote osteogenesis and repair bone defects. The ECM in bone tissue is a dynamic and conductive network that supports cellular activities essential for bone regeneration. Sodium alginate (SA), a natural polysaccharide, is widely used for its biocompatibility and gel-forming properties, while Polyaniline (PANi) offers electrical conductivity, enhancing cell proliferation and differentiation. Incorporating drugs can enhance scaffold functionality by providing additional bioactive properties and supporting tissue regeneration.
Materials and Methods: Sodium alginate was dissolved in deionized water, followed by the addition of an appropriate drug amount. Aniline was then added to the mixture, and finally, ammonium persulfate (APS) was introduced to initiate the polymerization of aniline. This polymerization process led to the formation of a conductive SA/PANi hydrogel without the need for additional crosslinkers, as the polymerized aniline acted as the crosslinking agent. Various parameters, including polymer concentrations and polymerization conditions, were optimized to achieve the desired hydrogel. The composite hydrogel's properties were characterized using Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and conductivity tests.
Results: FTIR analysis revealed distinct spectral features confirming the successful polymerization of aniline and gelation of sodium alginate within the composite hydrogel scaffold. Additionally, shifts and broadening of peaks associated with sodium alginate indicated its structural rearrangement and hydrogen bonding interactions with the polymerized aniline. SEM images displayed a uniform and porous structure in the SA/PANi hydrogel scaffold. Conductivity tests confirmed the hydrogel's good electrical conductivity.
Conclusion: A conductive drug-loaded SA/PANi hydrogel scaffold was developed, presenting a promising candidate for bone tissue engineering.
Keywords: Bone Tissue Engineering, Alginate, Polyaniline, Conductive Hydrogel, Osteogenesis

Ps-120: Features of Electrspun Nanofibers Mounted on Each Other Containing Collagen-Based Hydrogel for Tissue Engineering Applications

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Objective: Electrospun nanofibers, which can mimic the nanostructure of an extracellular matrix within native tissue, have extensively been applied in tissue engineering-based approaches.
Materials and Methods: To do this experimental study, hybrid materials containing synthetic and natural polymers were electrospun and mounted on each other aimed at imitating the structure of native ECM and improving the aspects of applied materials (natural and synthetic polymer) in combination with each other and compatibility of fabricated constructs. Simultaneously, collagen-based hydrogels prepared in the HCL solution were loaded into the interface of these mounted nanofibers, followed by crosslinking in the EDC/NHS solution and freeze-drying. In addition, one construct containing the desired hydrogel without applying nanofibers was considered the control group. In the following, corresponding features, including cell compatibility, porosity, and *in vivo* study, were investigated.
Results: The SEM imaging revealed the fibrous structure of electrospun nanofibers and multilayered structures of fabricated constructs. In addition, it was revealed that the porosity of all multilayered constructs was ≥ 85%, and an HDF cell-seeded construct with two layers of nanofibers within its structure had the best cell interaction, confirmed by FESEM imaging. *In vivo* studies of subcutaneously implanted samples demonstrated lower inflammatory responses in constructs containing one and two layers of nanofibers compared to the control one.
Conclusion: Based on these results, it could be concluded that the electrospun nanofibers accompanied by collagen-based hydrogels could be the proper choice for simulating the native tissue structure and improving the compatibility of implanted constructs.

Keywords: Nanofiber, Electrospinning, Collagen- Based Hydrogel

Ps-121: Uterine Fluid Derived Exosomes Enhance Endometrial Receptivity by Upregulating Leukemia Inhibitory Factor and Downregulating Mucin-16 Genes in Endometrial Cells

