

ROYAN

International Hybrid Twin Congress
Reproductive Biomedicine & Stem Cells
7 - 9 September, 2022 Tehran - Iran

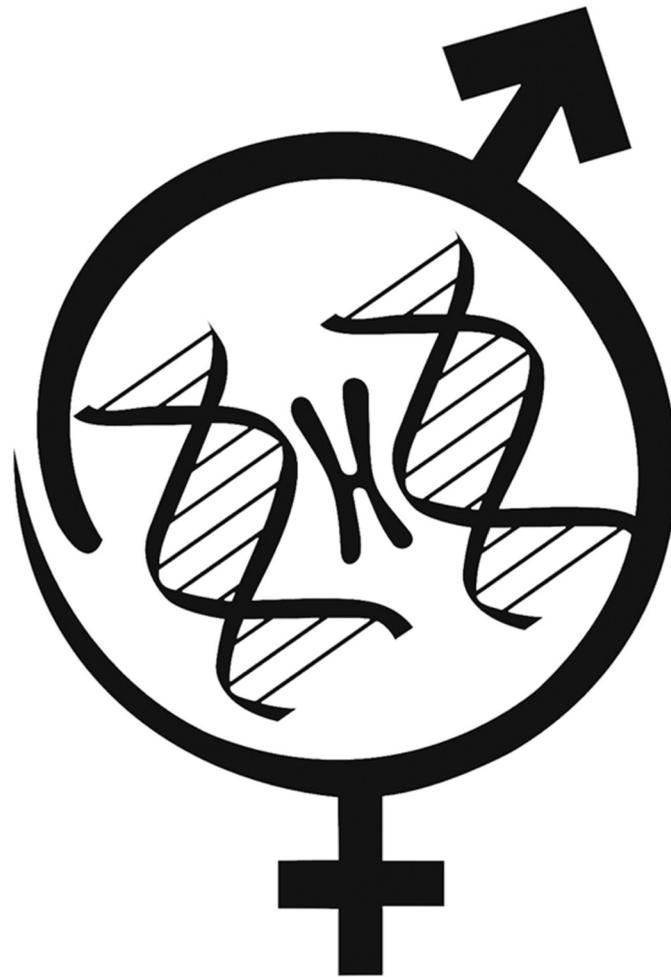


23rd Congress on
**Reproductive
Biomedicine**

Abstracts of
Royan International Hybrid Twin Congress

23rd Hybrid Congress on Reproductive Biomedicine
7-9 September 2022

17th Seminar on Nursing and Midwifery
7-8 September 2022



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran



**Abstracts of the
23rd Congress on Reproductive Biomedicine
17th Seminar on Nursing and Midwifery**

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Congress Chairperson



Bahar Movaghar

It is our pleasure to welcome you to the 23rd Royan International Congress on Reproductive Medicine and ART. Royan congress aims to provide a platform for academic scientists and researchers to encourage the exchange of the latest information on issues affecting fertility. After holding 22 International Congresses and successful holding of the 22nd congress as a virtual congress, this year the organizing committee has decided to hold the 23rd congress in a hybrid form that combines “in-person” sessions with “online” sessions on 7-9 September, 2022.

The program includes plenary and panel sessions with invited speakers, oral and poster presentations and pre congress workshops, all focusing on infertility diagnosis and treatment. The scientific committee intends to hold a comprehensive and useful program, including the male and female infertility, clinical embryology and reproductive genetics and also impact of COVID-19 on male and female infertility.

We will prepare some congress sessions as pre-recorded sessions if needed.

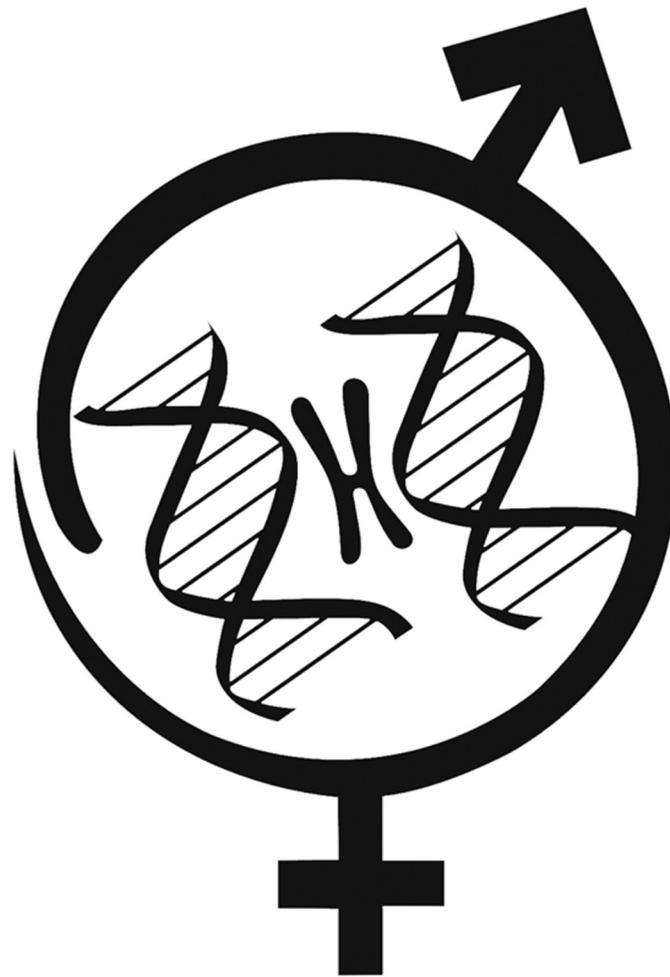
We invite all the scientists and researchers to discuss their research findings in a global platform and to exchange information, generate ideas, establish collaborations and connect with people from a wide range of nations and specialties.

We are looking forward to meeting you all, virtually and onsite, in Tehran 2022.

**Sincerely Yours,
Bahar Movaghar, PhD
Chairperson of the 23rd Royan International
Congress on Reproductive Biomedicine (2022)**

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Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Invited Speakers

Andrology

I-1: New Generation Artificial Intelligence Powered Andrology Diagnostics Offers Total Solution for Male Infertility

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I-2: Metabolic Checkpoint for Gametes and Embryo Reproductive Competence

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Selectivity is a main feature of animal and human reproductive processes. In male gametogenesis, hundreds of millions of sperm are released in a single ejaculate whereas only one is necessary to achieve a fertilization. In females, a wide cohort of primordial follicles is activated at each ovarian cycle with only a few or a single one (e.g. humans) reaching the final maturation and reproductive competence. In human assisted reproduction, the *in vitro* cultured embryos undergo a strict selection and a large majority of them arrest their development before reaching the blastocyst stage. Mechanisms explaining these arrests include a failure of the activation of the zygote genome, excess of oxygen radicals and endoplasmic reticulum stress and aneuploidy. These failures can be explained by a deficit of metabolism in supporting the chromatin re-modeling, the bioenergetics and the redox balance, all of them dependent on the function of the one carbon metabolism (OCM).

The OCM is the epicenter of cellular metabolism as providing the activated carbon units for transmethylation and the reducing equivalents (glutathione – GSH) for the redox balance. Activated methyl groups are necessary for the epigenetic regulation of DNA and histones, to regulate protein functions and to sustain key cellular syntheses including carnitine, creatine and CoQ10. GSH, besides distributing reducing power to the whole endogenous antioxidant system provides ROS neutralization within mitochondria and is strictly related to the energetic efficiency. In turn, the OCM is strictly dependent on the feed of micronutrients from the diet and other environment signals, which fits with its role in epigenetics.

In sperms, a massive and timed methylation of DNA and histones is necessary for the protamine transition and for the final nuclear compaction allowing resistance to ROS generated at time of swimming and acrosomal reaction. The administration of methyl donors known to feed the OCM to ART-resistant infertile men resulted in a significant improvement of sperm nuclear maturation, which correlated with the achieved pregnancies. *In vitro*, we showed that micronutrients in support to the OCM supported the mitochondrial function of alive human sperms. Oocytes necessitate a huge reserve of methylation capacity to exert the DNA repair function and the epigenetic re-methylation of the whole genome of the zygote. The administration of micronutrients to ladies with very low ovarian reserve appeared to re-activate the release of AMH and to

generate spontaneous pregnancies. These effects vouched for an improved oocyte quality as well as to a better priming to their post-fertilization job.

To further elucidate the underlying mechanisms, more recently we used an *in vitro* maturation model of bovine oocytes to investigate the effect of a micronutrient support on their developmental potential. Sibling bovine oocytes were randomly assigned to 24-hour *in vitro* maturation with or without the addition to the culture medium of micronutrients at physiologic concentrations. These micronutrients included methyl donors (methylfolate, methylcobalamin, betaine), a cysteine donor (L-cysteine) and cofactors for the involved enzymes (vit.s B2, B3, B6 and zinc). There were no differences in the MII rate between the groups of oocytes, however the supplemented oocytes showed lower DNA fragmentation, and higher mitochondrial mass and DNMT3a protein expression. The fertilization rates were as well overlapping, but zygotes from supplemented oocytes exerted a far larger methylation of the female pro-nuclei, confirming the boost to the methylation metabolism from micronutrients. Thereafter, the rate of maturation to blastocyst that was doubled in zygotes from supplemented oocytes. In summary, the short (24 hours) exposure of oocytes to the metabolic enhancers within the critical time window of final maturation, although exerting no effect on their maturation and fertilization rate, was already enough to dramatically improve their potential to develop.

Based on these evidences, we propose the occurrence of an OCM-based metabolic check point for the selection of the best gametes and embryos. In the context of evolution, this allows the mothers-to-be to further invest in reproductive efforts only on those gametes and embryos exerting the genetic/metabolic pattern better fitting with the available environment/feed. In the case of our bovine embryos the conditioning environment was the culture medium and its enrichment allowed a larger rate of oocytes to produce competent zygotes and viable blastocysts. The same is likely to happen in gametogenesis: Only the best suited follicle/oocyte(s) will be ovulated after an ovarian cycle and only the best suited sperm will be able to swim faster and in the right direction to achieve the fertilization.

These concepts are of paramount importance to understand the metabolic demand of reproduction and to clarify the role of diet and of dietary integration in support to fertility as well as to improve the yield from *in vitro* generation of embryos.

I-3: Oxidative Stress and The Structure and Function of Human Spermatozoa

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The integrity of the genetic material of the paternal and maternal gametes and of their respective epigenetic information is the guarantee of reproductive success, of the health of the offspring and of the sustainability of the species. The oxidative metabolism that drives the life of all aerobic cells is at the crossroads of the responses to the different environmental stimuli to which organisms are exposed, whatever their nature, physical, chemical or biological. Gametes are no exception to this situation and, due to their characteristics, spermatozoa are particularly exposed to oxidative alterations. To date, the links between oxidative damage to the spermatozoon and functional

impacts (motility, gamete interaction, capacitation) have been clearly established. However, it is necessary to consider another level of oxidative alteration of the male gamete that concerns the paternal nucleus. In this presentation, combining basic research on mouse models and clinical investigations, a presentation of the oxidative damage on the sperm nucleus will be made as well as its consequences. The situation in the andrological clinic, diagnostic and potential therapeutic aspects will also be discussed.

I-4: Why Bother about Male Infertility? Implications for The Male, Female and The Children.

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The management of infertility is traditionally in the hands of gynecologists with special interest in reproductive medicine. In majority of cases, assisted reproductive techniques (ART) as *in vitro* fertilization (IVF) and In intracytoplasmic sperm injection (ICSI) are used to help the childless couple with becoming parents and the investigation of the male is limited to searching for spermatozoa in seminal fluid, epididymis or testis. However, in 50% of cases, impairment of male reproductive function is a contributing factor to the infertility problem. Furthermore, only 25-30% of all ART treatments result in a child birth. Therefore, there is a need for finding alternative, male-related methods of infertility management, aiming to improve the efficiency of current therapies but also saving the female partner from potentially dangerous hormonal stimulation in cases where the major problem is poor semen quality.

Furthermore, recent research has clearly shown that men with impaired fertility are at higher risk of early mortality and morbidity due to non-communicable diseases as diabetes, metabolic syndrome, osteoporosis, cardiovascular conditions and some cancers. This is an additional reason to offering the male in an infertile couple a more detailed examination, which can help identifying men to whom preventive measures should be offered due to highly increased risk of morbidity and mortality. Last but not least, pathological conditions which are over-represented in subfertile men, e.g. sperm DNA damage, may have negative impact on risk of pregnancy-related pathologies as well as on perinatal characteristics of children conceived using ART. Therefore, more focus on the male partner is not only needed for improvement of outcome of infertility management but also to secure health of fathers, mothers and their children.

I-5: SARS-CoV-2 and The Impact of COVID-19 on Male Reproductive Health and Implications on The Use of ART

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More than two years after the World Health Organization (WHO) declared the new coronavirus disease (COVID-19) a pandemic, one of the most intriguing unsolved questions is the higher disease burden for males, including hospitalization in intensive care units (ICUs) and death, by the new acute respira-

tory syndrome coronavirus 2 (SARS-CoV-2). Unlike previous beta coronavirus, this new entity spreads strategically in silence for more extended periods before clinically symptomatic and eventual identification. Recently, a study containing data on common metabolic disorders, hospitalizations and disease prevalence by age, sex, and ethnicity reported that worse COVID-19 outcomes are associated with four underlying medical conditions: obesity, diabetes, hypertension, and heart failure. At a first glance, such outcomes might look like those from other acute infections or chronic health conditions, where male-sex issues represent trigger-points for poor prognosis. Historically men take less care of their own health than women, particularly in countries with high socioeconomic inequalities, where the workload and socio-cultural values impact men's self-care awareness. Our population-based study determined the role of the male sex as a risk factor for COVID-19 deaths in Sao Paulo, and to what extent socioeconomic vulnerability and individual health issues can interfere with such a risk. The COVID-19 manifests mainly by respiratory symptoms. However, as the pandemic progressed, this paradigm has changed as cumulative evidence has shown that COVID-19 is a systemic condition. Therefore, understanding multiple organs' involvement is crucial to comprehend SARS-CoV-2 infection's pathophysiology fully. It is believed that the systemic involvement is because of angiotensin-converting enzyme 2 (ACE2), the cell surface receptor for the SARS-CoV-2, and the transmembrane serine protease 2 (TMPRSS2), responsible for priming the viral S protein to facilitate the virus's entry, are present in various organs and tissues. The Nucleocapsid structural viral protein is remarkably immunogenic and hugely expressed during infection. High IgG antibodies against Nucleocapsid protein levels were detected in the serum of COVID-19 patients, confirming its pivotal antigen role for a T lymphocyte response in a vaccine microenvironment. Our hitherto study evaluates histological changes in rats' testes, epididymis, prostate, and seminal vesicles and analyzes hormone levels after solely Nucleocapsid protein inoculation. Conclusively, our data suggests testicular hormonal imbalance mediated by the SARS-CoV-2 Nucleocapsid protein that could be linked to reported post-COVID-19 syndrome hypogonadism. Recognizing the expression throughout the human body is essential to understand the clinical manifestations and predict potential lesions in organs often overlooked, that includes the testis and the spermatozoa as well as the ovary and the oocyte on the other side, which directly leads to the question of whether the embryo might be at risk, in special after assisted reproductive techniques, where a few thousand or even one single sperm is used for fertilization.

I-6: Obesity and Male Infertility and The Effect of Obesity on ART Outcomes

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I-7: Myth and Facts of Sperm DNA Fragmentation in Assisted Reproduction

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Obesity is seen as a global health issue affecting more than one-third of the world's population. Cardiovascular disorders, type 2 diabetes, cancer (including prostate cancer), neurodegeneration, and accelerated aging are complications of obesity. Also, erectile dysfunction, poor sperm quality, and subclinical prostatitis in males are the other common complications of male obesity. Important mediators of obesity that may alter the male reproductive system include hyperinsulinemia, hyperleptinemia, chronic inflammation, and oxidative stress while being little understood. Obesity is known to impair male fertility and reproductive potential, specifically by altering the hypothalamic-pituitary-gonadal axis, disrupting testicular steroidogenesis, and causing metabolic dysregulation, including insulin, cytokines, and adipokines. Importantly, obesity and its underlying mediators have a deleterious effect on sperm concentration, motility, viability, and normal morphology. Furthermore, obesity impairs chromatin condensation, DNA fragmentation, and promotes apoptosis and heritable epigenetic alterations. This presentation examines the effects of obesity on the male reproductive system and fertility, as well as the mechanisms involved. In addition, ART outcomes of obese men, strategies for weight management, changes in lifestyle, prescription medications, and complementary and alternative medicine for the treatment of subfertility caused by obesity are discussed.

I-8: Is There A Role for Antioxidative Management in Male Infertility Treatment?

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The mechanism for male infertility, which contributes to half of the overall infertility, is generally acknowledged to imply medical, psychological and social concerns. Notwithstanding, such psycho/emotional stressful moments have not exclusively to be addressed under the solitary perspective of the affected men suffering from alterations on different levels of their sperms, but even more, the complex dimension for the couple has to be considered. Nowadays several causes for male infertility could be identified, reaching from endocrine disorders, inflammatory or immunological factors, lifestyle together with exposure to different noxae, and environmental factors up to a plethora of several genetic or epigenetic factors. Furthermore, along with more accurate diagnostic techniques, in the last decade, science focused on molecular and biochemical factors leading to male infertility. It has been shown that several of these factors are interfering with male fertility due to the altered production of several reactive species counteracting the male antioxidative capacity on the other side. The focus of the newest scientific research lies in a deeper understanding of the concept of oxidative stress and its modulation capacities. Several publications are reporting conflicting results, and even actual guidelines by the European Association of Urology or the American Urological Association/American Society for Reproductive Medicine probably do not reflect the newest scientific knowledge. Based on existing research results some claimed therapeutic options must be investigated carefully, whereas other positions might

be revised.

Apparently, antioxidative stewardship is of the utmost importance and consists of strategies to optimize the selection, dosing, route of administration, duration and timing of antioxidative therapy to maximize clinical cure in sub- or infertile men. Unfortunately, up to date, there are no well-performed RCT's investigating the specific familiar environment and lifestyle of the infertile couple, nor the net benefits of any antioxidative therapy prescribed to both partners in the long-term. It's still unclear if under such specific settings any AOX therapy might even increase the live-birth rate significantly.

This presentation aims to impart knowledge to the attending audience about the latest research findings, the markers of oxidative stress and their assessments. The advantages and disadvantages of any antioxidant therapy must be weighed, and its correct use must be discussed by stringent indications.

I-9: Could Artificial Oocyte Activation following ICSI Improve Fertilization, and Pregnancy in Couples with Male Factor Infertility

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Introduction: The intracytoplasmic sperm injection (ICSI) has been capable of significantly improving male factor infertility, but fertilization failure after ICSI still occurs in 1–5%, and the primary reason for this failure is a lack of oocyte activation. For these cases, artificial oocyte activation (AOA) by a chemical agent or an electrical pulse following ICSI is recommended. In most fertility centers, this procedure is performed for couples with previous failed fertilization and/or severe teratozoospermia like globozoospermia. Therefore, this presentation aims to report the clinical results of the ICSI-AOA technique, and the status of the health of children born through this procedure.

Methods: This presentation covers the clinical outcomes of the ICSI-AOA technique and the health of children through meta-analysis, clinical trials, and case report studies during a period of recent 15 years. It also covers fifteen of our published papers regarding ICSI-AOA.

Results: According to background literature and our results, couples with previous failed fertilization and/or severe teratozoospermia and/or globozoospermic men may benefit from ICSI-AOA in terms of fertilization, which in turn, may improve the implantation and pregnancy rates. However existing conflicts in the literature on the effect of AOA on fertilization rate may have resulted from case selection, limited sample number, and type of agent used to induce AOA. Therefore, to resolve these ambiguities, prospective, randomized clinical trials are needed.

Conclusion: Regarding the health of children born through this technique, it has been reported that the health of these children is likely not jeopardized by this technique. Interestingly, recent clinical studies suggest that the normal ICSI cycle may also benefit from this technique and molecular analysis has shown that the molecular signature of ICSI-AOA is close to IVF-derived embryos as compared with those of ICSI to IVF.

I-10: How to Find The Cause of Male Infertility Factor?

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Male infertility accounts for approximately 40% of infertility factors. We aim to explain how to find the cause of male factor in infertile men, referred to an infertility treatment center.

First, a comprehensive and thorough medical history including familial diseases, genetic disorders, and incurable diseases is necessary. Surveys related to the infertility history in the family should be considered. Age is an important factor in the first visit and during the treatment.

In addition to infertility, other complaints, such as chronic headaches, cough or sinusitis, high blood pressure and so on, should be considered. Information related to the patient's occupation is of great importance.

The interview should contain these questions: Is a couple's infertility primary or secondary? Are Patient's weight and height proportionate? Do couples consume any specific drug, substance, or alcohol? Do signs of puberty, correct sexual orientation and phenotype appear?

In the next stage, the accurate physical exam of different organs, including cardio-pulmonary system, genitalia and so on, should be done. Consequently, according to the medical history and clinical findings, paraclinical and imaging evaluations are considered.

Let's consider some examples:

- In a man who does not meet tanner criteria of puberty, hormonal profile must be checked, since hormonal disorder is suspected.
- In a patient suffering chronic migraine, and semen analysis is abnormal, and the level of prolactin hormone of blood is higher than normal level, pituitary disorder is suspected.
- An infertile patient who has chronic cough and nasal and pharyngeal discharge should be assessed for Kartagener syndrome.
- A non- palpable vas deferens and azoospermia increases the odds of unilateral kidney agenesis by 15%, hence, pelvic and abdominal ultrasound examination is helpful for correct diagnosis.
- For a patient admitted with secondary infertility who has abnormal semen analysis and his finding of physical examination is in favor of varicocele, scrotum should be evaluated using doppler sonography.

In conclusion, using correct medical history, and physical examination, and accurate diagnostic methods, the exact cause of male factor is detectable.

Animal Biotechnology

I-11: Transcriptome Profiling of *Nlrp2*-null Mice Oocytes

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Background: The early mammalian development, relies on maternal-effect genes, which orchestrate the oocyte-to-embryo

transition. Subcortical maternal complex (SCMC) compromises multiple proteins encoded by maternal-effect genes and is indispensable for early embryogenesis in mouse and human. It is essential for important mechanisms including spindle positioning and symmetric division of zygote. *Nlrp2* is a component of SCMC and maternal deletion of *Nlrp2* causes subfertility noted by early embryonic loss and abnormal DNA methylation at few loci. However, how *Nlrp2* and other SCMC alter the transcriptome and methylome is still little known.

Materials and Methods: Deep RNA-Seq was used to assess transcription in pooled cumulus-oocytes complex (COC) from *Nlrp2*-KO and WT mice.