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Objective: Endometrial exosomes carry bioactive agents to uterine epithelial cells and trophectoderm to promote implantation. This study investigates the probable molecular mechanisms by which exosomes improve endometrial receptivity.
Materials and Methods: Exosomes were isolated from uterine fluid by Gradient ultracentrifugation and characterized by dynamic light scattering (DLS), Transmission Electron Microscopy (TEM), and western blotting. Endometrial Ishikawa cell line were treated with isolated exosomes and *in vitro* implantation assay was performed to evaluate the effect of exosomes on the receptivity potential of endometrial cells. Finally, the expression of several endometrial receptivity markers was evaluated by Real-Time polymerase chain reaction (PCR).
Results: DLS graph and TEM imaging showed that the isolated exosomes had a cup-shaped or spherical morphology with a mean size of 91.8 nm and zeta potential of -9.75 mV. Relatively strong immunoblotting bands for exosome-specific protein markers (CD-9 and CD-81) confirmed the isolation of exosomes. *In vitro* implantation assay revealed that treatment of endometrial cells by uterine exosomes enhances the receptivity potential of endometrial cells 1.5 ± 0.5 folds relative to the control group. Gene expression analysis showed that treatment of endometrial cells with uterine-derived exosomes results in upregulation and downregulation of Leukemia Inhibitory Factor (LIF) and Mucin-16, respectively; however, the expression of Trophinin and Insulin-Like Growth Factor-Binding Protein 1 (IGFBP1) was not affected.
Conclusion: These findings confirmed the vital role of exosomes in endometrial receptivity and showed that regulation of LIF and Mucin-16 expression is one of the probable mechanisms by which exosomes affect endometrial receptivity.
Keywords: Exosomes, Endometrium, Receptivity

Ps-122: An Injectable Type II Collagen/Hyaluronic Acid Hybrid Hydrogel A Step Towards An Extracellular Matrix-Inspired Scaffold for Cartilage Regeneration

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Objective: The extracellular matrix (ECM) plays a vital role in regulating cellular functionality and tissue regeneration, providing both compositional and architectural cues. In this study, Type II collagen (COL-II) and hyaluronic acid (HA), as the main components of cartilage, were grafted with tyramine (HA-Ty and COL-II-Tyr) to design an injectable bioinspired hydrogel scaffold for cartilage regeneration.
Materials and Methods: COL-II was purified from chicken sternums, and COL-II-Tyr and HA-Tyr conjugates were synthesized. The conjugates were crosslinked in the presence of horseradish peroxidase enzyme (HRP) and a cytocompatible concentration of Hydrogen Peroxide (H_2O_2), encapsulating human Bone Marrow-Derived Mesenchymal Stromal cells (hBM-MSCs). The gelation time, degradation rate, swelling, morphological and rheological properties of the hydrogels were evaluated, and the effect of COL-II-Tyr content on the viability, adhesion, proliferation, gene expression, and extracellular matrix deposition of hBM-MSCs was assessed.
Results: Increasing the HA-Tyr content led to higher storage modulus and reduced hydrogel shrinkage, resulting in increased swelling. Encapsulated hBM-MSCs survived well in all the hydrogels. Incorporating COL-II-Tyr into HA-Tyr hydrogels created a more favorable microenvironment for hBM-MSCs chondrogenic differentiation. Compared to HA-Tyr alone, the hybrid HA-Tyr/COL-II-Tyr hydrogel promoted enhanced cell adhesion, spreading, proliferation, and upregulation of cartilage-related gene expression.
Conclusion: The injectable HA-Tyr/COL-II-Tyr hybrid hydrogels benefits from the enhanced mechanical strength of HA and cell adhesive, proliferative and chondrogenic conductive properties of COL-II, mimicking the natural cartilage extracellular matrix. These results highlight the promising potential of this hybrid system as a biomaterial scaffold for cartilage regeneration applications, delivering cells in a supportive and bioactive microenvironment.
Keywords: Biomimetic Materials, Cell Encapsulation, Tissue Engineering

Ps-123: Harnessing the Regenerative Potential of Stem Cells: A Systematic Review of Emerging lncRNA-Driven Therapies for Alzheimer's Disease

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Objective: Alzheimer's disease (AD) is a severe neurodegenerative condition marked by progressive cognitive decline and neuronal loss. Despite extensive research, current treatments only provide symptomatic relief, necessitating more effective therapeutic approaches. Recent advancements in stem cell biology and Long Non-Coding RNA (lncRNA) research offer promising avenues for regenerative therapies in AD.
Materials and Methods: This review aims to consolidate literature on lncRNA-driven stem cell therapies for AD. Through

comprehensive searches in PubMed, Embase, and Web of Science, studies published between January 2010 and April 2024 were identified using relevant keywords. Eligible studies underwent rigorous screening, with data extraction performed by two independent reviewers

Results: From 287 initial articles, 42 met inclusion criteria. These studies investigated various stem cell types, such as mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), and neural stem cells (NSCs), for AD treatment. LncRNA-modulated stem cells demonstrated therapeutic potential in AD animal models. For instance, preconditioning MSCs with lncRNAs enhanced their neuroprotective and anti-inflammatory properties, while iPSC-derived neural progenitor cells overexpressing specific lncRNAs promoted neuronal differentiation and survival.