Results: The transcriptome profiles of WT and *Nlrp2*-KO showed distinctive differences.

We identified 231 differentially expressed genes (DEGs) (adjusted P value < 0.05), out of which 123 were upregulated and 108 were down-regulated genes. The GO analysis of DEGs revealed that they are involved in critical biological processes such as metabolism of proteins, gland morphogenesis and post-translational protein modification. By mapping against the oocyte specific annotations, we detected 228 differentially abundant transcripts (DATs), some of which came from genes that were not identified as DEGs. 55.7% of DATs and 67.5% of DEGs overlap with determined hypermethylated/hypomethylated domains within the oocyte genome. This finding highlights the impact of transcription on methylation establishment in oocytes.

Conclusions: This is the first report of transcriptome profiling in ovulated oocytes from mice with inactivation of a SCMC member. Our results clearly show that maternal loss of *Nlrp2* significantly change the gene level and transcript abundance in oocyte.

I-12: On-target Knockin of Large Constructs into The Mouse Genome via Combining CRISPR/Cas9 and Nocodazole

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On-target integration of large cassettes via homolog-directed repair (HDR) has several applications. The knock-in efficiency of large constructs is a challenging issue in mammalian genome. Nocodazole is an antineoplastic agent that induces arresting with cell cycle G2/M-phase. At this phase the highest rate of homology directed repair (HDR) is expected compared to the insertions/deletions (indels) rate. In this study, we made several large constructs (12.0-13.0 kb) which included the CRISPR/Cas9 system as part of the large DNA donor (5.0-7.0 kb) harbored by two homology arms specific for the promoter region of the Venus transgene. In this system, random indels directed by the CRISPR/Cas9 system did not knock out the Venus expression. However, only insertion of the CRISPR/Cas9-contained constructs into the promoter region could induce the Venus knockout. We used this strategy to evaluate targeted integration efficiency into a transgenic murine embryonic fibroblast (MEF) cell line carrying a single copy of the Venus transgene. We established a detection assay by which HDR events could be discriminated from the error-prone non-homologous end-

joining (NHEJ) events. Cell pre-treatment with nocodazole did not affect the integration rate. However, direct inclusion of nocodazole into the electroporation medium could significantly improve the HDR rate, on average 24 % efficiency. In conclusion, the results of this study showed that using cell synchronization reagents in the electroporation medium can efficiently induce HDR rate in the mammalian genome.

Keywords: Cell Synchronization, CRISPR/Cas9, HDR, Large Constructs, Nocodazole

I-13: CRISPR Conservation: Synthetic Biology with Ecological Therapeutic Applications

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Since colonisation by Europeans in 1788, Australia's biodiversity has been decimated due largely to habitat loss and the introduction of multiple invasive vertebrate pest species. In an effort to halt and even reverse loss of biodiversity in Australia, our laboratory is applying CRISPR editing to multiple vertebrate species in three different ways. First, we are developing genetic biocontrol strategies (e.g. gene drive) for eradicating invasive vertebrate pest populations. Second, we are enhancing the genome of a threatened species – the northern quoll – to provide defence against a toxic, non-native toad species. Third, we are developing the resources necessary for the eventual resurrection of a species that became extinct over 85 years ago: the thylacine (Tasmanian tiger).

I-14: Enhancement of Developmental Competence in Goat SCNT with Sequential IVM by CNP, PGE2 and AREG

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Developmental competence of *in vitro* matured cumulus oocyte complexes (COCs) in conventional IVM (C.IVM) is lower than *in vivo* matured COCs. This difference is related to unsynchronized nuclear and cytoplasmic maturation. Therefore, exposure of COCs to granulosa secreted factors in a two-step or capacitation IVM (CAPA-IVM) system has been proposed to arrest COCs in germinal vesicle stage for longer and better cytoplasmic maturation. So far, this approach has not been applied to goat. First experiment was done to define best time and concentration to retain oocytes in GV stage (CAPA) using CNP. In 1000nM of CNP for 8h, we observed GV arrest, reduction of lipid droplets, increased the expression of ATGL and PLIN2 involved in lipolysis and lipogenesis, respectively., in second experiment stimulation of maturation with prostaglandin E2 and amphiregulin for 18h was done and their optimal concentrations based on blastocyst formation rates with *in vitro* fertilization were defined as 1 and 600 nM, respectively. The third experiment was *in vitro* and *in vivo* development of SCNT embryos in CAPA-IVM. Despite similar blastocyst formation rate in IVF and SCNT between (CAPA-IVM) and C.IVM, their quality was higher in CAPA-IVM, which reflected itself as

higher expression of NANOG in SCNT blastocyst and insignificantly higher pregnancy rate.

Keywords: CAPA-IVM, Conventional IVM, C-type Natriuretic Peptide, Oocyte, SCNT

I-15: The Application of Animal Models in Preservation of Male Fertility

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Spermatogonial stem cells (SSCs) are a rare group of cells in the testis that undergo self-renewal and complex sequences of differentiation to establish and maintain spermatogenesis throughout adulthood. The difficulty of unequivocal identification of SSCs and complexity of replicating their differentiation properties *in vitro* have prompted the introduction of novel *in vivo* models such as germ cell transplantation, testis tissue xenografting, and testis cell aggregate implantation. Owing to these unique animal models, our ability to study and manipulate SSCs has dramatically increased, which complements the availability of other advanced assisted reproductive technologies and various genome editing tools. These animal models can advance our knowledge of SSCs, testis tissue morphogenesis and development, germ-somatic cell interactions, and mechanisms that control spermatogenesis. Equally important, these animal models can have a wide range of experimental and potential clinical applications in fertility preservation of prepubertal cancer patients, and genetic conservation of endangered species. Moreover, these models allow experimentations that are otherwise difficult or impossible to be performed directly in the target species. Examples include proof-of-principle manipulation of germ cells for correction of genetic disorders or investigation of potential toxicants or new drugs on human testis formation or function. The primary focus of this presentation is to highlight the importance, methodology, current and potential future applications, as well as limitations of using these novel animal models in the study and manipulation of male germline stem cells.

I-16: Varicocele and Autophagy: Crosstalk between Oxidative Stress, Metabolic Disorders and Autophagy

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According to several epidemiological reports, clinical varicocele (VCL) is observed in 10% to 20% of the general male population with a higher prevalence of 35%-40% in men with primary infertility and up to 80% in men with secondary infertility. The VCL-related pathophysiology in testicular tissue, including decreased testicular endocrine status, heat, oxidative, nitrosative, and endoplasmic reticulum-related stresses and pro-inflammatory cytokines overexpression at testicular and semen levels are shown previously. Increased oxidative stress (OS) associated with metabolic failure has been found to accelerate autophagy-related signaling in male infertility problems. Therefore, assessing the cross-talk between metabolic interactions,

oxidative stress, and autophagy-related signaling has gained high interest in varicocele cases.

The current review is trying to summarize the recent findings reported to date in VCL rats.

In order to get a better understanding of the subject, it is important to know that diploid cells of germinal lineage, primarily spermatogonia stem cells, consume a large amount of 6-carbon carbohydrates. Nonetheless, Haploid cells primarily utilize lactate produced by Sertoli cells. When energy supply is low, germ cells undergo a decrease in pentose phosphate pathway flux, which then reduces cellular GSH-dependent antioxidant power via disruption of the NADP⁺/NADPH ratio. Therefore, the testicular tissue can activate autophagy in response to nutrient deprivation, metabolic impairment, and oxidative stress.

Considering that oxidative stress is a major feature of reproductive pathophysiology in VCL conditions, metabolic disorders can play a crucial role in fostering OS conditions that lead to massive autophagy. Accordingly, autophagy can induce intensive apoptosis in testicular tissue by virtue of its nature of the action.

Keywords: Autophagy, Metabolic Disorders, Oxidative Stress, Spermatogenesis, Varicocele

I-17: Lab on A Chip Devices for Fertility

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Microfluidic methods offer several advantages over their traditional macro-scale counterparts by extending the possibility of biomedical research based on the idea of miniaturization. These miniaturized platforms provide opportunities to manipulate cells and biological processes at the single-cell level and develop nature-inspired technologies for diagnostic and therapeutic applications. For example, in the context of fertility, microfluidics can match the geometry of micro-confined regions within the female reproductive tract, thus, presenting opportunities for fundamental understanding of the even of fertilization and biomimicry-based selection of sperm that reflect the *in vivo* process. In this talk, I will provide an overview of our work in developing microfluidic technologies for sperm analysis and selection.

I-18: CRISPR-mediated gene-edited farm animals at the FLI in Germany

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For farm animals such as cattle, pigs, horses and sheep, informative gene maps are now available that form the basis for the development of new breeding concepts and targeted genetic modifications. The discovery of the CRISPR/Cas system and its adaption as a tool for gene editing in mammalian cells has dramatically changed the capabilities of scientists to alter the genome of farm animals. Genome editing in farm animals has a variety of applications for agriculture, biomedicine and basic research. At the Friedrich-Loeffler-Institut, the federal research institute of animal health, we focus on animal models for improving animal welfare by working on the generation of pigs

resistant against African Swine Fever, male pigs that show a sex reversal after knockout of the HMG box of the SRY gene and generating polled dairy cattle by genome editing. Dehorning of cattle raised concerns regarding animal welfare as it causes stress and pain for the calves and is a potential risk of infections. The celtic mutation a 208bp duplication and 6 bp deletion originating from beef cattle was introduced into the horned locus on Chr.1 of Holstein Friesian fibroblasts by CRISPR/Cas induced homology-directed repair. The cells were subsequently used as donor cells for somatic cell nuclear transfer and lead to the birth of a single cloned calve with a polled phenotype. Wild boar lung cells transfected with a CRISPR/Cas vector targeting the CPL204 gene of the African Swine Fever Virus (ASFV) were almost completely protected against ASFV infection. The knockout of the HMG box domain of the porcine SRY gene led to the birth of male pigs with a female phenotype. Though no difference could be detected between male sex reversed (MSD) and normal female pigs at the age of 3 months, MSD pigs did not show any formation of follicles on the ovaries and had an immature uterus compared to controls at the age of 9 months. Xenotransplantation is considered a promising solution to overcome the continuous organ shortage in allotransplantation. Due to its physiological and anatomical similarities to humans, the pig is considered the best available xeno-organ donor. Besides, significant improvements in the past, long-term xenograft survival is still challenging and needs extensive genetic modifications of the pig genome. The major epitopes causing rejection of the pig organ are sugar molecules expressed on the surface of pig cells. Therefore, we used CRISPR/Cas9 to knockout the responsible genes such as GGTA1, CMAH and B4GalNT2. In addition, the generated pigs carry a cassette of five transgenes (human CD46, CD55, CD59, A20 and human heme oxygenase) to overcome incompatibilities between the human and porcine coagulation system and to reduce endothelial cell activation located in combination at a single site on Chr. 6 of the porcine genome. The feasibility to efficiently alter the genome of pigs has dramatically prolonged the survival times of porcine heart xenografts in a pig-to-baboon setting up to 195 days, making xenotransplantation a realistic option to overcome the shortage of suitable allotransplants. This year, the first pig-to-human heart xenotransplantation has been performed. The patient survived for two months. One reason for the relatively short survival of the heart might be related to a porcine CMV infection, highlighting the importance to protect xeno-organs from CMV.

Embryology

I-19: Male Fertility Preservation in Non-oncological Disease

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Male fertility preservation has been widely used over the last few decades, and become one of the essentials of assisted reproductive technology (ART).

Currently, sperm cryopreservation is the most efficient approach in male fertility preservation using an ejaculated specimen. However, this option is inaccessible for patients with nonobstructive azoospermia, ejaculatory dysfunction, and prepubertal patients. Testicular tissue cryopreservation is available

in certain post pubertal patients but remains experimental in pre-pubertal patients. There is promising data in animal models regarding testicular tissue and spermatogonial cells (i.e. SSCs) cryopreservation. The preservation of SSCs and testicular tissue is now being introduced at more centers. However, there are still a considerable number of safety concerns with autotransplantation of testicular tissue. While fertility preservation is commonly used in cancer patients undergoing gonadotoxic chemotherapy, the indications for fertility preservation in men have extended beyond the oncologic setting to include autoimmune diseases, gender dysphoria, Chronic medical condition, HIV positive, pre-vasectomy, pre-Surgical interventions which may result in direct damage to spermatogenesis, Posthumous sperm retrieval, social sperm banking and even military deployment. Men exposed to toxins or those whose work conditions can adversely affect spermatogenesis are also candidates for fertility preservation. Infertility in men with nononcological medical conditions results primarily from exposure to gonadotoxic agents. Nonetheless, the underlying disease might also contribute to impaired sperm function.

Cryopreservation is an essential process in fertility preservation; however, it can potentially damage sperm cell structure and eventually leading to impaired sperm function. Future research should look into techniques for improve the efficacy of fertility preservation methods.

On the other hand, early recognition of the potential need for fertility preservation is critical in order to provide patients with counseling and comprehensive information about fertility problems.

I-20: Abdominal Fat Accumulation and Reproductive Health in Female

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I-21: Indication and Success of Ovarian Tissue Cryopreservation

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Ovarian tissue cryopreservation is recommended as an alternative innovative strategy for fertility preservation when oocytes or embryo vitrification are not feasible. The technique is invasive but safe and success rate after transplantation reached 30% to 50%, with more than 150 babies born worldwide. Although the efficiency of the procedure after ovarian tissue transplantation has been demonstrated, several questions remain regarding the most efficient, safe and time-saving procedures for clinical application. The evaluation of the clinical context remains also a crucial step at the time of diagnosis to offer the procedure to patients who will really benefit from it and with the highest potential of success after ovarian tissue transplantation. The presentation will discuss these technical and clinical challenges, as well as the recent progresses and the future options.

I-22: Artificial Intelligence as A strategy for Single-Embryo

Transfer

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The ultimate success of an assisted reproduction treatment is delivering a healthy baby to her healthy mother. Recent literature evidence suggests that single-embryo transfer is the safest path to achieve this goal. However, many wet-lab technologies to identify the best embryo to transfer are often expensive and out of reach of patients. In this presentation, we will discuss how artificial intelligence-based tools might be a simple, non-invasive, and inexpensive alternative for embryologists, clinicians, and patients to make a more informed decision for single-embryo transfer.

I-23: Challenges of Oncofertility and Fertility Preservation in Cancer Patients in The Point of View of Oncology Aspects

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Infertility is a major problem affecting children, adolescents, and young adults (AYAs) with cancer, either due to the disease itself or because of oncologic treatment. Best time about fertility planning is before cancer treatment. Fertility is affected by; type of cancer, age, types of treatment options, curability or life expectancy of cancer. Therefore, due to a better treatment and new options, like immunooncology, fertility preservation requires many different researches for better effect.

Type and dose of chemotherapy drugs, site of radiation field in radiotherapy, old age, cutability of cancer, specification of type of cancer: for example, hormone status in breast cancer patients and longtime planning in hormone therapy, are some of important factors in choice of treatment based on oncofertility aspects.

Fertility preservation planning is an important and essential part of treatment, therefore close communication between specific specialist in this field, education for patients, new research based on new treatment and their effects in fertility and effect of preservation therapies on cancer diseases our important subject.

I-24: Medical Management of Endometriosis

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Women with pelvic pain, suspected endometriosis, and no other indication for surgical treatment can be managed effectively with empiric medical treatment without establishing a surgical diagnosis. 395 Initial empiric medical therapy usually involves treat-

ment with Non-steroidal anti-inflammatory drugs (NSAIDs) and oral contraceptives (OCs; combined or progestin only). If treatment with NSAIDs and OCs does not significantly improve pain, second- and third line medical therapies or GnRH agonist and Antagonist. Traditional medical therapies for endometriosis have been based on Sampson's theory of retrograde menstruation and implantation and on the simple premise that ectopic endometrium may be expected to respond to treatment in much the same way as normal eutopic endometrium. Consequently, the objectives of treatment have been to reduce or eliminate cyclic menstruation, thereby decreasing peritoneal seeding and the likelihood that new implants will develop, and to suppress the growth and activity of the endometrium, anticipating that the same would occur in the endometriotic tissue derived from it. Interventions that reduce ovarian estradiol production are the most reliable ways to cause atrophy of endometriotic lesions and the most effective treatment for pain. These simple operational concepts have shaped medical treatments for endometriosis for decades, but our growing understanding of the pathogenesis of endometriosis at the molecular level is now beginning to suggest new treatment strategies aimed at the mechanisms of disease.

Established medical therapies for the treatment of pain associated with endometriosis include estrogen-progestin contraceptives, progestins, GnRH analogues and GnRH Antagonist. Typically, OCs are the first line of therapy with GnRH antagonists becoming the optimal second-line therapy in those who fail OC treatment or have troubling progestin-related side effects. Treatment decisions must be individualized, after carefully considering the severity of symptoms, the extent of disease, the desire for future pregnancy, age, side effects, and costs. Research is ongoing for the development of new therapeutic agents.

I-25: Developmental Programming of Metabolic Dysfunctions by Testosterone Excess

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Developmental programming is a primitive mechanism that is evident during sex determination. Its role is becoming apparent in the developmental origin of diseases. Because steroids orchestrate the communication between the intrauterine environment and the developing fetus, inappropriate exposure to sex steroids or environmental steroid mimics during critical windows of differentiation could disrupt the developmental trajectory of organ systems leading to adult dysfunctions. Experimental manipulation of the prenatal steroid environment provides a powerful tool for understanding mechanisms that underlie developmental malprogramming of metabolic systems. This presentation will address how prenatal exposure to excess testosterone perturbs the metabolic homeostasis in female sheep, a precocious model of translational importance to Polycystic Ovary Syndrome (PCOS), a major infertility disorder affecting reproductive aged women. Our studies provided causal evidence that in utero exposure to excess testosterone leads to peripheral insulin resistance and tissue-specific changes in insulin sensitivity with liver and muscle but not adipose tissue being insulin resistant. In addition, prenatal testosterone excess also programs defects at the adipocyte level manifested as reduced adipocyte size reflective of defects in adipogenesis, ectopic lipid accumulation that includes a Non-alcoholic fatty liver disease (NAFLD) liver phenotype, and hyper-

tension. Prenatal and postnatal intervention with either antiandrogen (flutamide) or insulin sensitizer (Rosiglitazone) partially prevented or ameliorated the prenatal testosterone-programmed changes in mediators of insulin sensitivity and adipocyte defects suggesting that both pathways are critical for the programming and maintenance of the prenatal testosterone-induced changes in insulin sensitivity and adipose defects. Because prenatal testosterone-treated sheep completely recapitulates the reproductive and metabolic characteristics of women with PCOS, these findings from the sheep model of PCOS phenotype are of translational relevance.

I-26: Lab Strategies Towards Single Embryo Transfer

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It is a given that single embryo transfer is a more acceptable practice in IVF so as to reduce the chance of harming both mother and offspring. In this presentation I will describe new non-invasive strategies that will allow us to transfer viable single embryos.

A number of strategies will be discussed. These include: (1) the use of Artificial intelligence in conjunction with time lapse imaging of embryos; (2) the use of image recognition software and artificial intelligence to examine static blastocyst images; (3) the use of DNA screening methods to ascertain the ploidy status of blastocysts from their surrounding culture media; (4) Assessment of metabolic function using Fluorescence Lifetime Imaging Microscopy (FLIM); (5) the use of other imaging systems such as Hyperspectral imaging and (6) the use of mass spectrometry to assess embryo culture media.

Finally, the use of ranking strategies will be discussed so as to choose the best single embryo in order to transfer with either a fresh or frozen transfer.

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I-27: Fertility Preservation in Boys

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Cytotoxic therapies and/or testicular irradiation are the main cause of germ cell loss and can hamper spermatogenesis permanently. These therapies are currently not only used to treat malignant disorders, but also for benign haematological conditions that need bone-marrow transplantation. Hence, preservation of reproductive potential has become an important quality of life issue in prepubertal boys and in adolescent and young adults (AYA). In AYA, cryopreservation of sperm is possible once both spermarche and oigarche are a fact. Besides these two prerequisites, counselling of AYA remains critical in a fertility preservation program. Prepubertal boys cannot benefit from sperm banking as active spermatogenesis is not present. As an alternative, testicular stem cell banking is being intro-

duced into more and more clinics as a preventive measure. Yet, this strategy should still be regarded as experimental given the lack of any report on successful transplantation. Therefore, also here counselling towards both child and parents is crucial.

I-28: Toward Single Embryo Transfer, The Power of One

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The twin birth rate has increased by more than 75 % since 1980, mainly due to the use of assisted reproductive technology (ART). Transfer of multiple embryos increases the risk of twin or higher order pregnancies which is associated with significant health risks and maternal and neonatal complications. Despite of physical, emotional, and financial stresses, multiple pregnancies also might increase the incidence of depression and anxiety disorders and all efforts in *in vitro* fertilization (IVF) clinics should emphasize on this goal, the delivery of a healthy single baby, with fewer twin and higher-order births.

Per single IVF attempt, overall birth rate is higher with double embryo transfer (DET), however the cumulative live birth rates with elective single embryo transfer (eSET) followed by transfer of a single frozen embryo were similar to those seen with a single DET, with significantly lower twin birth rate associated with SET. Multiple barriers exist toward application of SET as a routine procedure in IVF practices, however strategies like patient education and decision aids, counseling and advice from physicians and nurses, financial incentives and using prediction tools, would help patients to choose single embryo be transferred. Engagement of comprehensive chromosomal screening (CCS) technologies into the routine IVF procedures would help clinicians to assess embryos for aneuploidy before transfer and can increase the implantation and pregnancy rates and in the case of transfer of good quality embryo on Day 5, the live birth rate even can be as good as 70%.

eSET is considered as the most effective method for reducing multiple pregnancies resulting from IVF and for patient age of <36 with good prognosis, eSET should be consistently encouraged. In the absence of public policies to reduce multiple pregnancies, individual IVF centers can develop mandatory SET policies for all or certain subsets of their patients.