Conclusion: This systematic review underscores the promising prospects of lncRNA-driven stem cell therapies for AD. By leveraging stem cells' regenerative capabilities and lncRNAs' regulatory functions, these innovative strategies hold promise for neuronal repair, neuroinflammation reduction, and potentially halting cognitive decline in AD. However, further research is imperative to address safety, scalability, and clinical translation challenges.

Keywords: Stem Cells, lncRNA, Alzheimer

Ps-124: Using A Novel Acellular Dermal Matrix (Syn Derm) with Boron/MP for Healing Postoperative Wounds from Removing A Tumor on The Skin

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Objective: Improper healing may result in large and unpleasant skin lesions or scars, particularly on the face, leading to psychological problems. Therefore, proper and effective wound healing is crucial. Using autograft skin repair for wounds is complex and can cause additional cost and pain for the patient. In this study, researchers have investigated using a novel allograft acellular dermal matrix (ADM) with boron and mineral pitch as a skin dressing for treating postoperative wounds from removing basal cell carcinoma (BCC) on the nose skin.

Materials and Methods: The methods are outlined step by step along with the report.

Results: Despite the deep wound, using acellular dermal matrix for healing and wound closure was effective.

Conclusion: The rapid closure of the wound in this particular case is promising and sufficient. The use of Syn Derm for fast healing of deep wounds involving difficult tissues, particularly ADM and Syn Derm, shows promising results on wound healing.

Keywords: Aacellular Dermal Matrix, Postoperative, Syn Derm

Ps-125: Investigating The Levels of Interferon Gamma and Tumor Necrosis Factor in The Cerebrospinal Fluid of Brain Tumor Patients Receiving Allogeneic Natural Killer Cells

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Objective: Types of brain tumors include primary tumors as well as metastatic tumors. Today, immunotherapy with Natural killer cells (NK Cells) and injection of these cells into the tumor site or spinal fluid can be a complementary approach to other existing treatments. However, due to the special condition of these tumors and the high cost of immunotherapy methods, early detection of treatment response or resistance is of particular importance. It seems that cerebrospinal fluid and its protein factors can be a good solution for early detection of treatment response or resistance. In this study, we investigated the relationship between tumor necrosis factor alpha (TNF-a) and Interferon Gamma (IFNγ) levels in patients with brain tumors treated with allogeneic natural killer cells.

Materials and Methods: Interferon gamma and Tumor necrosis factor were measured in 18 cerebrospinal fluid samples of brain tumor patients before and after immunotherapy with natural killer cells by ELISA method and the relationship between the levels of these two cytokines and proteins, LDH, glucose, WBC, NK cells, NKT cells, T cells and MRI results of the patients were evaluated before and after treatment by SPSS.

Results: Significant reductions in IFNγ and Tumor necrosis factors were observed in patients with high-grade malignant brain tumors who were immunotherapy with allogeneic NK Cells. The mean level of TNF-a decreased from 41.99 pkg/ml to 26.60 pkg/ml and the mean level of IFNγ decreased from 83.48 to 28.18 pkg/ml. These reductions were significant in the group that responded well to the treatment.

Conclusion: Given the association between cancer and inflammation and considering IFNγ and TNF-a as two pre-inflammatory cytokines, reduction of these two factors during the course of treatment with NK Cells may indicate a reduction in inflammation and tumor recovery. IFNγ and Tumor necrosis factor may be suitable cytokines for studying the response to treatment in patients with brain tumors treated with NK Cells.

Keywords: High-Grade Glioma, Interferon Gamma, Tumor Necrosis Factor Alpha, Allogenic Natural Killer Cells, Cerebrospinal Fluid

Ps-126: The Associations Between Age and Protein Receptors in Breast Cancer

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Objective: Both women and men are affected by breast cancer, which is one of the most common cancers in life. There is a cluster pattern of breast cancer in Iran. Among the provinces of Iran, there is a difference in the prevalence of age. The rates in the central provinces are lower than in the southeastern provinces of Iran. According to various studies, breast cancer occurs most commonly between the ages of 40 and 49 in Iran. Obesity can impact cancer pathways in two ways: one is through the motor pathway, and the other is through an increase in blood glucose levels. We aimed to evaluate the associations between age and receptor-defined breast cancer (luminal A, luminal B, triple negative, and HER2+) in the south of Iran. The majority

of molecular subtypes were Luminal A subtype (36%), followed by Triple Negative (13%), HER2+ (13%), and Luminal B (5%).
Materials and Methods: A total of 109 female patients with primary breast cancer were collected. 45% of cases were aged <45. logistic regression was used to estimate Odds ratios (OR) and 95% confidence intervals (95% CI).
Results: Breast cancer patients aged>or=45 years old, compared with those ages <45 years old, were at an increased risk of luminal A (OR, 1.7; P: 0.1; 95% CI, 0.78-4.1) and triple-negative tumors (OR =3.4; P=0.07; 95% CI, 0.9-13.1). However, Odds ratios did not appear to vary significantly by age. Age in itself is not an independent prognostic factor.
Conclusion: We did not observe a significant difference between age status (more equal to 45 and less than 45) in patients with Her-2 positive (P=0.6) and luminal B breast cancer (P=0.4).
Keywords: Breast Cancer, Protein Receptors, Age, HER2+

Ps-127: Engineered Scaffolds Combined with Exercise Improve The Treatment of Volumetric Muscle Loss

Zohour Soleimani M[1, 2, 3*], Bayati V[2, 3], Nejaddehbashi F[2, 3],, Orazizadeh M[2, 3], Khoshnam SE[2]

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Objective: Adult skeletal muscle is a stable tissue that does not undergo cell division. Small injuries can be repaired without causing any inflammatory responses or cell death. Skeletal muscle has a limited ability to regenerate after injury, which may cause volumetric muscle loss (VML). When skeletal muscle is injured, damaged, or exercised, satellite cells are activated from their quiescent state. They then proliferate and fuse into existing fibers to provide new myonuclei or return to quiescence. The intensity of the exercise is a crucial factor in the activation of satellite cells and muscle regeneration. The combination of human amniotic membrane (HAM) and silver nano particle (AgNps) with polycaprolactone (PCL) increases the properties of PCL fibers, which leads to the creation of scaffolds with potential application in skeletal muscle tissue engineering. functional recovery was assessed using the sciatic functional index and gait analysis test.
Materials and Methods: In the past, we fabricated PCL, PCL/AgNps, PCL/HAM, and PCL/HAM/AgNps scaffolds for skeletal muscle tissue engineerin. In this study, we utilized scaffolds combined with exercise to investigate their effect on the skeletal muscle model in rats. functional recovery was assessed using the sciatic functional index and gait analysis test. Hematoxylin and eosin staining was performed for histological tissue examination.
Results: According to *in vivo* results, Groups of PCL/HAM/AgNps and PCL/HAM combined with exercise showed significant improvement in motor abilities compared to groups without exercise.
Conclusion: Using PCL/HAM/AgNps, PCL/HAM combined with exercise can improve skeletal muscle performance.
Keywords: Volumetric Muscle Loss, Polycaprolactone, Human Amniotic Membrane, Exercise