Keywords: Multiple Pregnancy, Single Embryo Transfer, Aneuploidy, Patient Consultation

Female Infertility

I-29: Assisted Reproduction Techniques Challenges and Management Options

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Reproductive aging has become an important social and medical issue. Today, in our modern society, the proportion of women who delay childbearing until the fourth decade of life has in-

creased greatly over time. The reasons can be associated with the high education level, the career goals, the desire of economic independence of woman, the highly effective contraception, the legalization of abortion and, last but not the least, the common idea that assisted reproductive technology (ART) can compensate for the natural decline in infertility with age. This misperception about the “rejuvenation effect” of ART is due to the lack of knowledge of their reproductive systems and to growing popularity ART. This trend is presenting new challenges to fertility specialists who are witnessing an increase in the number of women seeking a pregnancy after 35 years. Routinely, a woman at 35 or more than 35 years old is defined woman with advanced maternal age (AMA). However the blastocyst aneuploidy rate increases after 35 but it becomes more pronounced after age 40 infact up to an embryonic aneuploidy rate of 90% when women are 44 years old. This phenomenon means that the percentages to obtain a live birth at 43-44 is approximately 5%. For this reason in women with AMA, rather than trying to conceive for one year prior to having an infertility evaluation, the infertility work up is recommended after 6 months of regular unprotected intercourse or as soon as possible if there are any known clinical conditions that can negatively impact reproductive potential. This evaluation will include the evaluation of ovarian reserve markers such as AMH and AFC, as well as all the possible factors that could have an impact on getting pregnant including not only uterine factor, thyroid function, coagulation disorders, previous chlamydial infections, tubal patency but also sperm quality. Of note, there are no specific additional tests or set guidelines for AMA patients candidate for IVF. However, ca. 25% of couples may be affected from unexplained infertility and the goal becomes to achieve a pregnancy even without treating any specific pathology (idiopathic infertility). Unfortunately, although the decrease of woman fertility with age is well known, many times an increasing number of women arrive too late for the first infertility counseling. Moreover, the growing use of ART and the incorrect information from mass media, determined in the people the wrong idea that the right moment of life for becoming parents can be delayed beyond the physiological fertile age. To provide the right treatment for the right patient at the right time is an important duty, which requires solid knowledge in the fields of ovulatory physiology and disorders, pharmacotherapy and surgical management, but also in IVF strategies, as advances in reproductive medicine are extremely rapid and often involve the introduction of new Methods, protocols, and technologies. Finally, although many different IVF add-ons are available, but very few are supported by strong scientific evidence of safety and effectiveness. For this reason, it is important to consider when you chose any add-ons: cost, risk and Evidence.

I-30: Update on The Management of Poor Ovarian Response in ART Cycles

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Despite the reasonable progress in the field of assisted reproductive technology (ART), some issues remain unsolved. Remarkably, the clinical management of 9-24% of patients undergoing ovarian stimulation with a poor ovarian response (POR) is still challenging among the fertility expert. Poor responders are categorized based on age, previous ovarian response or ovarian

reserve through Bologna criteria or the Poseidon concept. In the last years, it is assumed that combining two stimulations in the same ovarian cycle has beneficial effects on the number of retrieved oocytes in poor responders. However, a recent study showed that Dual stimulation with continuous follicle stimulating hormone administration is not superior to conventional stimulation with regard to number of MII oocytes. Recently, follicular activation using ovarian fragmentation or ART following long-term gonadotropins suppression as well as disruption of the Hippo signaling pathway and intraovarian injection of either stem cells or Platelet-Rich Plasma have been applied for treatment of patients with POR. Nevertheless, the transition of these Methods from experimental to clinical needs more investigations in both veterinary and human studies.

I-31: Diagnostic Accuracy of Magnetic Resonance Imaging, Transvaginal, and Transrectal Ultrasonography in Deep Infiltrating Endometriosis

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To determine the diagnostic accuracy of pelvic magnetic resonance imaging (MRI), transvaginal sonography (TVS), and transrectal sonography (TRS) in diagnosis of deep infiltrating endometriosis (DIE). This diagnostic accuracy study was conducted during a 2-year period including a total number of 317 patients with signs and symptoms of endometriosis. All the patients were evaluated by pelvic MRI, TVS, and TRS in the same center. The criterion standard was considered to be the laparoscopy and histopathologic examination. Of 317 patients being included in the present study, 252 tested positive for DIE. The sensitivity, specificity, positive predictive value, and negative predictive value of TVS was found to be 83.3, 46.1, 85.7, and 41.6%, respectively. These variables were 80.5, 18.6, 79.3, and 19.7% for TRS and 90.4, 66.1, 91.2, and 64.1% for MRI, respectively. MRI had the highest accuracy (85.4%) when compared to TVS (75.7%) and TRS (67.8%). The sensitivity of TRS, TVS, and MRI in uterosacral ligament DIE was 82.8, 70.9, and 63.6%, respectively. On the contrary, specificity had a reverse trend, favoring MRI (93.9, 92.8, and 89.8% for TVS and TRS, respectively). The Results of the present study demonstrated that TVS and TRS have appropriate diagnostic accuracy in diagnosis of DIE comparable to MRI.

Keywords: Deep Infiltrating Endometriosis, laparoscopy, Magnetic Resonance Imaging, Transrectal Sonography, Transvaginal Sonography

I-32: The Success of Various Endometrioma Treatments in Infertility: A Systematic Review and Meta-Analysis of Prospective Studies

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Background: Endometriosis is seen in 0.5%-5% of fertile

and 25%-40% of infertile women. To investigate this conflict between gynecologists that ovarian endometriomas should be removed or not before making any decision about pregnancy among infertile women, the authors decided to carry out a systematic review and meta-analysis to compare the effect of various available therapeutic Methods and notice the impact of these options on women's pregnancy rate.

Materials and Methods: This review is based on PRISMA recommendations with an electronic search using the following databases: PubMed, Scopus, Google scholar, etc, from 2000 to 2018, in the English language. The studies compare pregnancy rate based on four different treatment types of OMAs between infertile women: (surgery + ART, surgery + spontaneous pregnancy, aspiration ± sclerotherapy + ART, and ART alone).

Main findings: At least eight prospective studies were included, in which 553 infertile women were compared in terms of treatment Methods of OMAs before trying to become pregnant.

Conclusion: Treatments are usually based on the patient's clinical condition and must be individual, with the purpose of relieving pain, improving fertility, or both. The authors do not have not any significant difference between our four groups of study; however, the success of surgical procedure compared to other Methods was higher and the success of ART alone was the least.

I-33: Empty Follicle Syndrome (EFS)

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EFS is defined as the complete failure to retrieve oocytes after ovarian stimulation, monitoring of multiple follicular growth and ovarian puncture with follicular aspiration (OPU), despite apparently normal development of ovarian follicles and appropriate estradiol production by granulosa cells with incidence 5-7% in studies.

Two types of EFS have been described:

1. Genuine empty follicle syndrome (gEFS): which occurs in the presence of adequate circulating hCG level at the time of oocyte retrieval. The cause of gEFS is unclear, but specific genetic factor is probably involved (LH CGR, ZP1, ZP2), dysfunctional follicle genesis (aging, reduced ovarian reserve, obesity) and biologic abnormality in mature oocyte despite normal HCG: level treatment for gEFS in controversial.

2. False EFS: which is associated with no circulating hCG or a level below a critical threshold is most likely caused by errors with the trigger shot, wrong drag, timing, defect in the industrial production of some hCG batches, rapid clearance, in appropriate storage.

Diagnosis of EFS at oocyte retrieval

1. Tell- tale sign of EFS is very sparse granulosa cells in the follicular fluid

2. If after puncturing 3-5 follicle (≥ 14 mm) no oocytes are found is a probability of EFS; after then sensitive urine pregnancy test in preformed; if test is negative; using rescue trigger and re-schedule the retrieval 36 hours later.

I-34: Endometrioma Management in The Infertile Patient

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I-35: Why Endometriosis must be Considered as A Systemic Disease?

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Endometriosis is a chronic inflammatory disease defined as the presence of endometrial tissue outside the uterus, which causes pelvic pain and infertility. Nevertheless, the clinical presentation is varied, the presence of pelvic lesions is heterogeneous, and the manifestations of the disease outside of the female reproductive tract are very frequent but remain poorly understood. Endometriosis must now be considered as a systemic disease rather than a disease predominantly affecting the pelvis. The disease should be viewed as a public health problem with a major effect on the quality of life of women as well as being a substantial economic burden. In light of the considerable progress with diagnostic imaging (for example, transvaginal ultrasound and MRI), exploratory laparoscopy should no longer be used to diagnose endometriotic lesions. Instead, diagnosis of endometriosis should be based on a structured process involving the combination of patient interviews, clinical examination and imaging. Notably, a diagnosis of endometriosis often leads to immediate surgery. Therefore, rethinking the diagnosis and management of endometriosis is warranted.

I-36: Adjuvant Therapy in Recurrent Implantation Failure

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Reproduction and Embryology defines recurrent implantation failure (RIF) as more than 3 failed embryo transfers (ETs) with high quality embryos, or failed transfer of 10 embryos in multiple transfers. RIF affects around about 10% of patients undergoing ART. In women undergoing IVF treatment, suffering from RIF success rate remains around about 10%. The pathogenesis of RIF is poorly understood as three different factors are involved: Female and male partner as well as the embryo. Several approaches have been proposed to be effective; however, accumulative data have demonstarted that most of the treatment options do not have the evidence base. The current review aims to summarize the recent finding in managing RIF via interference with endometrial receptivity through co-treatments Including data on GH, PRP, GCSF, intralipid, Active Immunization with Partner Lymphocytes, letrozol, intrauterine HCG, corticoestroid therapy, endometrial scratching and IVIG.

I-37: Growth and Development of ART Children after GDM

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Background: Previous studies reported the relationship between using assisted reproductive technology (ART) and increased risk of gestational diabetes mellitus (GDM). Both pregnancies conceived by ART and GDM are associated with adverse pregnancy outcomes. Several investigations indicated the short-term outcomes of ART. However, there is little evidence about long-term outcomes. Therefore, the aim of this study was to compare the growth and development of children born via ART and spontaneous conception (SC) following GDM.

Material and Methods: This cohort study was the second phase follow-up of singleton pregnant mothers aged 20–42 years during 2014–2017, who conceived via ART or SC until the birth of neonates after GDM. In this phase (2019–2020), we followed children 3–5 years old born after ART or SC from GDM or non-GDM mothers which classified to four groups (ART, ART-GDM, GDM and SC). Past medical history was obtained from hospital files. The children's height and weight were collected by checking the health care files. The growth of children was compared with mean difference of the 50th percentile in Centers for Disease Control and Prevention (CDC) pediatric growth chart based on age and sex (at birth and 3–5 years). The development of children was assessed using Age and Stages Questionnaire (ASQ) by asking the mothers which classified to communication, fine motor, gross motor, problem-solving, and personal social relationship skills. One-way ANOVA, stepwise regression, and post hoc analysis were used. Statistical analyses were made by SPSS.

Results: In this survey, 423 children aged 3–5 years were followed for growth and developmental status including, 197 ART conceptions (102 subjects with and 95 without GDM diagnosis), and 226 spontaneous conceptions (116 subjects with and 110 without GDM diagnosis). The results of growth comparing to the 50th percentile in pediatric growth chart showed: the mean weight of girls at birth was under the 50th percentile in pediatric growth chart in all 3 groups except for GDM ($P < 0.05$). However, at 3–5 years the increasing trend was observed for all 3 groups except for SC. The mean weight of boys at birth was under the 50th percentile in pediatric growth chart in all 3 groups except for ART ($P < 0.05$). However, at 3–5 years the increasing trend was observed for all groups (GDM and SC were statistically significant). The mean height of girls at birth was approximately similar to the 50th percentile in pediatric growth chart. However, at

3-5 years the increasing trend was observed all groups except for GDM ($P < 0.05$). The mean height of boys at birth was approximately similar to the 50th percentile in pediatric growth chart in all 3 groups except for SC ($P < 0.05$). However, at 3-5 years the increasing trend was observed for all groups. The lowest height was observed in GDM. The results of ASQ scores showed: ART-GDM children had the lowest development in all skills, while the highest development skills were belonged to children after SC and the relationship were statistically significant.

Conclusion: At birth, the growth of ART and ART- GDM children was lower than the 50th percentile in CDC pediatric growth chart; however, this difference was compensated during 3-5 years old. ART-GDM children had the lowest development in all skills during 3-5 years old using ASQ scores. Further study is suggested to be done in a larger population and in a longer period.

Keywords: Assisted Reproduction Technologies, Gestational Diabetes Mellitus, Growth and development

I-38: Medical Management of Endometriosis

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Women with pelvic pain, suspected endometriosis, and no other indication for surgical treatment can be managed effectively with empiric medical treatment without establishing a surgical diagnosis. 395 Initial empiric medical therapy usually involves treatment with NSAIDs and oral contraceptives (OCs; combined or progestin only). If treatment with NSAIDs and OCs does not significantly improve pain, second- and third-line medical therapies or GnRH agonist and Antagonist. Traditional medical therapies for endometriosis have been based on Sampson's theory of retrograde menstruation and implantation and on the simple premise that ectopic endometrium may be expected to respond to treatment in much the same way as normal eutopic endometrium. Consequently, the objectives of treatment have been to reduce or eliminate cyclic menstruation, thereby decreasing peritoneal seeding and the likelihood that new implants will develop, and to suppress the growth and activity of the endometrium, anticipating that the same would occur in the endometriotic tissue derived from it. Interventions that reduce ovarian estradiol production are the most reliable ways to cause atrophy of endometriotic lesions and the most effective treatment for pain. These simple operational concepts have shaped medical treatments for endometriosis for decades, but our growing understanding of the pathogenesis of endometriosis at the molecular level is now beginning to suggest new treatment strategies aimed at the mechanisms of disease.

Established medical therapies for the treatment of pain associated with endometriosis include estrogen-progestin contraceptives, progestins, GnRH analogues and GnRH Antagonist. Typically, OCs are the first line of therapy with GnRH antagonists becoming the optimal second-line therapy in those who fail OC treatment or have troubling progestin-related side effects. Treatment decisions must be individualized, after carefully considering the severity of symptoms, the extent of disease, the desire

for future pregnancy, age, side effects, and costs. Research is ongoing for the development of new therapeutic agents.

I-39: Updates on The Management of Uterine Myoma in Infertility Treatment

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Uterine myoma or fibroid is the most common benign gynecological tumors in women of reproductive age.

Fibroids are hormone dependent smooth-muscle tumors with a wide heterogeneity in composition, size and number.

Most women with fibroids are fertile; however fibroids may affect fertility by distorting the pelvic anatomy and the intrauterine environment. The way by which myoma result in infertility remain to clearly understood.

Beside anatomical distortion, the possible mechanism impairing fertility are; endometrial function alteration increased uterine contractility and impairment of the endometrial and myometrial vasculatization and blood supply, alters the local hormone balance that could affect gamete transport and/or reduce embryo implantation.

Submucosal and intramural myomas with pressure effect on uterine cavity are associated with decreased pregnancy and implantation rates after ART cycles.

The management method is highly depends on the size, number and location.

I-40: Surgical Aspects of Adenomyosis

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Adenomyosis was described by Carl Rokitansky over 150 years ago. Whilst around 1920 he coined the term adenomyosis and placed the condition on the surgical map. For several decades, while endometriosis received most of the scientific attention, its first relative adenomyosis, received little or none. Even to these days, there is no coherent medical decision on how it is best to manage this condition in its various clinical expressions. In my presentation I propose a surgical road map based on safety outcomes especially for women who are in reproductive age and request fertility.

I-41: DIE-Related Infertility: How We Can Improve the Reproductive Outcomes

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Background: To study Pregnancy Rate (PR) in infertile patients with Deep Infiltrated Endometriosis (DIE) after different types of surgery – shaving/discoid resection or segmental bowel resection.

Materials and Methods: 144 infertile patients 20-45 aged with

DIE were operated in 2011-2018 years. All of them had typical bowel symptoms, 95 patients out of them were going to get pregnant. 51 patients who had shaving as surgery made the first group, 44 patients who had bowel resection made the second group.

Results: Cumulative PR after surgical treatment of colorectal endometriosis made 55.7, 54% (29) out of them was spontaneous pregnancy, 46% (27) - after IVF. For the both groups Spontaneous PR was the same. More frequent Spontaneous Pregnancy was in 20-30 aged patients and more often after colorectal resection (18% vs 27%). Effectiveness of IVF was identical in both groups (25,4% and 25%). PR in 36-40 aged patients was 8%. Patients over 40 didn't have pregnancy.

Conclusions: Surgery of DIE increased PR (spontaneous and IVF). However, the type of surgery (shaving or resection) doesn't make any difference on reproductive outcome and depends on the patient age and their ovarian reserve, whether they have pains, bowel symptoms, failed IVF and previous endometriosis surgery. Bowel resection is preferable for 20-35 aged patients instead of shaving.

I-42: Management of Adenomyosis and Infertility: ART

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I-43: Repeated Implantation Failure (RIF) and Recurrent Pregnancy Loss (RPL): Immunological Aspects

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Fetus is a semiallograft to maternal host, therefore immune tolerance is important to prevent fetus from rejection. However, immune activation also plays a role in placental development and fetal growth. Therefore, it is necessary to evaluate pregnancy in terms of the balance between immune activation and regulation. We have showed in a mouse system that Tregs are essential for the success of allogeneic pregnancies at the time of implantation. Interestingly, paternal antigen-specific Tregs, induced by seminal plasma priming, are accumulated in regional lymph nodes of the uterus one day before implantation. We have shown in mice that immature DCs inducing tolerance influx into the uterus from outside of the uterus and PDL2-positive immunosuppressive DCs remaining in the uterus are increased at implantation. We have shown that M2 macrophages increase in the uterus at implantation and removal of M2 macrophages at implantation period Results in implantation failure in mice. Interestingly, we also found that M2 macrophages were reduced in the endometrium at implantation period in IVF failure non pregnant cases. Thus, immunosuppressive drug, tacrolimus and high dose IVIG therapy, that increase Tregs, M2 macrophages and immature DCs may be effective in treating implantation failure where excessive inflammation is present. Recurrent pregnancy loss (RPL) is defined as a history of two or more miscarriages, the rate of live births is low in cases of four or more previous miscarriages. In addition, there are many cases of unexplained RPL cases. We performed randomized controlled trial of high dose IVIG therapy from 4 to 6 weeks 6

days gestation in unexplained RPL patients with a history of 4 or more miscarriages.

We found that IVIG therapy was particularly effective when patient had 6 or more previous miscarriages and when therapy was started before 6 weeks of gestation. Overall, immunological treatment that takes into account the immune status of each case of implantation failure and RPL is likely to be effective.

Genetics

I-44: Expanding The Scope of Preimplantation Genomic Testing in IVF

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Recent advances in sequencing technology and "polygenic risk scores" offer the potential to expand the scope of genetic testing to conditions that are increasingly relevant to the health of individuals and their growing families. We recently described an approach that combines molecular and statistical techniques to reliably infer inherited genome sequence in 110 embryos. Advantages, challenges and controversies of this approach will be discussed.

I-45: A Missing Motor in Human Meiosis

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I-46: Non-Coding RNAs in Ovarian Follicle Microenvironment and Their Altered Regulation in Reproductive Aging

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Human oocytes are prone to assembling meiotic spindles with unstable poles, which can favor aneuploidy in human eggs. The underlying causes of spindle instability are unknown. We found that NUMA (nuclear mitotic apparatus protein)-mediated clustering of microtubule minus ends focused the spindle poles in human, bovine, and porcine oocytes and in mouse oocytes depleted of acentriolar microtubule-organizing centers (aMTOCs). However, unlike human oocytes, bovine, porcine, and aMTOC-free mouse oocytes have stable spindles. We identified the molecular motor KIFC1 (kinesin superfamily protein C1) as a spindle-stabilizing protein that is deficient in human oocytes. Depletion of KIFC1 recapitulated spindle instability in bovine and aMTOC-free mouse oocytes, and the introduction of exogenous KIFC1 rescued spindle instability in human oocytes. Thus, the deficiency of KIFC1 contributes to spindle instability in human oocytes.

I-47: NGS Based Male and Female Infertility Panels

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Since NGS technology stepped in molecular genetic applications, it has become quite easy to incorporate exome and genome-scale technologies into our routine practice.

In today's technology, we have been using Next Generation Sequencing (NGS) based infertility panels, markers for azospermia cases, sperm DNA damage/ apoptosis tests for the evaluation of male infertility. NGS based infertility panels, Immunological factors, endometrial receptivity tests are mainly used for the evaluation of female infertility.

The genetic causes of male infertility are quite complex. Genetic factors are responsible for 15% of male infertility. A large number (at least 2000) of genes is thought to be responsible for spermatogenesis. With the highest frequency of known genetic factors contributing to men, spermatogenic numerical defects constitute 25%. Until now, a limited number of tests were used in the evaluation of male infertility. However molecular genetics and flow cytometric tests have been implemented for comprehensive infertility analysis. In this context by using NGS based infertility panels we can detect Kallman Syndrome, genes related to spermatogenic failures. Additionally novel genes related to male infertility can be evaluated. We designed targeted NGS based male infertility test panel (40 genes) for the genes related to spermatogenic failure/sperm maturation, hormones/receptors, congenital bilateral absence of vas deference and motility / fertilization failure. There are increasing number of publications about infertility genetics. Novel genes have been discovered especially related with spermatogenic failure. In this context it won't be easy to dedicate a targeted small NGS panel for infertility diagnosis.

The inability of limited gene panels to detect novel variants has led us to apply exome based comprehensive panels. Same scenario is also valid for female infertility. We were performing NGS based female infertility test panel (38 genes) for the genes related with primary ovarian insufficiency, sex reversal syndromes, hypogonadotrophic hypogonadism and oocyte maturation defects. Since NGS technology is not compatible to detect repeat expansion type mutations, a specific method like TP-PCR has been performed to detect Fragile X Syndrome. Since studies on infertility-related genes are very recent, the clinical relevance of many variants has not been fully demonstrated.

Most of the variants we have been predicting are class3 variants. We call these variants as variants of unknown significance due to lack of clinical correlation between the gene and the infertility. As association studies increase, variant classification knowledge will also be improved and the specificity of these NGS-based tests will also become stronger. Although rapid developments have been occurring in wet laboratory stages of exome-based tests, variant classification and clinical data association studies are not progressing at the same level. For this purpose, we have been using high technology bioinformatic solutions which give chance to achieve daily updated literature information for infertility related genes.

The importance of gene panels in male and female infertility diagnosis consists of the following matters;

- This test panel is a screening test to identify the etiology of male infertility
- It is aimed to reveal the cause of infertility and to receive specific and correct treatment for the infertile patients

- The inheritance of these mutations to the next generations can be prevented via Preimplantation Genetic Tests. Developments in the field of molecular Genetics lead to important improvements in Reproductive Medicine. Knowing the exact cause of infertility allows for better diagnostic decisions and enables enhanced counseling for couples.

I-48: New Discoveries in Genetic Etiologies of Infertility

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Imaging

I-49: How to Find The Cause of Male Infertility Factor?

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Male infertility accounts for approximately 40% of infertility factors. We aim to explain how to find the cause of male factor in infertile men, referred to an infertility treatment center.

First, a comprehensive and thorough medical history including familial diseases, genetic disorders, and incurable diseases is necessary. Surveys related to the infertility history in the family should be considered. Age is an important factor in the first visit and during the treatment.

In addition to infertility, other complaints, such as chronic headaches, cough or sinusitis, high blood pressure and so on, should be considered. Information related to the patient's occupation is of great importance.

The interview should contain these questions: Is a couple's infertility primary or secondary? Are Patient's weight and height proportionate? Do couples consume any specific drug, substance, or alcohol? Do signs of puberty, correct sexual orientation and phenotype appear?

In the next stage, the accurate physical exam of different organs, including cardio-pulmonary system, genitalia and so on, should be done. Consequently, according to the medical history and clinical findings, paraclinical and imaging evaluations are considered.

Let's consider some examples:

- In a man who does not meet tanner criteria of puberty, hormonal profile must be checked, since hormonal disorder is suspected.
- In a patient suffering chronic migraine, and semen analysis is abnormal, and the level of prolactin hormone of blood is higher than normal level, pituitary disorder is suspected.
- An infertile patient who has chronic cough and nasal and pharyngeal discharge should be assessed for Kartagener syndrome.
- A non- palpable vas deferens and azoospermia increases the odds of unilateral kidney agenesis by 15%, hence, pelvic and abdominal ultrasound examination is helpful for correct diagnosis.
- For a patient admitted with secondary infertility who has ab-

normal semen analysis and his finding of physical examination is in favor of varicocele, scrotum should be evaluated using doppler sonography.

In conclusion, using correct medical history, and physical examination, and accurate diagnostic methods, the exact cause of male factor is detectable.

I-50: Challenges of Radiological Diagnostic Methods in ART

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Ultrasound, which is an essential tool for the evaluation of women undergoing assisted reproduction treatment, is used for treatment and prediction of ovarian reserve and endometrial receptivity, direct monitoring of response to controlled ovarian stimulation, and facilitates oocyte retrieval and embryo transfer. Additionally, it is performed to screen for any pelvic pathologies.

Assessment of ovarian reserve

The commonly employed tests of ovarian reserve can be divided into static markers (estradiol, follicle stimulating hormone, inhibin-B and anti-Mullerian hormone), dynamic markers (tests of stimulation with clomiphene citrate, gonadotrophins and gonadotrophin-releasing hormone analog) and ultrasonographic markers. These items should be assessed using Imaging methods:

- Ovarian volume
- Antral follicle count
- Ovarian blood flow

Assessment of endometrial receptivity

Endometrial receptivity is the generic term to describe how the endometrium will respond to embryo implantation. The endometrium must play some part, however, as conception is unlikely in patients with a thin endometrium. Conception is unlikely in association with an endometrial thickness of 5 mm or less. Adequate endometrial blood flow is generally regarded as a marker of endometrial receptivity.

Follicular monitoring

Follicular development is evaluated in the normal menstrual cycle.

Main Pelvic pathologies are:

- Polycystic ovarian syndrome
- Ovarian cysts
- Hydrosalpinx
- Congenital uterine anomalies
- Other uterine pathologies

In conclusion imaging is used daily to monitor the response to treatment and to guide oocyte collection and ET in women undergoing IVF treatment. Ultrasound is an inexpensive and noninvasive method for evaluation of pelvic organs and is used widely in ART.

Andrology

O-1: Effect of Sperm *In Vitro* Incubation with Pentoxifylline and Pentoxifylline Oral Administration in Asthenozoospermic Patient; Evaluation of Sperm Parameters and Chromatin Condensation

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Background: Asthenozoospermia is defined as low sperm motility. One of the reasons is over loaded reactive oxygen species (ROS) in testicles and seminal plasma, which finally increases DNA fragmentation. The present study tries to summarize the results of two studies on *in vitro* incubation with pentoxifylline (PTX) and PTX oral administration in asthenozoospermic patients.

Materials and Methods: In first study, sample were collected from asthenozoospermic patients. They were divided into two groups of case and control and incubated for 45 min with PTX, then sperm parameters and chromatin condensation (by SCD test) were evaluated. When positive effect of PTX on sperm motility was achieved, in 2nd study 30 asthenospermic men were treated with PTX oral administration. Since the *in vitro* incubation with PTX, caused an increase in sperm DNA fragmentation, in 2nd study we orally administrated PTX with Vit E + folic acid for 3 mounts, to analyze whether antioxidant can ameliorate the negative effect of PTX on DNA fragmentation? So, before and after three 3 mounts, sperm parameters and sperm chromatin by Aniline blue (AB), Toluidine blue (TB) and Chromomycin A3 (CMA3) were evaluated. Also, TUNLE was used for apoptosis.

Results: *In vitro* study showed that sperm motility increased after 45 min incubation with PTX (P<0.01). Sperm DNA fragmentation was increased (P<0.0001) by SCD test. The results of 2nd study, showed that semen volume, sperm count and motility (p<0.0001) increased significantly, after three months administration of PTX+VITE+folic acid. AB, TB and CMA3 showed sperm DNA fragmentation was decreased after 3 mounts (not significantly). TUNLE test showed decrease of sperm apoptosis.

Conclusion: PTX may have effect on ROS production and can improve sperm motility, but probably has negative effect on sperm DNA fragmentation, so co-administration of PTX with antioxidant may ameliorate this side effect.

Keywords: Asthenospermia, Folic Acid, Pentoxifylline, Sperm Chromatin, Vitamin E

Embryology

O-2: Effect of A Synthetic Platinum-Based Complex on Autophagy Induction in Sertoli TM4 Cells

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Background: Platinum-based chemotherapy that are widely used in clinical, have major limitations and various side effects. Gonadotoxicity and sterility are the most common complications for cancer survivors, which is drug-specific and dose-related. We aimed to investigate the cytotoxicity effect of new synthesized Pt(II)-phosphane complexes containing heterocyclic thionate ligands (PCTL) on mice TM4 Sertoli cells with particular emphasis on the role of autophagy.

Materials and Methods: MTT assay was performed to evaluate the IC50 of PCTL and to analyze the TM4 cells' viability. Cells morphology was evaluated via invert microscope, and nuclei swelling or autophagic vacuoles formation were assessed by DAPI and MDC staining. Finally, the expression of Atg5, Beclin1, p62, BAX and BCL2 were analyzed by qPCR.

Results: The IC50 value of PCTL was 100 µM and cytotoxic effects were in a dose and time-dependent manner. Evaluation of TM4 Sertoli Cells morphology indicate that the cytotoxic concentrations of PCTL were significantly higher than cisplatin. The results of PCR showed an increase in the expression of the p62 dose-dependently in PCTL treated cells in compared to cisplatin. No significant difference was observed in the expression of Atg5 and Beclin1 genes compared to the control group. Furthermore, BAX increasing and BCL2 decreasing expression indicating the induction of apoptosis in treated PCTL TM4 cells; however, higher doses of PCTL need to induce the apoptosis in compare to cisplatin.

Conclusion: The results of the study indicate that the PCTL had less-lethal effects on TM4 sertoli cells in compare to cisplatin and probably did not induce autophagy in TM4 cells.

Keywords: Autophagy, Cisplatin, Gonadotoxicity, Platinum-Based Anticancer Agents, Sertoli TM4 Cells

O-3: Effects of Conditioned Media of Human Umbilical Cord Wharton's Jelly-Derived Mesenchymal Stem Cells on The Antioxidant Enzymes Gene Expression in Granulosa Cells of Healthy and Polycystic Ovary

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Background: Polycystic ovary syndrome (PCOS) is related to oxidants-antioxidants imbalance. Previous research has shown that conditioned media-derived mesenchymal stem cells play

a role in improving antioxidant capacity in the different cell cultures. Therefore, we investigated the effects of conditioned media of human umbilical cord Wharton's jelly-derived mesenchymal stem cells (hWJMSCs-CM) on the expression of antioxidant enzymes of human normal granulosa cells (N-GCs) and granulosa cells from polycystic ovaries (PCO-GCs).

Materials and Methods: Ovarian GCs were obtained during the oocyte retrieval procedure from 10 women with PCOS and from 10 healthy women who were referred to Shiraz Ghadir Mother and Child Hospital. To assess superoxide dismutase (SOD) and glutathione peroxidase (GPx) genes expression, total cellular RNA was extracted from GCs using a total RNA Purification Mini kit. Extracted mRNAs were reversely transcribed into first-strand cDNA using a cDNA synthesis kit. qRT-PCR was accomplished using SYBR Green PCR Master Mix. Samples were quantified by the comparative $\Delta\Delta C_t$ method with β -actin as an internal control. Experiments were performed in triplicate.

Results: Basal SOD and GPx genes expression by N-GCs was significantly higher than PCO-GCs. hWJMSCs-CM significantly increased SOD and GPx genes expression in N-GCs and PCO-GCs. In the presence of hWJMSCs-CM, GPx genes expression in the PCO-GCs was not significantly different from N-GCs.

Conclusion: hWJMSCs-CM had positive impacts on luteinized GCs through its effect on the expression of antioxidant enzymes. It can improve PCO-GCs to normal conditions by affecting the main aspects of oxidative metabolism.

Keywords: Antioxidant Enzymes, Conditioned Media, Granulosa Cells, Polycystic Ovary, Wharton's Jelly-Derived Mesenchymal Stem Cells

O-4: Crosstalk between Metabolic Interruption and Oxidative Stress in Hyperglycemia Condition; Evidence Revealed after Insulin Therapy in Rats Testicles

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Background: Diabetes mellitus (DM), most common metabolic disorders, negatively affects male fertility potential leading to irreparable spermatogenesis dysfunction through endocrine suppression and oxidative stress (OS) in the male gonads. While the OS is crucial to inducing DM-caused infertility in male subjects, its precedence and delay over hyperglycemic conditions in the testicular tissue remains a matter of controversy. Hence, the present study was conducted in order to explore the crosstalk between OS and metabolic disorders in the testicular tissue of HG-induced rats by analyzing the lactate synthesis and OS markers before and after insulin administration.

Materials and Methods: For this purpose, 18 mature male Wistar rats were divided into control (no DM induction and administration), hyperglycemia-Induced (HG) and Insulin-treated HG-induced (Ins+HG) groups (n=6/group). Following 6 weeks from HG induction and insulin treatment, the testicular antioxidant profile [total antioxidant (TAC), total oxidant status (TOS),

and malondialdehyde (MDA)], immunoreactivity of MCT-4 and MCT-1 in Sertoli and germ cells, and lactate level were examined, and compared between groups. The results were statistically analyzed using one-way ANOVA and Bonferroni post-hoc tests. The $p < 0.05$ was considered a statistically significant difference.

Results: The HG group exhibited a remarkable ($P < 0.05$) reduction in TAC, and increment in TOS and MDA levels versus the control group. Moreover, the immunoreactivities of MCT-1 and MCT-4 were decreased in the germ and Sertoli cells, respectively. Finally, the HG group represented a significant ($P < 0.05$) reduction in the testicular lactate concentration. In contrast, the insulin could remarkably ($P < 0.05$) up-regulate the TAC level and ameliorate the HG-increased TOS and MDA levels. Simultaneous with these alterations, the immunoreactivities of MCT-1 and MCT-4, as well as the lactate level, increased in the Ins+HG group.

Conclusion: The up-regulation of transporters MCT-1 and MCT-4 and elevated lactate synthesis in the Sertoli cells, simultaneously with improved antioxidant capacity of the testicles after insulin administration, indicate a substantial correlation between metabolic disruption and antioxidant capacity of the testicles under hyperglycemic conditions. Forasmuch the antioxidant capacity of testicular tissue increased as a result of the improved metabolic processes (induced by insulin administration).

Keywords: Hyperglycemia, Insulin, MCT-1, MCT-4

Ethics and Medical Law

O-5: The Right to Abortion in Surrogacy Contracts

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Background: When it comes to abortion, it can be argued that mother should be considered as one of the main decision-makers, if not the only one, especially when her life is in danger. Mother has usually been perceived to be the carrying person. However, surrogacy has changed this perception, since there might be two women involved in this process: one contributing the egg and the other carrying the resulting fetus. Even the woman who has intended to have a child might not play any biological role in the reproduction process. The question is that which of the involved women should decide on abortion in such cases. Whose right is it?

Materials and Methods: This is a theoretical research with a comparative approach. First, it deals with theoretical aspects of the issue and, then, it goes through various laws and judicial decisions in this regard.

Results: The main claim is that bodily integrity of the surrogate mother and her right to health care require us to prefer her decision on abortion over that of the intended parents. The latter should not even have a veto right over the said decision. On the other hand, the intended parents' right to reproduction should also be taken into account. Recently, legislators have tried to give attention to intended parents' rights through making a room for the applicant parents' entitlement to compensation, though it is always the carrying person who seems to be

considered as having the upper hand.

Conclusion: Abortion in surrogacy cases is faced with various conflicting claims and interests. Accordingly, legislators are to take into account rights and claims of all concerned parties striking a balance in this regard.

Keywords: Abortion, Bodily Integrity, Surrogacy

Female Infertility

O-6: High Intensity Interval Training and Endothelial Function in Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. Given that PCOS is associated with an increased cardiovascular disease (CVD) risk, and reduced endothelial functioning increases this risk, the clinical importance of lifestyle improvement on endothelial function in PCOS needs investigation. The main purpose of this study was to evaluate the effect of 8 weeks high intensity interval training (HIIT) on serum levels of VCAM-1, and E-selectins in women with PCOS.

Materials and Methods: Thirty females with PCOS aged between 18 and 40 years were randomly categorized to an 8-week high intensity interval training intervention (HIIT), or a control group. The HIIT program include 8 weekly sessions (3 sessions a week). After an overnight fast of 10 hours, fasting serum samples were collected before and after intervention. Statistical analysis was performed using SPSS software (version 23). P less than 0.05 was considered statistically significant.

Results: After 8-week intervention a significant reduction in serum E-selectin levels was observed in HIIT group, but not in control group. Regarding sVCAM1, no significant changes was observed before and after intervention in both groups.

Conclusion: These findings demonstrated that 8 weeks HIIT in PCOS patients could be prescribed to improve endothelial function.

Keywords: Endothelial Function, Exercise, High Intensity Interval Training Intervention, Polycystic Ovary Syndrome

O-7: Relationship Between Ovarian Reserve Markers with Uterine Myomas

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Background: Ovarian reserve is a woman's fertility potential. Poor ovarian response to stimulation may be due to aging, endometriosis, myoma, and pelvic inflammatory disease. These studies evaluate the effect of uterine myomas on ovarian reserve.

Materials and Methods: The present case-control study was performed on 240 women in both case and control groups from 2016-to 2017 in Royan Center. The case group consisted of 97 women with infertility, which had intramural myoma. The control group consisted of 143 women who had infertility and did not have this lesion. The sampling method was continuous and available. The data collection tool was a questionnaire in two parts of individual variables. The second part was related to both groups' ultrasound results (number of antral follicles and ovarian volume) and laboratory (FSH and AMH) done on the third day of the cycle. Data analysis was performed using SPSS software and t-test and a chi-square test.

Results: The result showed no statistically significant difference between the two groups in FSH ($P > 0.05$). Still, in AMH there was a statistically significant difference between the two groups ($P < 0.05$). Also, the results showed that the number of antral follicles in the right ovarian, the number of antral follicles in the left ovarian, and thickness were not statistically significant between the two groups ($P > 0.05$). But the variables of the size of uterine, echo uterine, and intramural fibroma was statistically significant ($P < 0.05$).

Conclusion: The results showed myomas with different mechanisms such as changes in uterine size and the effect on uterine lead to a decrease in ovarian reserve and infertility. Therefore, treatment and surgery can reduce these effects and improve the fertility of the affected women.

Keywords: Infertility, Myoma, Ovarian Reserve

Genetics

O-8: Identification of Candidate Genes in Recurrence and Non-Recurrence Endometrial Carcinoma Patients by an Integrative Analysis

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Background: Endometrial carcinoma (EC) is one of the most prevalent tumors of the female reproductive system. Although

numerous studies, including analysis of gene expression profile and cellular microenvironment have been reported in this field, pathogenesis of this disease remains unclear. The molecular profile of endometrial cancer has become an important tool in determining patient prognosis and their optimal adjuvant treatment. This study aimed to screen the candidate genes differentially expressed in recurrence and non-recurrence patients by bioinformatics analysis.

Materials and Methods: GEO database and GEO2R online tool were applied to screen the differentially expressed genes (DEGs) of EC from the microarray datasets. Protein-protein interaction (PPI) network for the DEGs was constructed to further explore the relationships among these genes and identify hub DEGs. Gene ontology and KEGG enrichment analyses were performed to investigate the biological role of DEGs. Besides, expression profile, and survival analysis of MFNG gene, as one of the top hubs DEGs, were also investigated using Gene Expression Profiling Interactive Analysis2 (GEPIA2) to further explore the roles of these hub gene in the mechanism of EC tumorigenesis.

Results: A total of 551 DEGs were screened out as the DEGs with 369 upregulated and 182 downregulated in EC. The gene ontology analysis showed that these genes were significantly enriched in cell communication, biological regulation, and localization, etc. The KEGG pathway analysis showed that DEGs were enriched in T-cell activation, leukocyte cell-cell adhesion, and leukocyte activation, etc. More importantly, MFNG, ZAK, SOCS2, WNT4, SMO, SMAD9, USP39, PRKACG, SF3A3, TRAF7 were identified as the hub genes of EC. Expression validation by bioinformatics analysis also proved the expression of MFNG was differentially expressed in EC, but overall survival was not altered significantly.

Conclusion: MFNG involved in the pathogenesis of EC and might be a candidate biomarker for distinguishing recurrence and non-recurrence patients.

Keywords: Athway, Bioinformatics Analysis, Biomarker, Differentially Expressed Gene, Endometrial Carcinoma

O-9: Increased Expression of LBX1, EN1 and ISL2 Genes in Plasma of Women with Endometriosis Compared to Women without Endometriosis

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Background: Endometriosis is a complex disease with nonspecific symptoms that leads delay diagnosis. Current diagnostic methods are based on ultrasound which cannot detect superficial/peritoneal endometriosis and laparoscopy which is an invasive procedure. There are no available biomarkers for the disease, making the diagnosis as difficult and challenging process. The previous study conducted at Royan Institute on genes involved in endometrial development, focusing on HOX genes, revealed that the mRNA expression levels of 3 HOX cofactors genes named EN1, LBX1 and ISL2 are significantly upregulated in eutopic tissues of endometriosis compared to the control group. Objective: To determine whether levels of EN1, LBX1 and ISL2 mRNA are higher in the plasma of women with endometriosis compared to healthy women.

Materials and Methods: Peripheral blood samples were collected from healthy women (with male factor) and endometriosis women in III and IV disease stages (n=4, in each group) after obtaining written consent according to the local ethical approval. The plasma fraction was separated by centrifuge. The RNA extraction was performed on the plasma samples of two groups, and the expression levels of candidate genes were evaluated by qRT-PCR technique.

Results: EN1, LBX1 and ISL2 genes showed significant increased levels in plasma of the endometriosis group compared to the control group.

Conclusion: The present study provides the first report of increased expression of EN1, LBX1 and ISL2 genes in the plasma of endometriosis patients, and suggesting them as potential biomarkers for the non-invasive diagnosis of endometriosis in the future.

Keywords: Endometriosis, EN1, ISL2, LBX1, Plasma

Andrology

P-1: Evaluation The Effect of Coenzyme Q10 on The Expression Level of Sperm TFAM Gene in Patients with Idiopathic Oligoasthenoteratospermia (OAT)

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Background: Coenzyme Q10 (CoQ10) is a mitochondrial electron transport chain component. It plays an important role in the human seminal fluid as an antioxidant, mitochondrial bioenergetics and metabolic process. Studies have shown that CoQ10 deficiency can lead to sperm damage, lower sperm motility, and sperm count. Therefore, the aim of this study was to evaluation the effect of coenzyme Q10 on the expression level of sperm TFAM gene in patients with idiopathic oligoasthenoteratospermia.

Materials and Methods: This study included 88 infertile patients with idiopathic OAT and 42 healthy men as control group and underwent semen analysis based on the WHO 2010. All participants received orally CoQ10 200 mg/daily for 3 months. After this time, sperm parameters and the expression level of the sperm TFAM gene were measured using real-time PCR in control group and infertile patients.

Results: Treatment with CoQ10 significantly improved sperm concentration ($P=0.002$), progressive motility ($P=0.02$), and total motility ($P=0.006$). The expression level of sperm TFAM gene was decreased as compared control patient ($P=0.001$).

Conclusion: Administration of orally 200mg/daily CoQ10 supplementation for 3 months could improve sperm parameters and decrease sperm TFAM gene expression in patients with idiopathic OAT.

Keywords: Coenzyme Q10, Male Infertility, Oligoasthenoteratospermia, Sperm, TFAM

P-2: The Effect of Dopamine D2 Receptor Inhibition on CatSper1 Gene Expression in Testes of Male Rats

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Background: Sulpiride is used as an antagonist of dopamine D2 receptors and as a type of antipsychotic drug to control some types of mental illness such as anxiety, morbid stress and schizophrenia. The aim of this study was to evaluate the effect of this drug and its potential benefits or harms on CatSper1 in the testes of male rats.

Materials and Methods: In this experimental study, male Wistar rats weighing 190 ± 10 were divided 24 adult were randomly divided into 4 groups of 6; Groups include saline control, sulpiride control (4mg / kg bw, ip), mental sulpiride and physical sulpiride. Induction of psychological or physical stress by placing rats in insulated or conductive chambers of electricity (0/5Hz, v40, 2mA (adjacent) for 60 minutes daily

(twice per minute for ten seconds), respectively. After 14 days, after dissecting the animals, the CatSper1 gene was evaluated in testicular tissue

Results: The expression of CatSper1 gene in the control group of sulfrid was reduced compared to the control of saline ($P<0.05$). In the group of physical sulpiride and mental sulpiride, there was a significant decrease compared to the control of sulpiride ($P<0.05$), but in the group of physical sulpiride and mental sulpiride, no significant difference was found ($p>0/05$). CatSper1 gene and testicular tissue damage were compared with the control group ($P<0.05$).

Conclusion: Drugs affect dopamine D2 receptor function and mRNA expression, reducing CatSper1 gene expression and testicular tissue damage. Sulpiride administration exacerbated the complications

Keywords: Antipsychotic, CatSper1, Dopamine, Sulpiride

P-3: Aspartame Induced Testicular Toxicity in Healthy Mice; Evidence for P53, BAX, Caspase-3, Bcl-2, and Hsp70-2 Genes Expression

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Background: Aspartame is the most famous and most widely used artificial sweetener that is widely used in various foods. There are many controversial reports of aspartame toxicity to various tissues. However, not enough data is available about the effects of aspartame on the reproductive system. The present study was performed to evaluate the effects of aspartame on the male reproductive system.

Materials and Methods: In this study, 36 adult male NMRI mice were randomly divided into four groups of nine. The three groups of the above-received aspartame at 40, 80, and 160 mg/kg body weight orally by gavage for 90 days, respectively. The control group also received 0.5 ml of physiological serum by gavage. Twenty-four hours after the last treatment, tissue samples were taken and used to express Bcl-2, BAX, P53, Caspase-3, and Hsp70-2 genes and proteins.