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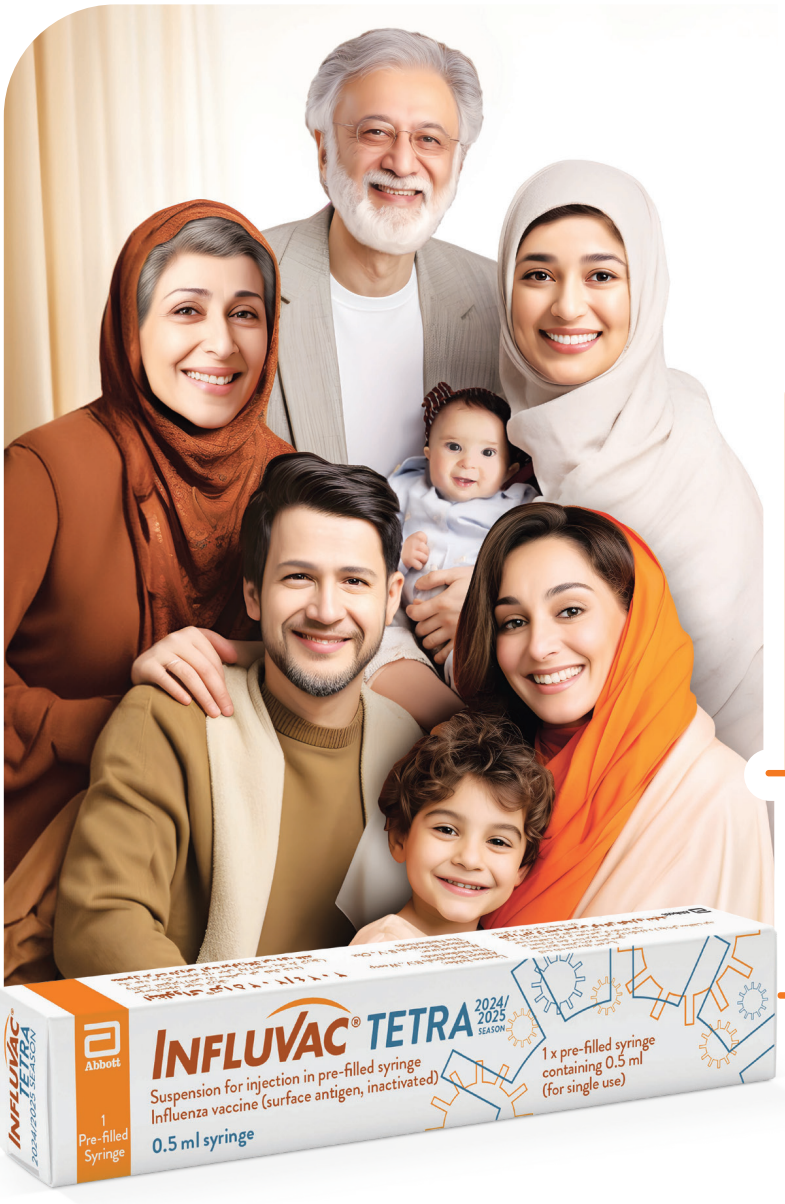

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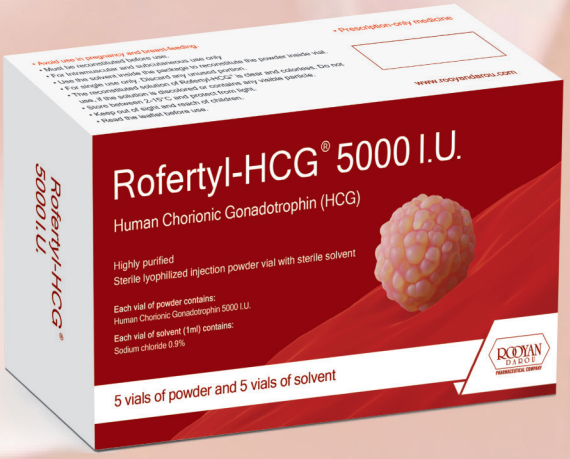

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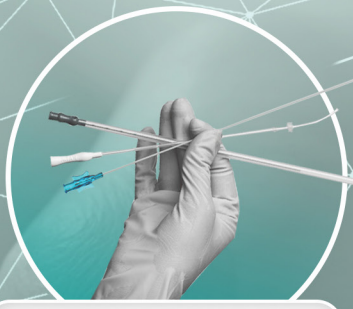

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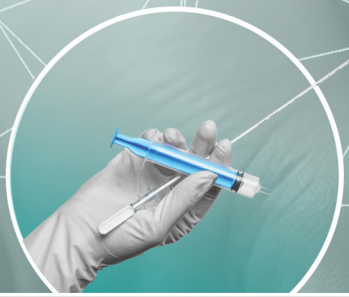

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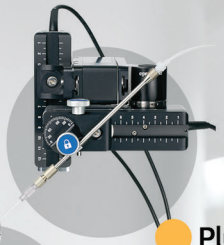

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