Results: Aspartame significantly reduced ($P<0.05$) the expression of Bcl-2 and Hsp70-2 genes and proteins in addition to a significant increase ($P<0.05$) in the expression of P53, BAX, and Caspase-3 genes and proteins.

Conclusion: Our findings revealed that long-term administration of aspartame at high doses adversely can induce changes in the expression of genes involved in cell death programming through the exertion of oxidative stress and induces cell degradation or apoptosis.

Keywords: BAX, Bcl-2, Caspase-3, Hsp70-2, P53

P-4: Correlation Analysis of Sperm Count with Telomere Length and Sperm Functional Tests

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Background: Telomeres are particular sequences at the ends of chromosomes that are known as one of the most important biological markers of genome integrity. Previous studies demonstrated a significant correlation between sperm telomere length with semen quality. In this study, we showed the relationship analysis between sperm count with sperm telomere length and sperm functional tests.

Materials and Methods: Semen samples were obtained from 80 men referring to from Isfahan Fertility and Infertility Center. Sperm concentration was assessed by sperm counting chamber through computer-assisted semen analysis software, and semen volume was estimated by weight. To calculate sperm count, semen volume was multiplied by sperm concentration. Telomere length (quantitative real-time PCR), protamine deficiency (chromomycin A3), DNA fragmentation (TUNEL assay), and lipid peroxidation (Bodipy probe) were assessed for each sample. Pearson correlation coefficient in SPSS was used for the relationship between sperm count with the aforementioned parameters.

Results: Positive significant correlations were observed between sperm count with telomere length ($r=0.35$; $P=0.005$), and sperm motility ($r=0.4$; $P=0.001$). In addition, we observed negative significant correlations between sperm count with abnormal sperm morphology ($r=-0.37$; $P=0.003$), protamine deficiency ($r=-0.54$; $P<0.001$), DNA fragmentation ($r=-0.32$; $P=0.007$), and lipid peroxidation ($r=-0.35$; $P=0.03$).

Conclusion: In semen samples of men with low sperm count, the level of oxidative stress and chromatin functional tests such as telomere length, protamine deficiency, and DNA damage were significantly high. While, in men with normal sperm count, these parameters were in normal ranges.

Keywords: DNA Damage, Oxidative Stress, Protamine Deficiency, Sperm Telomere Length

P-5: Assessment of Sperm Function in Advanced Glycation End-products (AGEs) Diet Mice Model

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Background: Advanced Glycation End Products (AGEs) are a complex group of molecules. Endogenous AGEs are produced in our body while exogenous AGEs are consumed through food, smoking and alcohol consumption. AGE could bind receptors of AGE (RAGE) and this binding has been associated with many diseases including diabetes, heart disease, kidney failure, Alzheimer's, as well as aging. AGE and RAGE signaling could induce lipid peroxidation, reactive oxygen species (ROS) production and trigger inflammation. Studies have shown that AGE and RAGE were significantly increased in spermatozoa of diabetic and hyperglycemic men. Since increased oxidative

stress is considered as one of the main causes of male infertility as well as the final consequence of AGEs diets, we aimed to induce the AGE model in C57bL mice and assessed the effect of AGE on sperm parameters.

Materials and Methods: In this study, ten C57bL male mice were divided into a control and AGE diet groups. After 35 days, all animals were sacrificed, and body weight was assessed. Sperm concentration, motility, and abnormal morphology were assessed after the extraction of sperm from the caudal left and right epididymis. Sperm concentration, and motility were assessed by CASA system. Sperm morphology was assessed by Eosin and Nigrosine staining. Comparison of body weight, and sperm parameters between two groups were analyzed by independent t test. A p-value less than 0.05 was considered statistically significant.

Results: The mean of final body weight and, food intake in the AGE group increased compared to the control group. Unlike the mean of sperm concentration and morphology which were no significant differences between the two groups, the mean of sperm motility was significantly lower in the AGE group than the control group ($P<0.05$).

Conclusion: The result of this study clearly shows that AGE diets can affect sperm motility more than other parameters. The diet period with AGE in these mice was about 35 days (a period of spermatogenesis in mice). It is possible that with increasing AGE diet period, damage to spermatogenesis and sperm function becomes more pronounced, which requires further studies in this area.

Keywords: Advanced Glycation End Products, Morphology, Motility, Sperm Concentration

P-6: The Effect of Apigenin Antioxidant on Human Sperm Quality after Frozen-Thawed Procedure

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Background: The sperm cryopreservation procedure can induce damaging alterations in sperm function. This process is usually associated with reduced viability and motility, and increased oxidative stress and DNA damage in sperm. Although several mechanisms can be the cause of this damage, the increase in oxidative stress during freeze-thaw is the most important factor impairing sperm function. Apigenin is a trihydroxyflavone that has multiple physiological functions, such as antioxidant, and anti-inflammatory activities. In this study, we aimed to assess the effect of Apigenin on human sperm quality after frozen-thawed procedure.

Materials and Methods: Following the administration of different concentrations of Apigenin during the freezing process (0.4, 0.2, 0.1, 0.05, 0.0 mM), sperm motility, viability, ROS production (DCF staining), lipid peroxidation (Bodipy staining), and DNA damage (Acridin orange staining) were assessed on 10 normozoospermic samples before and after the freezing/thawing process. One-Way ANOVA was used for comparison of study parameters between different concentrations of Apigenin.

Results: The analysis of data demonstrated that mean percentage of sperm motility and viability significantly were lower

after frozen-thawed procedure than before this procedure, and none of the Apigenin concentrations used in this study could improve these two parameters after the freeze-thaw process. In addition, we did not observe significant improvement in sperm function parameters including lipid peroxidation, ROS production, and DNA damage after the use of different concentrations of Apigenin during freeze-thaw. Only a concentration of 0.2 mM Apigenin improved sperm DNA damage compared to the control group, which was almost significant.

Conclusion: In this study, we could not observe the antioxidant effect of Apigenin during the freeze-thaw process on sperm function. Numerous studies using other concentrations of Apigenin on sperm function have been suggested to determine the antioxidant properties of Apigenin.

Keywords: Apigenin, DNA Damage, Freeze-thaw Procedure, Human Sperm, Viability, Motility, Oxidative Stress

P-7: Assessment of Sperm Chromatin Status and Oxidative Stress in Rat Varicocele Model

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Background: Infertility is one of the common global problems that affect a population of about 10-15% of couples. Of all infertility cases, approximately 40–50% of infertility is due to male factors. Varicocele, abnormal dilation of the scrotal venous pampiniform plexus, is known as the most important cause of male infertility. The increased testicular temperature in this condition can lead to decreased spermatogenesis and sperm production. Therefore, in this study, we aimed to assess the effect of varicocele induction on sperm parameters, chromatin structure, and, oxidative status in Wistar rats.

Materials and Methods: This study was performed on 20 male Wistar rats. The rats were classified into 2 groups: control(C) and induced-varicocele(V). After 2 months, rats were sacrificed and the left epididymis and testis were used for evaluation of sperm parameters, sperm DNA damage (Acridine orange staining), chromatin maturation (Chromomycin A3, and aniline blue staining), sperm lipid peroxidation (Bodipy probe assay), and testicular lipid peroxidation (Malondialdehyde assay) levels. All the parameters were compared between two groups by independent-samples t test. A p-value less than was considered statistically significant.

Results: Mean of sperm concentration and motility were significantly lower in varicocele induction group than control group ($P<0.01$). In addition, mean percentage of sperm abnormal morphology was significantly higher in varicocele induction group than control group ($P<0.05$). Regarding sperm functional tests, mean of sperm DNA damage, protamine deficiency, persistence histone, and lipid peroxidation were significantly higher in varicocele induction group than control group ($P<0.001$). Furthermore, the testicular lipid peroxidation level was significantly higher in varicocele induction group than control group ($P<0.001$).

Conclusion: According to these results, we demonstrated that varicocele status is associated with high levels of oxidative stress, and consequently a decrease in sperm quality. Antioxidant therapy or varicocelectomy surgery seems to be able to

help improve this condition.

Keywords: Oxidative Stress, Sperm Parameters, Varicocele Induction

P-8: Correlation Between Sperm DNA Fragmentation Index with Semen Parameters in 500 Oligozoospermic Samples

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Background: The integrity of DNA in sperm cells is essential for transferring genetic material to the next generation. According to literature, an increased level of sperm DNA fragmentation is related to a low level of fertilization, embryo quality, and pregnancy. The purpose of this study was to assay the correlation between semen parameters with sperm DNA fragmentation index (DFI) in 500 oligozoospermic men.

Materials and Methods: Semen samples were obtained from 500 oligozoospermic men referring to Isfahan Fertility and Infertility Center. Semen analysis and sperm DNA fragmentation were assessed according to WHO guideline and sperm chromatin structure assay (SCSA), respectively. Sperm concentration and motility were assessed by sperm counting chamber through computer-assisted semen analysis software, and semen volume was estimated by weight. Pearson correlation coefficient in SPSS was used for the relationship between sperm count with the aforementioned parameters.

Results: A positive significant correlation was observed between sperm DNA damage with the percentage of sperm abnormal morphology ($r=0.125$; $P=0.006$). In addition, negative significant correlations were found between sperm DNA damage with sperm concentration ($r=-0.168$; $P=0.000$), and motility ($r=-0.216$; $P=0.000$). We did not observe a significant correlation between semen volume with sperm DNA damage ($P>0.05$).

Conclusion: Our study demonstrated that a high level of sperm DNA damage is associated with a significant impairment in all the sperm parameters.

Keywords: DNA Fragmentation, Male Infertility, Oligozoospermia, Sperm Parameters

P-9: The Effect of Caffeine-Treated Mesenchymal Stem Cell Secretions on Sperm Nucleus Maturity, Testicular and Epididymis Weight and Testosterone Concentration in Male Rats with Type 2 Diabetes Mellitus.

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Background: Diabetes mellitus results from insulin resistance. The disease is associated with sexual disorders that result

from oxidative stress, apoptosis in the reproductive axis. In the study, the effects of secretions from mesenchymal stem cells and treated with caffeine on some reproductive aspects in male rats with diabetes were evaluated.

Materials and Methods: 32 male rats in the weight range of 190 ± 20 g were divided into healthy and diabetic control groups. Induction of diabetes by Streptozotocin /Nicotine amide method with a volume of 0.1 mM was performed once fasting at a distance of 20 minutes. Each group was divided into three subgroups: saline recipient, decaffeinated conditioned medium and caffeine treated conditioned medium (0.5 mM, n = 5). Two rats were used to extract stem cells. Intraperitoneal injection of solutions was performed three times during the treatment period (42 days) at the rate of 0.1 ml. At the end of the treatment, blood samples were taken to measure testosterone concentration and semen was sampled to assess sperm maturity; the weight of Vapudim testis was recorded after facilitation. **Results:** Diabetes significantly ($P < 0.05$) decreased testicular and epididymal weight, sperm nucleus maturation and testosterone concentration. Treatment of diabetic rats with simple or caffeinated conditioned medium significantly increased these cases ($P < 0.05$) compared with diabetic rats. The improving effects of caffeine conditioned medium in diabetic rats were more and better than the effects of simple conditioned medium. **Conclusion:** Treatment of diabetic rats with conditioned medium of simple or caffeinated mesenchymal stem cells reduced reproductive disorders in male rats.

Keywords: Caffeine, Conditional Medium, Diabetes, Reproduction, Stem Cell

P-10: Afamin Concentration Correlates Inversely with Vitamin E Levels and Antioxidant Status in Seminal Fluids of Iraqi Asthenospermic Patients

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Background: Afamin, a plasma glycoprotein, has previously been identified as an alternate carrier protein for vitamin E in extravascular fluids including seminal fluids. Afamin may be involved in antiapoptotic cellular processes caused by oxidative stress. Afamin and vitamin E work together to improve the survival of cortical neurons during apoptosis.

Materials and Methods: Semen samples were obtained from 60 fertile and 60 asthenospermic infertile men of matched age. After liquefaction of seminal fluid at room temperature, routine semen analyses were performed. The afamin was assessed by Elisa kit. Vitamin E, total antioxidant, total reactive oxygen species, and lipid peroxidation levels were measured in seminal plasma samples.

Results: Afamin concentration, total reactive oxygen species, and lipid peroxidation levels were significantly higher in subfertile men than in healthy men; while vitamin E and total antioxidant status were decreased in seminal plasma of asthenospermic specimens.

Keywords: Afamin, Asthenospermia, Lipid Peroxidation, Reactive Oxygen Species, Vitamin E

P-11: The Key Role Peroxiredoxin Enzyme in Attenuating Ferroptosis in Asthenospermic Patients

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Background: The presence of reactive oxygen species in the male reproductive tract are strongly linked to infertility. Many pathologies, including male infertility, are caused by oxidative stress. Recent data in germ cells has related oxidative stress to the activation of a novel cell death mode called ferroptosis. Peroxiredoxins (PRDXs) are antioxidant enzymes that have been linked to sperm function and male fertility. However, the impact of PRDXs on male fertility remains a key unsolved topic. As a result, we aimed to learn more about the role of these enzymes in sperm function and fertilization via study their correlation with ferroptosis.

Materials and Methods: The current study, which was conducted for one year, involved 90 fertile and 90 asthenospermic subfertile men belonging to Hilla City, Iraq. Semen samples that were obtained from the participants were utilized to assess peroxiredoxin-6 and peroxiredoxin-4 concentrations in parallel with measurement of the ferroptosis biomarkers such as glutathione peroxidase-4 and malondialdehyde concentration and reduced/oxidized protein thiol ratio.

Results and Conclusion: Peroxiredoxins activity and level are correlated with reduced/oxidized protein thiol ratio and linked inversely to ferroptosis and directly to semen quality.

Keywords: Peroxiredoxin, Ferroptosis, Glutathione Peroxidase-4, Glutathione Peroxidase-4, Reduced/Oxidized Protein thiol Ratio

P-12: Evaluation Of Genetic Variants in the Pmfbp1 Gene in Patients with Acephalic Spermatozoa Syndrome Referring to Royan Institute

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Objective: Acephalic spermatozoa syndrome is a rare form of teratozoospermia characterized by a large number of headless

and a small number of abnormal head-tail junction sperm in ejaculates which causes male infertility. This syndrome has genetic origin. PMFBP1 (polyamine modulated factor 1 binding protein 1) is a scaffold protein that localized in the mature spermatozoa head-to-tail linkage site and plays an important role in attachment of the head and tail of sperm during spermatogenesis.

Material and Methods: In the present study, 10 infertile men with acephalic spermatozoa syndrome as a case group and 10 men with normal spermogram as control group were recruited. DNA was extracted from peripheral blood and after primers designing, PCR reaction and sanger-sequencing were performed. The results of sequenced segments were analyzed by Finch TV and Blast.

Results: In the present study, 10 infertile men with acephalic spermatozoa syndrome as a case group and 10 men with normal spermogram as control group were recruited. DNA was extracted from peripheral blood and after primers designing, PCR reaction and sanger-sequencing were performed. The results of sequenced segments were analyzed by Finch TV and Blast.

Conclusion: In the present study we found no relationship between the genetic variants in exons 4 and 17 of PMFBP1 gene and acephalic spermatozoa syndrome. Since PMFBP1 is necessary for head-tail junction, it seems for a closer look, we should have more studies in other regions of this gene such as the other exons and regulatory areas.

Keyword: Male Infertility, PMFBP1 Gene, Acephalic spermatozoa syndrome

P-13: VASA Protein and Gene Expression Analysis of Human Non-Obstructive Azoospermia and Normal Cells

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Background: VASA, also known as DDX4, is a member of the DEAD-box proteins and an RNA binding protein with an ATP-dependent RNA helicase. The VASA gene expression, which is required for human germ cell development, may lead to infertility.

Materials and Methods: Immunocytochemistry (ICC) and immunohistochemistry (IMH) were used to examine the expression of VASA protein in the human testis sections of azoospermic patients, in-vitro and in-silico models. Some studies of fertile humans showed VASA expression in the basal and adluminal compartments of seminiferous tubules.

Results: However, our ICC and IMH in infertile humans showed expression of VASA in the luminal compartments of the seminiferous tubule. The immunohistochemical analysis of three human cases with different levels of non-obstructive azoospermia revealed a higher expression of VASA-positive cells. For this purpose, Enrichr and Shiny GO databases were used for pathway enrichment analysis and gene ontology. StRING and Cytoscape online evaluation were applied to predict proteins' functional and molecular interactions and then performed to recognize the master genes. According to the obtained results, the main molecular functions of the upregulated and downregulated genes include the meiotic cell cycle, RNA binding, and differentiation. StRING and Cytoscape analyses presented seven

genes, i.e., DDX5, TNP2, DDX3Y, POU5F1, SOHL2, DDX31, and SYCP3, as the hub genes involved in infertility with VASA co-function and protein-protein interaction.

Conclusion: Our results indicate that VASA is crucial in differentiating the testicular germ cells. Our findings suggest that VASA and its interacting hub proteins could help determine the pathophysiology of germ cell abnormalities and infertility.

Keywords: Azoospermia, Infertility, Seminiferous Tubules, VASA

P-14: Ameliorative Effects of Sildenafil Citrate on Sperm Biochemical Parameters during Cryopreservation in The Asthenozoospermic Men

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Background: Asthenozoospermia reduces sperm motility resulting in infertility. Reactive oxygen species (ROS), produced during cryopreservation, impair sperm function. The effect of Sildenafil Citrate (SC) supplementation on oxidative stress parameters during cryopreservation of semen samples of Asthenozoospermia men was assessed.

Materials and Methods: 30 semen samples were collected from Asthenozoospermic patients. Each sample was divided into three groups: Control (fresh), Freeze (treated with cryo-protectant), and Freeze+SC (treated with cryo-protectant+0.67μM SC solution). In each sample, the level of sperm Tumor necrosis factor alpha (TNF-α), Malondialdehyde (MDA) and sperm antioxidant enzymes, including Catalase (CAT), Glutathione (GSH), and Superoxide dismutase (SOD) were analyzed using Enzyme-linked immunosorbent assay (ELISA).

Results: Our results showed a significant reduction in the level of sperm antioxidant enzymes and also a significant increase in sperm TNF-α and MDA level in the freeze group compared to the control group (P<0.05). whereas in the Freeze+SC group, a significant increase in sperm antioxidant enzymes level and a significant decrease in sperm TNF-α and MDA level was observed compared to the freeze group (P<0.05).

Conclusion: SC supplementation in cryopreserved sperms of asthenozoospermic men, leads to a major improvement of post-thaw sperm quality and sperm biochemical parameters through reducing ROS and preventing the undesired effects of ROS on sperm function.

Keywords: Asthenozoospermia, Biochemical Parameters, Cryopreservation, Oxidative Stress, Sildenafil Citrate

P-15: Effects of Sperm Cryopreservation in Presence of Deep Eutectic Solvents on Sperm Fertility Potential and ROMO1 Gene Expression

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Background: The use of cryopreservation agents (CPAs) plays an important role in cell survival, as they prevent formation of ice crystals. Deep eutectic solvents (DES) can act as CPA. Reactive oxygen species (ROS) modulator (ROMO1) interferes with

the production of ROS. The reduction in the level of ROMO1 gene expression causes reduction in the ROS rate within cells and improves sperm features. Therefore, this study evaluates the effect of sperm cryopreservation in the presence of a specific type of DES including choline chloride and glucose (ChG) on the expression of ROMO1 gene and sperm quality.

Materials and Methods: Normozoospermia samples were collected from Mehr IVF center (Rasht, Iran). The semen samples were grouped as following: I. control (non-freeze), II. freeze with SpermFreezeTM (Fertipro Co.), and III. freeze with DES (ChG). After thawing process, the samples were evaluated for sperm quality, including assessment of chromatin integrity (toluidine blue), chromatin condensation (aniline blue), survival and acrosome reaction (triple staining), and evaluation of sperm membrane integrity (eosin-nigrosin staining). The expression of ROMO1 gene was detected by reverse transcription-quantitative PCR.

Results: The results show that there is not a significant difference in the sperm quality (such as chromatin integrity and condensation, and intact acrosome) and sperm parameters (such as viability, motility, morphology, concentration) following freezing with ChG solvent compared to group II ($P > 0.05$). Also, ROMO1 gene expression did not change in the ChG group compared to groups of I and II ($P > 0.05$).

Conclusion: The results of study indicate that ChG is able to be considered as CPA and use for sperm cryopreservation routinely.

Keywords: Deep Eutectic Solvents, Male Infertility, ROMO1, Sperm Cryopreservation

P-16: The Effect of Surface Roughness on Sperm Motility

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Background: Microfluidics for male infertility is a solution adopted in the recent years showing promising results in sperm quantification, quality analysis, manipulation and isolation. In microfluidic devices, sperm swim in a micro-confined environment comprising channel and walls that tries to mimic that of the female reproductive tract *in vivo* and thus the contact surface properties as surface roughness affect sperm motility. Despite the vital role of surface roughness at micro scales, few authors have investigated its effect on cells other than the sperm, and there is almost no research focusing on the effect of surface roughness on sperm motility.

Materials and Methods: In the present study, sperm motion against three distinct surfaces (severely etched, slightly etched and control) was investigated both numerically and experimentally.

Results: Our results showed that alteration in surface roughness parameters such as increasing average roughness (S_a), leads to the reduction of sperm velocity and in some cases, will end up in entrapment. Moreover, the results highlight that the number of trapped sperm directly depends on the roughness parameters.

Conclusion: Finally, the results suggest that whether intentional roughening or patterning of the surface or eliminating the surface roughness could be used as sperm sorting strategies where the weaker sperm are hindered or trapped in the former condition and the surface enhances sperm motion in the latter, which both are ongoing studies.

Keywords: Male Infertility, Microfluidics, Sperm Sorting, Surface Roughness

P-17: Protective Approaches of Fraxinus Excelsior Compounds on The Implantation Based on Infertility via Bioinformatics and Chemoinformatic Analysis

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Background: Recurrent implantation failure, endometrium variation, and polycystic ovary syndrome can lead to women's infertility. These occurrences might be due to genetic factors and physiological disorders. Phytochemical products are beneficial substances with various applications in everyday life. These natural products be complementary and alternative medicine in preventing and alleviating illness hallmarks synchronized with ordained medicine.

Materials and Methods: The present study marked hub genes involved in implantation-based infertility based on microarray data analysis in R programming language. Significant genes with differential expression were enriched in databases to manifest pivotal association with molecular signaling pathways. The protein-protein interactions network emphasizes the RPS6KB1 gene as a potential therapeutic target in the implantation-based infertility network. Moreover, the Iranian traditional medicine references defined that Fraxinus excelsior is prescribed for infertility in women besides other therapy. To determine the effects of F. excelsior compounds as complementary and alternative medicine and effective traditional herbal medicine, we performed Pharmacophore Modeling, QSAR, and Molecular Docking.

Results: Based on *in-silico* analysis, 21 hub genes with significant differential expression are involved in the mTOR, PI3K, IGF1R, and inflammation signaling pathways that could be associated with implantation-based infertility. Further, RPS6KB1, with the highest betweenness in the protein network, is marked as a potential therapeutic target. The chemoinformatic analysis described the effective prediction of the Fraxinus excelsior's components on the active site of RPS6KB1 protein based on QSAR, pharmacophore modeling, and molecular docking methods.

Conclusion: Consumption of the Fraxinus excelsior's practical components as a cocktail drug in complementary and alternative medicine could influence implantation-based infertility in women.

Keywords: Fraxinus Excelsior, Molecular Docking, Pharmacophore Modeling, QSAR, RPS6KB1

P-18: The Protection Effect of Naringenin on Apoptosis and Oxidative Stress Induced by Varicocele: An Animal Model

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Background: Varicocele is one of the most important causes of male infertility. Naringenin is a flavonoid with high potential of anti-oxidant and anti-apoptotic activity. The aim of present study was to investigate the effect of Naringenin to reduce the damage from varicocele in testes.

Materials and Methods: 24 male Wistar rats were divided into four groups: Control, Sham, VCL (varicocele induced rat) and N20+C (varicocele with Naringenin (20 mg/kg) treatment). After 21 days rats were scarified and the quality of tissue, oxidative stress, HSP70 expression and VEGF, BCL-2, caspase-3 and IL-6 genes expression in testes were evaluated.

Results: The expression of HSP70 in VCL group increased compared to Sham group and in N20+C group ($P<0.001$) was lower than VCL group ($P<0.05$). The MDA in VCL group increased and SOD and TAC decreased compared to Control ($P<0.01$) and there was no significant difference between N20+C and Control group. In VCL group the expression of VEGF ($P<0.05$), caspase-3 ($P<0.001$) and IL-6 ($P<0.001$) genes increased, and BCL-2 ($P<0.05$) decreased in compare to Control group. The expression of VEGF ($P<0.05$) and BCL-2 ($P<0.05$) increased, and caspase-3 ($P<0.001$) and IL-6 ($P<0.001$) genes decreased in N20+C group in compared to VCL group.

Conclusion: The expression of HSP70 in VCL group increased compared to Sham group and in N20+C group ($P<0.001$) was lower than VCL group ($P<0.05$). The MDA in VCL group increased and SOD and TAC decreased compared to Control ($P<0.01$) and there was no significant difference between N20+C and Control group. In VCL group the expression of VEGF ($P<0.05$), caspase-3 ($P<0.001$) and IL-6 ($P<0.001$) genes increased, and BCL-2 ($P<0.05$) decreased in compare to Control group. The expression of VEGF ($P<0.05$) and BCL-2 ($P<0.05$) increased, and caspase-3 ($P<0.001$) and IL-6 ($P<0.001$) genes decreased in N20+C group in compared to VCL group.

Keywords: Naringenin, Oxidative Stress, Varicocele

P-19: *In Vitro* Evaluation of Cell Viability in Mouse Sertoli Cells Exposed to Cannabinoid System Components, 2-Arachidonyl-Glycerol and Delta-9-Tetrahydrocannabinol: Friends and Foes of Male Fertility

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Background: Sertoli cells (SCs) are involved in testis biology and spermatogenesis process. The subsequent proliferation of immature SCs until puberty determines sperm production

capacity through adulthood. Endogenous cannabinoids like 2-Arachidonyl-glycerol (2-AG) play a crucial role in the maintenance of SCs homeostasis. However, previous reports mention that the exogenous cannabinoid Delta-9-Tetrahydrocannabinol (THC) might affect the male reproductive system and fertility by causing testicular cell deficiency, reduced serum testosterone concentration and affecting SCs development and functions. Nevertheless, fewer studies evaluated THC and 2-AG effects on SCs. Therefore, in this study, the role of different concentrations of THC and 2-AG on cultured SCs was investigated following 24 hours' exposure period.

Materials and Methods: SC were seeded in a 96-well plate at a density of 10000/per well. Then, varying concentrations of 2-AG (0, 1, 2, 3, 4, 5, 10 μ M) and THC (0, 0.1, 0.5, 1, 5, 10, 50 μ M) were added, and cells were incubated for 24 hours Cell viability was ascertained by MTT assay.

Results: Cellular viability increased in cells exposed to low concentrations of 2-AG and decreased in cells exposed to THC and high concentrations of 2-AG. Accordingly, the cellular viability significantly increased from $100 \pm 4.9\%$ to $133 \pm 13\%$ in cells exposed to 2 μ M 2-AG ($P<0.01$), while significantly decreased from $99 \pm 4.6\%$ to $74 \pm 3.9\%$ in cells exposed to 50 μ M THC ($P<0.01$).

Conclusion: These results showed that 2-AG in concentrations close to its physiological levels promoted cell proliferation and THC suppressed the proliferation of SCs based on a concentration-dependent manner. This study contributes to a better understanding of how cannabinoids modulate testis hemostasis by affecting SCs viability.

Keywords: 2-AG, Cell Viability, Male Fertility, Sertoli Cell, THC

P-20: Antioxidant Effects of L-Proline on Human Sperm Cells during The Freezing-Thawing Process

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Background: Sperm cryopreservation is an essential aspect of male fertility preservation. However, it is widely reported that the cryopreservation process induces detrimental impacts on sperm functions.

Materials and Methods: The study included forty normozoospermic males who received antioxidants (zinc + vitamin C). Each sample was aliquoted to 4. In aliquots 1 to 4, experimental concentrations of L-proline (0, 1, 2, 4 mmol/L) were included in the freezing medium. Sperm parameters (motility, viability, and morphology) and sperm chromatin and DNA integrity, including Toluidine blue (TB), Chromomycin A3 (CMA3), Aniline blue (AB), and sperm chromatin dispersion (SCD), were assessed. The levels of nitric oxide (NO), reactive oxygen species (ROS), total antioxidant capacity (TAC), and lipid peroxidation (LPO) were measured in the sperm freezing medium.

Results: 2 and 4 mmol/L of L-proline maintained sperm motility and viability higher than in the control groups. The MDA,

NO, and ROS levels were significantly lower in the 2 and 4 mmol/L L-proline group compared to the control group. Supplementing 2 and 4 mmol/L of L-proline improved the TAC levels compared to the control group. 4 mmol/L of L-proline significantly reduced TB and CMA3 tests compared to the control group. A notable decrease in AB and SCD levels was observed in 2 and 4 mmol/L of L-proline groups compared to the control group.

Conclusion: L-Proline inclusion in the sperm freezing medium preserves sperm quality and chromatin and DNA integrity by modulating oxidative stress and enhancing antioxidant levels.

Keywords: Antioxidant, Human Spermatozoa, L-Proline, Oxidative Stress, Sperm Cryopreservation

P-21: Sperm Medium Supplementation: Effects of L-Proline on Human Sperm Function *In Vitro*

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Background: Incubating spermatozoa is a routine procedure in infertility centers, so supplementation of sperm culture with antioxidants is recommended to preserve sperm quality.

Materials and Methods: Sperm samples were collected from the normozoospermic man who received antioxidants (vitamin E and D). Each sample was divided into four portions: increasing concentrations of L-proline (0, 1, 2, 4 mmol/L) were added to the sperm culture. Sperm parameters and chromatin integrity, including Aniline blue (AB), Toluidine blue (TB), Chromomycin A3 (CMA3), and Sperm chromatin dispersion (SCD), were evaluated. The levels of reactive oxygen species (ROS), lipid peroxidation (LPO), and total antioxidant capacity (TAC) were determined in the sperm medium. All assessments were performed after 0, 1, 4, and 24 hours of incubation.

Results: 2 and 4 mmol/L L-proline significantly improved preservation of motility and viability after 24 hours of incubation, and 2 mmol/L of L-proline maintained normal morphology compared to the control group. The level of ROS significantly diminished in the 4 mmol/L L-proline group compared to the control group. 2 and 4 mmol/L L-proline decreased MDA levels after 4 and 24 hours of incubation. 2 mmol/L of L-proline increased maintenance of TAC levels compared to other groups. Supplementing 2 and 4 mmol/L L-proline significantly improved the AB, TB, and CMA3 levels. 2 mmol/L L-proline significantly reduced SCD levels compared to the control group.

Conclusion: The inclusion of L-proline in the human sperm medium maintains sperm parameters and chromatin quality during different incubation times.

Keywords: Chromatin Integrity, Human Spermatozoa, L-Proline, Oxidative Stress, Sperm *In vitro* Incubation

P-22: Effect of Microparticle Based on Polystyrene on Histomorphometric Factors of Testicular Tissue *In Vivo*

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Background: Microplastics (MPs), as a new sustainable environmental contaminant, could cross nutritional barriers and accumulate in various body tissues. However, the histological and histomorphometric effects of polystyrene-MPs (PS-MPs) on testicular tissue and their mechanism are unclear.

Materials and Methods: In this study, 36 adult male NMRI mice were randomized into four groups of 9. The three groups received microplastics at 0.01, 0.1, and 1 mg/kg BW orally for 42 days by gavage. The control group also received 1 ml of distilled water by gavage. Tissue samples were taken 24 hours after the last treatment. Testicular tissue samples were obtained from mice, tissue procedures performed, and slides stained with H&E.

Results: The present study showed that factors GSI index, The number of Leydig cells, the height of the germinal epithelium, Seminiferous tube diameter, The mean diameter of seminiferous tubules, Sertoli cell proliferation, Spermatoocyte proliferation, Positive tubular differentiation coefficient, Positive meiosis coefficient, and Johnson index had a significant decrease ($P < 0.05$) and factors the thickness of the testicular capsule, the percentage of degenerated cells, and Intermediate connective tissue thickness significantly increased compared to the control group ($P < 0.05$).

Conclusion: Our findings showed that administration of PS-MPs to mice for 42 days caused extensive changes in the size and tissue structure of the rat testis, affecting the sperm production process, sperm quality, and ultimately fertility.

Keywords: PS-MPs, Histomorphometry, Seminiferous, Spermatoocyte

P-23: High-Dose and Chronic Nano-Micelle Curcumin Consumption Impairs Sperm Progressive Motility by Disrupting the Sperm Redox System; An Experimental Study

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Background: Curcumin and its nano-micelle form (NMC) have been shown to highly exert antioxidant effects, even leading to reductive stress in several tissues. Aside from its ability to boost antioxidant capacity, chronic and high-dose consumption of NMC is also capable of causing reductive imbalance in the testicles, oxidative DNA damage in germ and somatic cells, and consequently, a decrease in sperm count and motility. It is shown that redox balance plays an essential role in maintaining progressive sperm motility among the various mechanisms regulating sperm motility. Thus, in order to examine the pathophysiology of NMC against sperm motility, the present study aimed to investigate whether NMC targets the reductive bal-

ance or not?

Materials and Methods: Twenty-four mature male Wistar rats were divided into 4 groups, including control (received the same volume of solvent, orally) and 7.5 mg/kg, 15 mg/kg, and 30 mg/kg NCM-received groups (orally, n=6/each group). Following 48 days, the epididymal sperms were collected, and total oxidant status (TOS), antioxidant capacity (TAC), Glutathione (GSH)/Glutathione disulfide (GSSG) relative ratio, catalase, and superoxide dismutase (SOD) levels, as well as Malondialdehyde (MDA) contents, were analyzed. In addition, sperm motility was compared between groups. The results were statistically analyzed using one-way ANOVA and Bonferroni post-hoc tests. The P<0.05 was considered a statistically significant difference.

Results: The sperm motility was decreased in all NMC-received groups when compared to the control rats. The NMC, in a dose-dependent manner, decreased sperm TAC and increased TOS leading to a significant reduction in TAC/TOS ratio in all NMC-received groups (P<0.05). In contrast to decreased TAC and increased MDA content, the NMC-received rats exhibited a remarkable (P<0.05) increment in the SOD and catalase levels. This situation was more evident in 30 mg/kg NMC-received rats. Additionally, the GSH/GSSG ratio was increased in all NMC-received groups.

Conclusion: In light of reduced TAC, elevated levels of SOD and catalase, and a notable increment in GSH/GSSG ratio, we can conclude that chronic and high-dose consumption of NMC results in a reductive imbalance in the sperm. As a result of these biochemical alterations, high-dose NMC can adversely affect progressive sperm motility.

Keywords: Nano-Micelle Curcumin, Rat, Reductive Imbalance, Sperm Motility

P-24: Impaired DNA Demethylation in Germ Cells Closely Depends on Suppressed TET Proteins Expression Level in An Experimental Varicocele Condition

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Background: Failed dynamic DNA methylation and demethylation collaboratively with a genomic integrity has been shown to remarkably affect the sperm potential in maintaining male fertility in varicocele subjects. Ten-Eleven Translocation proteins (1-3) (TET 1-3) are known to activate the DNA demethylation process by oxidizing 5-methylcytosine (5mC) in the pachytene spermatocytes, round and elongated spermatids. The TET enzymes role in pluripotency, differentiation, development, and meiosis is well documented. The current study was aimed to investigate the effect of varicocele on expression levels of TETs (1-3) in the testicular tissue.

Materials and Methods: For this purpose, 12 male mature Wistar rats were divided into control and varicocele-induced groups (n=6/group). The experimental varicocele was induced by partially ligating the left renal vein. Following four months, the left testicles were dissected out and the mRNA levels of TET proteins (1-3), as well as the TET (1-3) + spermatocytes and spermatids, were evaluated by immunohistochemical staining techniques.

Results: In the varicocele-induced group the mRNA levels of TET 2, and 3 were decreased, however, the TET1 enzyme mRNA expression was increased. Moreover, the mean distributions of TET 1, 2, 3+ spermatocytes and spermatids were decreased in the varicocele-induced group.

Conclusion: Minding TET1 role in regulating pluripotency of spermatogonia, its high mRNA levels could be related to maintenance of pluripotency of the stem cells. However, developing full spermatogenesis requires all TET enzyme types. Thus, failed spermatogenesis in varicocele conditions could be due to the lack of TET proteins that leads to impaired DNA methylation/demethylation balance among all germ cell types.

Keywords: Varicocele, TET Proteins, Spermatogenesis, DNA Demethylation

P-25: Expression of Cystathionine β-Synthase and Cystathionine γ-Lyase in Infertile Men: A Case-Control Study

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Background: Transsulfuration pathway (TSP) leads to the generation of several antioxidants including glutathione (GSH) and hydrogen sulfide (H₂S). The cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) are the important enzymes responsible for GSH and H₂S production, so the loss of CBS and CSE can lead to oxidative stress. Spermatozoa are particularly vulnerable to oxidative stress. Therefore, the expression of CBS and CSE mRNA in infertile men were evaluated to clarify their possible role in male infertility.

Materials and Methods: In this case-control study, assessment of sperm concentration, motility, and morphology was performed on 20 men with varicocele (VC), 10 men with oligozoospermia (OLG), 10 men with asthenozoospermia (ASt), 10 men with teratozoospermia (TRA), 10 men with oligoasthenoteratozoospermia (OAT), and 15 fertile men. RNA was isolated from spermatozoa for analysis of CBS and CSE genes by real-time polymerase chain reaction (RT-PCR).

Results: A significant decrease in sperm concentration was observed in all infertile groups compared to fertile men (P=0.000). The sperm motility was lower in ASt (P=0.000) and OAT (P=0.000) groups compared to fertile men. The spermatozoa abnormal morphology in ASt (P=0.001), TRA (P=0.004), and OAT (P=0.000) groups were higher than in fertile group. The expression levels of CBS mRNA in all infertile groups (P=0.001) were significantly lower than in fertile men. No significant differences were detected in the expression of CSE mRNA in infertile groups compared to fertile men.

Conclusion: The down-regulation of CBS mRNA in spermatozoa is implicated in male infertility which may be associated with a disturbance of antioxidants biosynthesis including GSH and H₂S.

Keywords: Cystathionine β-Synthase, Cystathionine γ-Lyase, Male Infertility, Oxidative Stress

P-26: Sperm Protamine Deficiency in Different Groups of Infertile Men

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Background: Proper packaging of chromatin through the replacement of histones with protamines is one of the most important events that take during the process of spermiogenesis. Any defect in this process can lead to sperm DNA fragmentation which results in reduced embryo quality and pregnancy. Considering the importance of this protein, we aimed to assess sperm protamine deficiency in different populations of infertile men with a high sample size.

Materials and Methods: Semen samples of 941 infertile men and 181 fertile men were collected from Isfahan Fertility and Infertility Center. The infertile population consisted of infertile men candidates for ICSI (n=206), infertile men with no underlying disease (n=330) and varicocele (n=371), and globozoospermic men (n=34), while fertile men were healthy men with proven fertility (n=181). We assessed the extent of protamine deficiency by chromomycin A3 (CMA3) staining technique.

Results: The mean percentage of sperm protamine deficiency was significantly higher in infertile men than the fertile individuals (P<0.001). Also, we observed significant differences in sperm protamine deficiency between the fertile group with each of the infertile subgroups (P<0.001). Concurrently, we did not observe a significant difference in the mean percentage of sperm protamine deficiency values between infertile subgroups (P>0.05).

Conclusion: The results of this study clearly show that in infertile men with various causes, sperm protamine deficiency can be a major factor in infertility.

Keywords: Chromomycin A3, Male Infertility, Protamines, Spermatozoa

P-27: High-Fat Diet Consumption Further to Total Body Weight Can Negatively Affect The Sperm Quality; An Experimental Trial

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Background: The aim of this study was to compare the impact of long-term administration of a high-fat diet in two different groups, one with increased weight about 8 times to control group and one which increased weight about 3 times compared to control group.

Materials and Methods: Immature male mice were randomly divided into 2 groups as: Control (received normal diet) and experimental group (EG), which received a high-fat diet. After 22 weeks, the experimental group was subdivided into mice with body weights 3 times higher than control (EG1) and those with body weights 8 times higher than control mice (EG2) (n=6/each group). Following 22 weeks, the body weight, testis weight, gonadosomatic index (GSI), and adiposity index (AI) were evaluated and compared between groups. In addition, the

sperm count, motility, viability, and chromatin integrity as well as DNA damage were examined.

Results: The body weight and AI were significantly increased in all high-fat diet-received mice (EG1 and EG2) compared to the control group. However, the GSI was decreased in these groups. The testicular weight of the EG2 mice was higher compared to the control and EG1 groups. The EG2 group exhibited a remarkable increment in the sperm count versus control and EG1 mice. The percentage of hyperactivated sperms was significantly higher in EG1 when compared to the control and EG2 groups. In addition, the EG1 represented higher sperm viability versus other groups. The EG2 mice showed a remarkable (P<0.05) increment in the percentage of sperms with impaired chromatin condensation. All high-fat diet-received mice showed severe DNA damage versus control group (P<0.05).

Conclusion: Our findings show that despite the body weight, chronic consumption of a high-fat diet can negatively affect sperm parameters. However, the severity of damages can be altered depending on body weight ratio after chronic high-fat diet consumption.

Keywords: AI, Body Weight, GSI, High-fat Diet, Sperm Quality

P-28: Evaluation of Sperm Cryopreservation Using Deep Eutectic Solvents on Gene Expression of Spaca3 and Sperm Quality

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Background: It is possible to reduce the fertility potential of the individual, which can use the technique of sperm freezing to preserve male fertility potential. In this way, the aim of this study was to investigate the effects of sperm cryopreservation in the presence of Deep Eutectic Solvents (DESS) on the expression of sperm gene such as SPACA3 (The protein from this gene plays an important role in the binding of sperm to the oocyte.) and its relationship with semen quality and parameters.

Materials and Methods: In this study, normozoospermic samples were collected from Mehr IVF center (Rasht, Iran). The semen samples were grouped as following: I. control (non-freeze), II. freeze with SpermFreezeTM (Fertipro Co.), III. freeze with DES (EtP), and IV. freeze with DES (GlyP). After thawing process of samples, RNA extraction was performed for evaluation SPACA3 gene expression by reverse transcription-quantitative PCR. In addition, chromatin structure, chromatin condensation, viability, and acrosome reaction of sperm were determined for each group.

Results: The results show that cryopreservation using DES could preserve the semen quality (such as chromatin integrity and condensation, and intact acrosome) and sperm parameters (such as viability, motility, morphology, concentration). So that, there is not significant difference compared to I (P>0.005) and II groups (P>0.05). It has also shown that the presence of these solvents did not alter the expression quality of sperm genes such as SPACA3 in normozoospermia samples.

Conclusion: This study shows that DES solvents were introduced as high performance sperm cryopreservation.

Keywords: DES, Male Infertility, SPACA3, Sperm Freezing, Vitrification

P-29: Reduced Sperm Oxidative Stress Level in Varicoceles Rats after Alpha-lipoic Acid Therapy

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Background: Alpha-lipoic acid (ALA) is an antioxidant with important properties that is soluble in both water and lipid. Previous studies demonstrated the efficacy of ALA in several diseases related to oxidative damage through the scavenger of reactive oxygen and nitrogen species, and also restoring some antioxidants such as glutathione, vitamins C and E. Therefore, in this study, we aimed to assess the effect of ALA on the level of sperm lipid peroxidation in varicoceles rats after 2 and 4 months by oral gavage (300 mg/kg of body weight).

Materials and Methods: In this study, surgical varicocele was induced on 30 male Wistar rats. Ten of these rats received ALA for four months from the time of varicocele induction. While, for ten other rats, treatment with ALA was performed two months after varicocele induction for 2 months. The remaining 10 rats did not receive the ALA. Simultaneously, two sham (n=10) and control (n=10) groups were considered. All the rats were sacrificed and sperm was extracted from the epididymis and the level of sperm lipid peroxidation was assessed by Bodipy probe assay through flow cytometry. The percentage of sperm lipid peroxidation was compared between groups by the SPSS One-Way ANOVA procedure to analyze data.

Results: The mean of sperm lipid peroxidation significantly was higher in varicocele group than control and sham groups (P<0.05). When varicoceles rats were treated with ALA for two or four months, the mean of sperm lipid peroxidation significantly reduced compared to varicocele group that did not receive ALA (P<0.01).

Conclusion: The results of this study clearly demonstrated that induction of varicocele in rat model can result in oxidative stress, and treatment with ALA as an antioxidant soluble in water and lipid lead to a reduction of lipid peroxidation.

Keywords: Alpha-Lipoic Acid, Lipid Peroxidation, Oxidative Stress, Varicocele

P-30: Altered Methyltransferase Gene Expression, Mitochondrial Copy Number, and 4977-bp Common Deletion in Subfertile Men with Variable Sperm Parameters

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Background: Semen parameters have been found to poorly predict reproductive success yet are the most prevalent diagnostic tool for male infertility. There are few but conflicting

reports regarding the correlation of DNMT genes expression, mitochondrial DNA copy number (mtDNAcn), and deletion (mtDNA_{del}) with different sperm parameters.

Materials and Methods: Semen samples from 30 men with unknown male infertility and normal sperm parameters (experimental group I), 30 infertile patients with at least two abnormal sperm parameters (experimental group II), and 30 fertile normozoospermic men (control group) were collected. After semen analysis, total RNA and DNA were extracted. The isolated DNA was used for assessing the respective mtDNAcn and the presence of common 4977bp deletion in mtDNA by applying real-time quantitative PCR and multiplex PCR, respectively. Synthesized cDNA from total RNAs used to quantify DNMT1, DNMT3A, and DNMT3B transcripts in study groups by using real-time quantitative reverse-transcription PCR.

Results: Significantly higher proportions of mtDNAcn were found in experimental group II. DNMT1 was significantly downregulated in both experimental groups and 4977bp deletion was not detected. Progressive motility and normal morphology were significantly and negatively correlated with mtDNAcn. A significant positive correlation was detected between sperm parameters and DNMT1 mRNA levels.

Conclusion: Infertile men with different sperm parameter qualities showed elevated mtDNA content. Abnormal sperm parameters are associated with DNMT1 gene expression, that indicates the possibility of changes in some epigenetic aspects of spermatogenesis in subfertile men with different sperm parameters.

Keywords: Methyltransferase, Mitochondrial DNA Content, Sperm, Transcript

Evaluation of Human Sperm Parameters After Cryopreservation Process With Follicular Fluid Treatment in Asthenozoospermia Patients

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Objective: Follicular fluid (FF) is a liquid composed primarily of hormones, enzymes, anticoagulants, electrolytes, reactive oxygen species and antioxidants. The aim this study to describe the possible effects of human follicular fluid on the sperm parameters after cryopreservation process

Material and Methods: Samples of 20 patients with asthenozoospermia referred to Qom University Jihad Infertility Treatment Center were evaluated in three groups: before cryopreservation (control), cryopreservation, cryopreservation + Follicular fluid (FF). The Sperm parameters such as motility, morphology, and viability were assessed using WHO, Papanicolaou, and eosin-necrosin staining, respectively, to evaluate the integrity of the plasma membrane using the HOST technique, as well as to evaluate the degree of DNA fracture by the Halo sperm technique.

Results: The results showed that incubation of Follicular fluid (FF) with freezing medium of sperm (cryopreservation + Follicular fluid (FF)) increases sperm motility and viability (P <0.05). Also, a significant increase in sperm motility was achieved after incubation of Follicular fluid (FF) with sperm sample (cryopreservation + Follicular fluid (FF)) (P <0.05). The results of the present study showed that incubation of Follicular fluid (FF) with freezing medium of sperm significantly reduces the amount of DNA damage (P <0.05). Plasma membrane integrity and sperm mitochondrial membrane potential also in-

creased significantly in cryopreservation + Follicular fluid (FF) group ($P < 0.05$). However, sperm morphology did not show a significant difference ($P < 0.05$).

Conclusion: Our results show that Follicular fluid (FF) can reduce the destructive effects of the freeze-thaw process

Keyword: Asthenozoospermia, Antioxidant, Follicular fluid, Cryopreservation

Animal Biotechnology

P-31: Sex-Specific Effects of High-fat Diet on Insulin Resistance and Anxiety-Like Behavior in Wistar Rats

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Objective: Peripheral metabolic disturbances are associated with an overall range of clinical health consequences such as hyperglycemia, hyperinsulinemia and hyperlipidemia, and could contribute to the development of neuropsychiatric diseases and anxiety-like behaviors. This study investigates whether 16-week high-fat diet (HFD) consumption causes insulin resistance and anxiety-like behavior in a sex-dependent manner in rats when compared with normal chow diet (NCD) intake.

Materials and Methods: After weaning, twenty male and female rats were randomly fed HFD (55.9% fat) or NCD (10% fat) for 16-weeks. During the experiments, body weight, food intake and calorie intake were recorded weekly. In the eighth week of the study, animals' blood samples were collected through the retro-orbital sinus for measurement of glucose, insulin, and lipid profile. Also, at week 8 and 12 of the study, the glucose tolerance test was performed by intraperitoneal injection of D-glucose and collecting blood via tail vein at 0-, 30-, 60-, and 120-min intervals. Finally, anxiety-like behavior was evaluated by elevated plus maze (EPM) test in the week 16.

Result: Despite a significant decrease in the cumulative weekly food intake, the gain in body weight and calorie intake were not different in HFD compared to NCD treated rats in both sexes. Glucose tolerance test results after 8 weeks showed that HFD significantly increased the blood glucose levels at 30 and 60 min in male but not female HFD rats when compared with NCD groups. At week 12, an increase in blood glucose levels at 30 min was observed in male but not female HFD rats when compared to their respective control groups. The fasting blood glucose and serum insulin were increased significantly in male but not female HFD rats, therefore, male rats fed HFD showed a significant increase in the HOMA-IR index, an index of insulin resistance. The analysis of results related to lipid profiles showed that the LDL-cholesterol concentration was remarkably increased in HFD rats compared to control group in both sexes. Evaluating anxiety-like behaviour showed that HFD rats spent significantly less time and fewer entries in the open arm of the EPM compared to rats fed the NCD in both sexes, indicating increased anxiety-like behaviour.

Conclusions: Our results suggest that high-fat diet induces peripheral insulin resistance in male rats, however, increases anxiety-like behavior in both sexes.

Keywords: Anxiety-Like Behavior, Metabolic Deficits, Nutrition

P-32: Mitoquinone Improves Rooster Sperm Quality Indicators During Liquid Storage

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Background: Rooster sperm cellular membrane is chilling-sensitive due to high proportion of polyunsaturated fatty acids that are stalwartly vulnerable to lipid peroxidation. The present study was performed to determine the effects of different levels of mitochondria-targeted antioxidant "MitoQ" on the rooster's cooled sperm quality indicators.

Materials and Methods: Semen samples were collected from 10 roosters, diluted in Lake extender, assigned into 5 groups according to the MitoQ concentrations (0, 1, 10, 100, 1000 nM MitoQ) and stored at 5°C up to 48 hours. Total motility, progressive motility, viability and membrane integrity were evaluated at 0, 24 and 48 hours of cooling storage periods.

Results: Our findings depicted that extender supplementation with MitoQ had no effect ($P > 0.05$) on cooled semen samples quality parameters at time 0, while at times 24 and 48 hours storage, samples contained 100 nM MitoQ presented higher ($P \leq 0.05$) total motility, progressive motility, viability and membrane integrity compared to the other groups.

Conclusion: In conclusion, supplementation of Lake Extender with 100 nM MitoQ could be a helpful strategy to conserve cooled semen quality in rooster.

Keywords: Cooled Semen, Mitochondria, Mitoquinone, Rooster, Sperm Quality

P-33: Mouse Spermatogonial Cells Differentiation in Decellularized Human Placenta Matrix

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Background: Natural scaffold production from extracellular matrix (ECM) components is a promising strategy for *in vitro* spermatogenesis.

Materials and Methods: The human placenta was decellularized and the neonatal mouse spermatogonial cells (SCs) were cultured three-dimensionally (3D) on decellularized placenta. The expression of differentiating markers (Sypc3, Acrosin and Protamine 1) was determined after 3 weeks of culture.

Results: The level of Sypc3 and Prm1 genes expression was up-regulated in 2D and 3D culture and the highest level was for cells in the 3D group. There was an increment in Acrosin gene expression in the 3D culture group. The percentage of Prm1 positive cells in the 3D group (36.4%) was significantly higher than in the 2D group (10.96%) after 35 days of culture.

Conclusion: We suggested the placental scaffold as a promis-

ing bio-scaffold that has high potential for mouse *in vitro* spermatogenesis.

Keywords: Decellularization, Scaffold, Placenta, Spermatogenesis, Differentiation

P-34: Exercise Training Ameliorates The Sperm Motility by Boosting β -Defensin Expression and Antioxidant Status of Epididymis after Nandrolone Consumption in Rats

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Background: Nandrolone decanoate (ND) administration has been reported to significantly induce germ cell apoptosis, suppress testicular endocrine status, and decrease the sperm morphology and motility ratio. On the other hand, moderate-intensity exercise training protocol (MICT) is shown to boost anti-apoptotic proteins expression, systemic and testicular endocrine statuses, and sperm quality. The current study was aimed to investigate the protective effect of MICT on ND-induced detrimental effects on sperm parameters by exploring possible changes in β -defensin (1-4) expression and testicular antioxidant status.

Materials and Methods: For this purpose, 24 mature male Wistar rats were divided into sedentary control, MICT (E-sole), ND-administrated (10 mg/kg, 3 times/week, for 40 days, ip), and E+ND-induced groups. Following 40 days, the testicular oxLDL and carbonyl groups (CG, markers for lipid and protein peroxidation), β -defensins (1-4) expression levels, and sperm count, motility, and DNA integrity were examined and compared between groups.

Results: Observations revealed that ND could significantly ($P<0.05$) increase testicular oxLDL and CG versus control rats. Moreover, it could remarkably ($P<0.05$) suppress the epididymal β -defensins (1-4) expression and decrease the sperm count, motility, and DNA integrity. However, >>exercise training could diminish testicular oxLDL, CG, and boost β -defensins (1-4) expression, and ameliorate sperm count, motility, and DNA integrity ($P<0.05$).

Conclusion: Minding that testicular antioxidant status cooperates with β -defensins to protect sperm motility, nuclear maturation, and fertilization potential, we can conclude that exercise could improve sperm motility and DNA integrity by inhibiting oxidants-induced lipid and protein peroxidation in testicles and β -defensins (1-4) expression level in the epididymis.

Keywords: β -defensin, Exercise Training, Nandrolone Decanoate, Sperm Parameters

P-35: Protective Effects of Equisetum Arvense Methanolic Extract on Epididymal Structure in Diabetic Male Mice

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Background: Diabetes mellitus is a major metabolic disorder that has adverse effects on male reproductive system. This dis-

ease damages the epididymal tissue and leads to male infertility. Recently, herbs have been a popular source of medicine for prevention of diabetes. Thus, the current study aimed to evaluate the protective effects of Equisetum arvense methanolic extract (EE) on diabetes-induced detrimental effects on histological structure of epididymis and cauda epididymal sperm.

Materials and Methods: Twenty four mature male mice were divided equally in four groups: control-sham, diabetic (induced by streptozotocin, 50 mg/kg for five days), diabetic+EE (250 mg/kg, orally, daily gavage) and diabetic+EE (500 mg/kg, orally, daily gavage). After 45 days, the animals were euthanized. Then, cauda epididymis was analyzed for histopathological alteration. Also, epididymal sperm were collected to evaluate the sperm characteristics.

Results: Epithelium thickness and diameter of the cauda epididymal tubule and lumen were decreased remarkably ($P<0.05$) in the diabetic group compared with the control-sham group, whereas the EE-treatment decreased significantly ($P<0.05$) the adverse effects of diabetes. The percentage of sperm with abnormal morphology was significantly ($P<0.05$) lower in treated groups than in the diabetic group. Although the sperm count was decreased in diabetic group, it was not statistically significant ($P>0.05$) with those in diabetic+EE. Sperm motility and viability were increased significantly ($P<0.05$) in the treated groups compared with the diabetic group.

Conclusion: The EE inhibited diabetes-induced damages on epididymal tissue and sperm quality, which may have been associated with hypoglycemic and antioxidative activities in this plant.

Keywords: Diabetes, Equisetum Arvense, Epididymis, Sperm

P-36: The Effects of Acrylamide on Fertilization Potential and Sperm Parameters in Mice

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Background: Today, with changing of lifestyle, the tendency to use packaged food, street food and fast food has been increased. Many of these foods contain Acrylamide. One of the most important side effects of Acrylamide is toxicity in the reproductive system. In this study, the long-term effects of Acrylamide on *in vitro* Fertilization (IVF) and sperm parameters were investigated.

Materials and Methods: Sixteen male mice (age: 6-8 weeks) were divided into two groups. The control group has received normal saline (0.2 ml/day) and treated group received Acrylamide (50 mg/kg, 0.2 ml/day) orally for 45 days. At the end of the term, sperms were extracted from the tail of the epididymis and fertilization process was performed in HTF + 4mgBSA medium and embryonic growth stages were studied during 120 hours of incubation. Also, sperm count, motility, viability, sperm chromatin quality, and DNA integrity were evaluated. Two proportion method by Minitab software and T-test by SPSS software were used for statistical analysis ($P<0.05$).

Results: There was a significant decrease in the percentage of fertilization, 2cell-embryos and blastocysts resulting from *in vitro* fertilization and a significant increase in the number of arrested embryos in treated group by Acrylamide compared with the control group ($P<0.05$). Also in treated group by Acrylamide, sperm count, motility, and viability of sperms, the num-

ber of sperms with DNA damage, abnormal morphology, and with immature nucleus showed a significant increase compared with the control group ($P < 0.05$).

Conclusion: The study showed long-term oral administration of Acrylamide has negative effects on fertility potential and sperm parameters.

Keywords: Acrylamide, Fertilization Potential, Mice, Nucleus Maturation, Sperm Quality

P-37: Vitamin D Deficiency Modulator Regulation of Steroidogenesis in Male Mouse

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Background: The action of vitamin D (VitD) on male reproductive performance has been controversial mainly because it is difficult to distinguish between what is mediated by calcium/phosphorus homeostasis and what is due to genomic and non-genomic effects. In this regard, rodent models in which VitD deficiency (VDD) was artificially induced without creating an imbalance in calcium and phosphorus homeostasis showed that sperm structure and function were affected and that the intensity of the alteration followed the severity and duration of the VDD. To show the genomic and non-genomic effect of VDD on spermatogenesis.

Materials and Methods: Testicular extracts from mouse models of VDD with different degrees of severity, obtained either through the use of a vitamin D-deficient diet or paricalcitol injections, were analyzed by QRT-PCR to assess the steady-state RNA levels of the major steroidogenic players, including CYP24A, CYP19A1, 17 β HSDH1, 3 β HSDV1, as well as estradiol, testosterone, LH and FSH levels. In addition, testicular histomorphometric and sperm structural and functional parameters analyses were carried out.

Results: we show that the accumulation of transcripts of each monitored steroidogenic actor decreases linearly with the severity of the VDD. We also show that already in situations of low and medium VDD, the estradiol and testosterone content of testicular extracts is strongly decreased.

Conclusion: VDD exerts potent effects on testicular steroidogenic support of spermatogenesis, independently of its well-known calcitropic action. Therefore, VDD-mediated reduction of steroidogenesis is clearly an aspect of the loss of testicular spermatogenic efficiency in vitamin D deficiency, a very common situation worldwide.

Keywords: Sperm, Spermatogenesis, Vitamin D

P-38: Protective Role of Aloe Vera on Testis Tissue in Em-

bryos of Diabetic Mothers

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Background: Negative effects of diabetes have been shown on the male reproductive system, broadly. Testis plays a vital role in male reproductive system. One of the possible disorders of maternal diabetes is the effect on male reproductive system. The use of medicinal herbs is recommended in reducing complications of during pregnancy. The Aloe Vera extract causes a significant decrease in blood glucose levels. This study was carried out to investigate the effects of the Aloe Vera extract (AVE) on testis histomorphometry in rat embryo from diabetic mothers.

Materials and Methods: Forty adult female rats were divided into four groups: control group (without treatment), diabetic group (50 mg/kg of streptozotocin /IP), AVE group (400 mg/kg/orally) and diabetic+AVE group (400 mg/kg/orally). All four groups were being pregnant via natural mating. After in gestational days 18th and 20th, mother rats were anesthetized, and embryos have been transferred into 10% buffered formalin. After applying histological techniques, different histological parameters have been evaluated.

Results: The results of testis tissue showed that numbers of spermatogonia, Sertoli and Leydig cells and diameter of seminiferous tubules were significantly decreased in 18- and 20-day embryos of diabetic group with comparison to control group ($P < 0.05$). On the contrary, Aloe Vera extract resulted in a significant increase in number of spermatogonia, Sertoli and Leydig cells and seminiferous tubule diameter compared with diabetic group in 18 and 20 day embryos ($P < 0.05$).

Conclusion: This study showed that Aloe vera extract could have positive and protective effects on testis tissue if used as a hypoglycemic agent in diabetes.

Keywords: Aloe Vera, Diabetic Mothers, Histomorphometry, Testis, Rat

P-39: Effect of Nano-Micelle Curcumin on Autophagy in Testicular Tissue of Wistar Rats

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Background: Curcumin exerts beneficial therapeutic effects. However, chronic and high dose consumption of curcumin is able to exert deleterious side effects on male reproductive system. The curcumin-related effects have been improved by converting the curcumin to nano-micelle form named as nano-micelle curcumin, which is able to alter its beneficial or deleterious effects. In the current study, Wistar male rats were used to uncover the possible dose-dependent effect of nano-micelle curcumin on spermatogenesis, intracellular metabolomics, and autophagy process.

Materials and Methods: For this purpose, 24 male rats ($200 \pm$

20 g) were randomly divided into control, 7.5 mg/kg, 15 mg/kg, and 30 mg/kg nano-micelle curcumin-received (orally) groups. Following 48 days from nano-micelle curcumin administration, the histomorphometric, histochemical (intracytoplasmic carbohydrate and lipid accumulation in germ and somatic cells), and the expression levels of Atg7, Beclin-1, p62, LC3-I in the testicular tissue have been evaluated.

Results: Observations revealed that nano-micelle curcumin, in a dose dependent manner, could decrease the Leydig and Sertoli cells number, germinal epithelium's height, tubular diameter, and the percentages of seminiferous tubules with positive tubular differentiation (TDI) and spermiogenesis (SPI) compared to the control group. Furthermore, the apoptotic Leydig cells number was increased ($P<0.05$) and the number of Leydig cells with intracytoplasmic carbohydrate accumulation and steroidogenic activity were dose-dependently decreased in the nano-micelle curcumin-received groups ($P<0.05$). In comparison with control rats, the total number of Sertoli cells containing intracytoplasmic carbohydrate was decreased and the number of apoptotic Sertoli cells as well as cells with high accumulation of lipid droplets were increased in the nano-micelle curcumin-received groups ($P<0.05$). Finally, the nano-micelle curcumin-received groups exhibited a remarkable ($P<0.05$) increment in the expression levels of autophagy-related genes.

Conclusion: Our data shows that overdose consumption of the nano-micelle curcumin (ignoring its appropriate pharmacological dose) is able to up-regulate the autophagy-related genes expression by altering the metabolic interactions in the testicular tissue.

Keywords: Autophagy, Nano-Micelle Curcumin, Rat, Testicular Tissue,

P-40: Effect of Lactobacillus Reuteri on Heat Stress-induced Effect on HSP70-2a and HSP90 Heat Shock Proteins Expression in Rat Testicles

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Background: Heat stress has been shown to repress spermatogenesis and spermiogenesis processes, resulting in fertility problems in male subjects. The probiotics are shown to promote the spermatogenesis development, endocrine potential of the testicles, and semen quality by using animal models. Heat shock proteins are key responders proteins in maintaining the homeostasis of the molecular mechanisms in the germ and somatic cells of testicular tissue in heat stress circumstances. Moreover, these proteins are known to protect sperm DNA integrity by regulating chromatin-histone exchange process. Therefore, the current study was performed to examine the effect of lactobacillus reuteri probiotic (LRP) on heat shock proteins Hsp70-2a and Hsp90 expression levels in testicular tissue and to explore chromatin condensation and DNA integrity of sperm cells after heat stress condition.

Materials and Methods: For this purpose, 30 mature male Wistar rats were divided into control (n=6) and heat stress-induced (n=24) groups. Then, the heat stress group subdivided

into 37°C and 40°C sole (n=6/ each group) as well as lactobacillus reuteri probiotic-treated (1mg/rat LRP+37°C and 1 mg/rat LRP+40°C, n=6/ each group) groups. The heat stress was induced by using water bath (20 min/day, for 48 days) and the LRP was administrated orally for 48 days. Thereafter, the animals were euthanized, testicles were dissected out and the Hsp70-2a+ and hsp90+ immunoreactivities in spermatids were analyzed. Moreover, the percentage of sperms with normal chromatin condensation and DNA integrity were analyzed. One-way ANOVA and bonferroni post-hoc tests were used for statistical analyses.

Results: Observations revealed that as the temperature increased, the mean distribution of Hsp70-2a+ and Hsp90+ spermatids were decreased. However, the LRP-treated groups showed a significant ($P<0.05$) increment in Hsp70-2a+ and Hsp90+ spermatids per seminiferous tubules. Finally, the percentages of sperms with normal chromatin condensation and DNA integrity were increased in the LRP-treated groups. This situation was more evident in 37°C+LRP group.

Conclusion: Heat stress is able to detrimentally affect the chromatin-histone exchange process by suppressing Hsp70-2a and Hsp90 expression at spermatids, which in turn is able to result in severe DNA damage.

Keywords: DNA Damage, Heat Shock Proteins, Heat Stress, Probiotic

P-41: The Study of Taraxacum Officinale (Dandelion) Flower Extract Effect in Infertility Induced by Cadmium Chloride in Wistar Rats on Testicular Tissue

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Background: Taraxacum officinale extract (TOE) has bioactive phytochemicals effects which can protect DNA against damage of reactive oxygen species (ROS).

Materials and Methods: 40 male Wistar rats were randomly allocated into eight groups: the control, Cd, TOE extract (100, 200, 400 mg/kg/body weight) and chloride cadmium-TOE (100, 200, 400 mg/kg/body weight). Rats were intraperitoneally injected with TOE extract once daily for 28 days. Serum levels of MDA and SOD were measured. Motility, viability and total count of sperm, were measured. Testis histopathology for investigation of Johnson score were done.

Results: Result shows that number of spermatogonia, spermatocytes, spermatids have been significantly reduced in Chloride cadmium group compared to those in control group ($P<0.05$). However, compared to the chloride cadmium group, the chloride cadmium- TOE extract group showed increase in the number of the cell types. Motility value ($P<0.05$) and dead sperm rate ($P<0.001$) was respectively increased and decreased in dandelion treatment group compared with chloride cadmium group. Serum level of SOD and MDA was respectively increased and decreased in chloride cadmium- TOE at 400 mg/kg group compared with another groups ($P<0.01$).

Conclusion: Our results indicate that *T.officinalis* extract had protective effect on testis probably by scavenging free radicals and so reducing toxicity caused due to oxidative stress.

Keywords: Cadmium Chloride, Dandelion, Infertility, Testicular Tissue, Rat

P-42: Hyperketonemia and Serum Levels of Oestrogen and Progesterone at First Insemination in Holstein Cows

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Background: Negative energy balance in the dairy cows has various reasons such as metabolic stress, lower body condition score, blood glucose is reduced and hormonal changes; especially glucagon levels are increased. Increased glucagon levels lead to glycogenolysis, lipolysis, beta-oxidation of fats, gluconeogenesis, and ketogenesis (such as acetic acid, acetone, and beta-hydroxybutyrate (BHBA)) in the liver. In addition, negative energy balance is associated with attenuation in the concentration of LH surge and reduces the chance of pregnancy at first insemination. The aim of this study was to evaluate of hyperketonemia on the serum levels of oestrogen, progesterone and pregnancy rate at first insemination.

Materials and Methods: This study included a total of 70 Holstein average 3 multiparous cows in one of the industrial dairy farms in Isfahan province, Iran. 10 mL of jugular vein blood were collected at each sampling time two times: first, 7–14 days of postpartum to measure the BHBA, and second; at first insemination, to measure estrogen and progesterone hormone levels. All statistical analyses were carried out by IBM SPSS Statistics version 20 and the statistical difference was presented by $P < 0.05$.

Results: In this research indicated that cows with BHBA levels greater than 600 $\mu\text{mol/L}$ had lower oestrogen levels ($P=0.03$) and higher progesterone levels ($P=0.07$) at the first time of insemination. Pregnant cows also had significantly lower serum BHBA ($P=0.03$) and higher oestrogen levels ($P=0.009$) than nonpregnant cows at first insemination.

Conclusion: The present study confirms the negative effects of the higher ketone bodies on oestrogen level and Pregnancy rates at the first insemination in hyperketonic cows.

Keywords: First Insemination, Holstein Cows, Hyperketonemia, Oestrogen, Progesterone

P-43: The Effect of Achillea Millefolium Hydroalcoholic Extract on Testicular Tissue of Male Wistar Rats

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Background: *Achillea millefolium* (AM) has been consumed

in folk medicine. Studies have shown this plant can alleviate complications such as inflammation, hemorrhages, and pain. In addition, one of the organs vulnerable to inflammation is the male reproductive system. Therefore, the present study aimed to evaluate the efficacy of the hydroalcoholic extract of the plant after chronic exposure.

Materials and Methods: In this research, twenty adult male Wistar rats were randomly divided into control groups and normal rats received a hydroalcoholic extract of AM (a dose of 300 and 500 mg/kg) by intraperitoneal injection for 21 days. At the end of this study, blood was collected in tubes containing anti-coagulants to determine testosterone levels, and testicular tissue was sent to the laboratory for histopathological examination.

Results: The use of the hydroalcoholic extract of AM at a dose of 300 mg/kg has shown that this dose of AM extracts caused a significant increase in testosterone, LH, and FSH hormones levels and enhancement of testicular capsule thickness, Leydig cells population, and intensity of interstitial tissue. In addition, Tubular Differentiation Index (TDI) and Spermatogenesis Index (SPI) were significantly increased in the testis of the AM treatment group compared to the control group ($P=0.03$) while a dose of 500 mg/kg could not increase these hormones levels and had no significant effect on testicular tissue.

Conclusion: According to the findings of this study, AM at a dose of 300 mg/kg increased testosterone, LH, and FSH hormones levels and the positive effects on testicular tissue.

Keywords: *Achillea Millefolium*, FSH, LH, Testicular Tissue, Testosterone

P-44: Chronic and High-dose Consumption of Nano-Micelle Curcumin Negatively Affects Lactate and Glucose Transportation Process in Rats Testicular Tissue

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Background: Chronic and high dose consumption of nano-micelle curcumin (NMC) has been shown to negatively affect the testicular antioxidant status, spermatogenesis development, sperm quality, and pre-implantation embryo development. The current study tried to uncover a new aspect of NMC-induced impact in the testicular tissue by more focusing on lactate and glucose transportation in Sertoli-germ cell network.

Materials and Methods: For this purpose, 24 mature male Wistar rats were divided into control (normal saline, $n=6$) and NCM-received groups ($n=18$). The NCM group subdivided into 7.5 mg/kg, 15 mg/kg, and 30 mg/kg NCM-received rats ($n=6$ rat/group, orally). Following 48 days, the testicles were dissected out, the mRNA levels of monocarboxylate transporter (MCT-4, a lactate transporter on Sertoli cells), and glucose transporters 1 and 2 (GLUTs 1, 2) as well as GLUT+ cells (Sertoli and germ) distribution in the seminiferous tubules were analyzed and compared between groups. The data were statistically analyzed by one way ANOVA and continued by bonferroni test.

Results: Observations showed that NMC, mainly at higher doses, could significantly ($P < 0.05$) decrease MCT-4, GLUT-1, and GLUT-2 mRNA levels in the testicular tissue compared to the control rats. Moreover, the 15 mg/kg and 30 mg/kg NCM-received rats revealed a remarkable ($P < 0.05$) reduction in the GLUT-1+ and GLUT-3+ germ and Sertoli cells per seminiferous tubules with the same criteria.

Conclusion: Our data showed that chronic and high-dose consumption of NMC is able to significantly affect the glucose and lactate transportation in the testicular tissue by suppressing the expression levels of lactate and glucose transporters.

Keywords: GLUT, MCT-4, Nano-Micelle Curcumin, Rat, Spermatogenesis

P-45: Comparing the effect of broccoli extract, broccoli in combination with soy isoflavones and diphereline on endometrial implants and antioxidant markers in female rat model of endometriosis

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Background: Endometriosis is a gynecological disorder characterized by the growth of endometrial tissue outside the uterine cavity that is associated with chronic pelvic pain, subfertility and an increased risk of ovarian cancer. The purpose of the study was to investigate the effect of broccoli extract (BE) alone and in combination with soy isoflavones on endometrial implants in female rat.

Materials and Methods: In this study, endometriosis was induced surgically in 40 mature female rats. The rats were divided into 5 groups that were treated by oral gavage for 6 weeks with 0.5 ml of saline 0.9%/day (control group), BE (3000 mg/kg/day), soy isoflavones (50 mg/kg/day), BE/soy isoflavones (BE 3000 mg/kg/day + soy isoflavones 50 mg/kg/day) and diphereline as standard medications (3 mg/kg) intramuscularly. At the end of treatments, the volume and histopathology of the endometrial implants were compared among the 5 groups.

Results: The levels of antioxidant markers including Superoxide dismutase (SOD) and Malondialdehyde (MDA) in serum were also evaluated between the groups. The volume of the implants with treatment by BE/soy isoflavones were not significantly different from diphereline. The histopathological grade of endometrial implants in BE/soy isoflavones and diphereline group were significantly decreased comparing to the control group. There was no significant change in the histopathological score in rats treated with BE/soy isoflavones compared to diphereline group.

Conclusion: BE in combination with soy isoflavones decreased the growth and histopathologic grades of auto-transplanted endometrial implants. This herbal product may have the potential to be used as an alternative medication for the treatment of endometriosis.

Keywords: Broccoli, Diphereline Endometriosis, Soy Isoflavones

P-46: Effects of Omega-3 Fatty Acids and Vitamin E on mRNA Levels of Desaturase and Elongase in Ovarian and

Adipose Tissue of Laying Hens

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Background: Positive effects observed following the consumption of omega-3 fatty acids (FA) have been reported. However, little information exists about mechanisms and the genes related to FA metabolism in ovary when FA supplemented in diet. Our aim was to investigate the effects of omega-3 FA and vitamin E on the expression of genes involved in FA metabolism in laying hen as a model in ovarian studies.

Materials and Methods: Sixty-eight 29-week-old Bovans White hens were divided into four groups: basal diet+sunflower oil (C); C+alpha-tocopherol (E); basal diet+fish oil (n-3), and n-3+alpha tocopherol (n-3+E). After feeding animals for six weeks, ovarian and adipose tissue (AT) samples were collected. Gas chromatography was utilized to determine the FA profiles, and desaturase index (monounsaturated FA:saturated FA ratio) was calculated. qPCR was employed to evaluate the expression levels of desaturase and elongase.

Results: The desaturase index in AT and ovary was not affected by experimental diets. Fatty acid desaturase (FADS1) expression significantly (P=0.03) increased in the group receiving fish oil as omega-3 source. Moreover, mRAN abundance of FADS3 was higher in the n-3+E group than other experimental groups (P<0.01). Expression levels of elongate family such as ELOVL2 and ELOVL4, ELOVL5 in ovary were unaltered by treatments. Dietary inclusion of FA and vitamin E did not affect mRNA levels of genes in AT.

Conclusion: Omega-3 FA and vitamin E alter the expression level of genes involved in FA desaturation in the ovarian of laying hens, which warrants further studies.

Keywords: Desaturase, Elongase, Laying Hen Ovary, Omega 3, Vitamin E

P-47: Growth Factor Improves *In Vitro* Maturation of Domestic Cat Oocytes

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Background: Domestic cat is the only non-endangered species among felids and could serve as an oocyte donor to preserve the endangered feline species. Nevertheless, *in vitro* maturation (IVM) is suboptimal in cats, despite major progress over recent years. In this study, we have assessed the effects of growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) combination and insulin-like growth factor 1 (IGF1) and Epidermal growth factor (EGF) combination on IVM of cat oocytes.

Materials and Methods: Following spay surgery, cumulus oophorus complexes (COCs) were harvested from ovaries and randomly allocated to four groups including control (basic medium), IE (basic medium with 10 µg/ml IGF1 and 10 µg/ml EGF), GB (basic medium with 200 ng/ml GDF9 and 200 ng/ml BMP15) and IE+GB (basic medium with 10 µg/ml IGF1 and 10 µg/ml EGF plus 200 ng/ml GDF9 and 200 ng/ml BMP15) for IVM at 38 °C for 24hours. Maturation and degeneration of oocytes were evaluated by stereomicroscope.

Results: The proportion of matured oocytes in IE (13/73: 17.11%), GB (9/65: 13.85%), and IE+GB (8/58: 13.79%) groups was greater than that in the control group (2/73: 2.74%; $P<0.05$). IGF1 and EGF combination positively impacted feline oocytes IVM ($P=0.03$); however, GDF9 and BMP15 combination did not influence IVM rate ($P>0.05$). Degeneration rate did not differ among the control (16/73: 21.92%), IE (12/73: 16.44%), GB (16/65: 24.62%) and IE+GB (11/58: 18.97%) groups ($P>0.05$).

Conclusion: In conclusion, the present study showed incorporation of IGF1 and EGF into IVM medium could enhance the maturation rate of feline oocytes.

Keywords: Domestic Cat, EGF, IGF1, IVM, TGF β

P-48: Carbon Quantum Dots Are Able to Rebalance Carbohydrate and Lipid Foci Storage in Germ and Somatic Cells; An Experimental Trial

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Background: Carbon quantum dots (CQDs) are nanoparticles (less than 10 nm in size) of carbon. Indeed, CQDs are widely used in various industrial and biological fields, including bio-imaging, drug delivery, catalysis, optronics, and fingerprint recovery. The effects of CQDs on spermatogenesis are not fully explored. The main energy sources for developing germ cells are carbohydrates and fatty acids, and any alteration in their concentration or intracellular storage will have adverse effects on germ cell development. The current study was considered to uncover the possible dose-dependent effect of CQDs on carbohydrate and lipid foci storage in germ and somatic cells of rats testicular tissue.

Materials and Methods: For this purpose, 24 mature male Wistar rats were divided into control (received the same volume of solvent), 5.2 mg/kg, 10 mg/kg, and 40 mg/kg CQDs (intraperitoneally, n=6/each group) groups. Following 5 weeks, the rats were euthanized and the testicles were dissected. The periodic acid Schiff (PAS) and Sudan black-B staining techniques were used to examine the intracytoplasmic carbohydrate and lipid foci storage in the germ and Sertoli cells per one seminiferous tubule. One-way ANOVA and bonferroni post-hoc tests were used for statistical analyses.

Results: Observation revealed that CQDs, dose-dependently, could reduce the germ and Sertoli cells carbohydrate storage. Accordingly, the mean distributions of PAS⁺ spermatogonial and Sertoli cells were decreased ($P<0.05$) in CQDs-received groups. This situation was more evident in 40 mg/kg CQDs-received rats. In addition, the mean distributions of Sertoli cells with intensive intracytoplasmic lipid foci storage were increased in 10 mg/kg, and 40 mg/kg CQDs-received groups versus control and 5.2 mg/kg CQDs-received rats ($P<0.05$).

Conclusion: CQDs are capable of adversely affecting carbohydrate and lipid storage in germ and Sertoli cells, which can negatively impact metabolic interactions in these cells. An increased lipid storage in Sertoli cells is also associated with increased cellular damage in seminiferous tubules.

Keywords: Carbon Quantum Dots, Carbohydrate, Lipid, Spermatogenesis

P-49: Assessment of Apoptotic Related miRNA in Asthenozoospermic and Normozoospermic Bull Semen

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Background: We aimed to evaluate the expression of 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 eosin staining for viability. Three miRNAs related to apoptosis were evaluated with qRT-PCR.

Results: Results revealed that the percentage of progressive motility and viability were significantly higher in NS group ($P<0.05$). The curvilinear velocity (VCL), straight-line velocity (VSL), and average path velocity (VAP), were significantly higher in NS group. There were significant differences between the two groups of study regarding miRNA-related expression (miR-296-3p, miR 455-3p, and miR-345-3p).

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, some miRNA such as miR-296-3p and miR-345-3p were noteworthy correlation with apoptosis status.

Keywords: Apoptosis, Asthenozoospermic, miRNA, Normozoospermic

P-50: The Role of Rosmarinic Acid Loaded to Serum-derived Exosome as A Factor to Resucing Inflammation in Mice with Induced Endometritis

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Background: The aim of this study was to evaluate the role of Rosmarinic acid loaded to exosome to reducing uterine inflammation in mice with induced endometritis with LPS.

Materials and Methods: For this purpose, Balb-C mice serum was first obtained and then exosome derived from serum by ultracentrifugation technique. Then, using sonication method, 5 and 10 mg doses of Rosmarinic acid were loaded into the exosomes. After evaluating the quality and amount of drug loading system, in 6 experimental groups, the mice induced endometritis with LPS. They were treated (control-inflammation-Rosmarinic acid-exosome-drug + exosome-drug loaded in the exosome). Then, the incidence of inflammation and the effectiveness of the drug in uterine tissue were evaluated by histological analysis, and the expression of inflammatory genes such as, NLRP3, IL-18 and TNF- α was measured by qPCR.

Results: The results showed that pathological lesions, particularly inflammatory cell infiltration and expression of inflammation related genes attenuated in all treatments, which were more remarkable in the higher dose groups (10 mg/kg) ($P < 0.05$). Importantly, the best improvement effect was found in the RLE10-group. In comparison, the lowest treated impact was observed in the Exo5 and R5 groups.

Conclusion: As a result, in the high doses of Rosmarinic acid groups with exosome and loading of this drug with exosome, they could have a better effect in reducing inflammation in mice with endometritis.

Keywords: Drug-loading, Inflammation, qPCR, Rosmarinic Acid, Sonication

P-51: The Protective Role of Sambucus Nigra Extract in Spermatogenesis

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Background: Sambucus nigra, commonly known as elderberry (EB), is a member of the family of berries that is rich in anti-oxidative compounds and has been noticed for a few years for its potential beneficial effects on the male reproductive system.

Materials and Methods: We divided 30 mice into control, hyperthermia, and hyperthermia receiving EB diet (duration = 35 days groups). 43 °C water bath for 30 min was used to induce hyperthermia. Thereafter, the mice were sacrificed, and sperm parameters analysis was conducted on their sperm samples. We also performed immunohistochemistry (against caspase-3 and TNF- α) histopathology and serum hormone level (for testosterone, LH, and FSH) experimentations.

Results: Our findings revealed that an EB-containing diet can significantly improve both stereological and sperm parameters, such as the number of Leydig cells, spermatogonia, primary spermatocyte, and round spermatid. We have also found that the level of testosterone was improved in the serum of the EB receiving group. Additionally, the level of expression of caspase-3 and TNF- α was significantly dropped in the EB receiving

group compared to the controls.

Conclusion: An EB-containing diet can be recognized as an alternative therapeutic option for improving the spermatogenesis of azoospermic mice who were exposed to hyperthermia.

Keywords: Elderberry, Hyperthermia, Spermatogenesis, Mice, Sambucus Nigra

P-52: Effect of Mito-Tempo on Quality Parameters of Buck Sperm during Semen Cooling Process

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Background: Utilization of antioxidants in semen diluent, protects cells from oxidative damages. Supplementation of cooling medium with mitochondria targeted antioxidants could be a beneficial way to improve semen quality during chilling process. The current study was aimed to assess the influence of using Mito-TEMPO as a mitochondrial targeted antioxidant on quality parameters of buck semen during cooling processes.

Materials and Methods: In this experiment, 5 Saanen bucks were used and semen samples were collected twice a week. After semen collection and primary evaluation, diluted semen samples were added to extenders containing 0, 1, 10, 100, 1000 μ M Mito-TEMPO. The prepared samples were stored at 5 °C up to 48 hours. Chilled sperm viability, mitochondrial membrane potential and membrane functionality were assessed during 0, 24 and 48 hours.

Results: In results, at time 0, no difference was observed among groups but at 24 and 48 h storage, higher ($P \leq 0.05$) sperm mitochondrial activity, membrane functionality and viability were observed in 10 and 100 μ M Mito-TEMPO groups than the other groups.

Conclusion: Therefore, supplementation of cooling medium with Mito-TEMPO (10 and 100 μ M) could be an efficient method to improve the quality of buck's semen during storage period.

Keywords: Buck, Mito-TEMPO, Sperm Chilling

P-53: Effect of L-carnitine in Freezing Extender on Motility, Viability and Lipid Peroxidation of Ram Semen

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Background: Ovine semen cryopreservation is not efficient for artificial insemination. L-carnitine (LC) has been evaluated in cryopreservation media to improve the sperm parameters of ram after freezing-thawing process. The purpose of this study was to analyze the effects of different concentrations of LC in extender for frozen-storage of ram semen. Motility characteristics, abnormal morphology, viability and lipid peroxidation

were evaluated after cryopreservation.

Materials and Methods: Semen samples were collected from 5 Zett rams, twice a week, then diluted in the extenders that contained different concentrations of LC as follows: Tris extender without LC (control), Tris containing 1 mM (L 1), 2 mM (L 2), 4 mM (L 4) and 10 mM (L 10) LC.

Results: Supplementation of Tris with 4 mM LC produced higher significant of total motility (41.6 ± 1.2 %), progressive motility (22.5 ± 1.4 %), viability (48.9 ± 1.7 %) and lower significant of lipid peroxidation (2.67 ± 0.11 nmol/ml) compared to other groups. The lower significant of motility, progressive motility, and viability were observed in frozen-thawed sperm in extender containing 10 mM LC (30.0 ± 1.2 %, 7.5 ± 1.4 % and 31.1 ± 1.7 %, respectively) compared to others. Morphology was not affected by different concentrations of LC.

Conclusion: Our results revealed that supplementation of Tris extender with 4 mM LC significantly improved the ram sperm quality after cryopreservation.

Keywords: L-carnitine, Ram, Sperm Freezing

P-54: The Effect of Acesulfame-K Administration during Pregnancy on Spermatogenesis

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Background: Today consumers prefer low-calorie foods and beverages in order to experience good-tasting sweet food and beverages that are safe for the teeth and lower in calorie content; diabetics and obese people can also use these foods. The aim of this study was to investigate the effect of acesulfame-K administration during pregnancy on spermatogenesis.

Materials and Methods: 4 groups of rats were intraperitoneally injected with Ace-K doses of 50, 100, 200, 400 mg/kg body weight during pregnancy. After the lactation, male newborn rats were divided into two groups: "treated group" which were received Ace-K as same doses and method as their mothers and "untreated group" was kept without injections until puberty. The blood samples were collected and LH, FSH, and testosterone were measured. Weight changes and testis weight to body weight ratio were examined.

Results: Testosterone were significantly increased in "treated group" at 50, 200, and 400. FSH were significantly decreased in "treated group" at 50, 100 and in "untreated group" in 50, 200, 400. Neonatal weight gain until puberty period was significantly higher in "treated group" at 50 and 200. There was a significant difference in weight gain of pregnant rats at a dose of 200.

Conclusion: In this study changes in hormones levels did not necessarily indicate the negative effects of Ace-K on fertility.

Keywords: Acesulfame-K, Hormones, Male Rat, Pregnancy, Spermatogenesis

Embryology

P-55: Cytarabine Effects on Caspase-3 Gene Expression on BALB/C Mice Ovary Tissue

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Background: The increasing use of chemotherapy drugs increased the risk of infertility. Apoptosis is a major event in the germ cell during the Folliculogenesis and Spermatogenesis stages. Apoptosis occurs during Folliculogenesis and most follicles become atresia with apoptosis and a few dominant of follicles will mature. The number of viable germ cells, being regulated in such a way, is essential for the growth and maturation of germ cells. Caspase-3, is the major cause of apoptosis and is often used as a marker for the effectiveness of cancer treatment. In the current research the effect of anticancer antimetabolite drug, Cytarabine, is investigated on ovarian tissue and caspase-3 gene expression.

Materials and Methods: Twelve adult female mice of BALB/C were divided into a control and test groups that received 100 mg/kg of Cytarabine in the form of IP (Intraperitoneal injection) in single dose. After two weeks, ovarian tissue sections were stained with Hematoxylin-Eosin. Quantitative evaluation of the caspase-3 gene expression was done with the Real-time PCR method and the data were analyzed with RESt software.

Results: All types of follicles decreased which is in the result of apoptosis increasing. Cytarabine also led to a significant increase in the expression of caspase-3 gene in ovarian tissue ($p < 0.05$).

Conclusion: As the increment of Caspase-3 expression significantly increased the apoptosis and subsequently destroyed DNA, RNA, proteins and other cell components the increased expression of Caspase-3 in ovary tissue should be considered in Cytarabine application.

Keywords: Caspase, Cytarabine, Ovary

P-56: Evaluation of Human Spermatogonia Stem Cell Differentiation on Decellularized Testicular Tissue

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Background: In males, the division and differentiation of, spermatogonial stem cells (SSCs) cause to the production of gametes and fertility. *In vitro* culture of SSCs and improvement of culture methods facilitate the study of spermatogenesis, treatment of infertility with male causes and genetic modification through male gametes. In this study, we tried to create similar body conditions for the SSCs by culturing the cells on a three-dimensional substrate obtained from decellularized testicular tissue.

Materials and Methods: After taking the testicular tissue from the brain dead patient and transferring it to the laboratory, the testicular tissue cells were isolated from the tissue by enzymatic method. To propagate SSCs, the cells were cultured in a specific culture medium for 4 weeks. After confirming the identity of the SSCs, they were cultured on 2D and 3D media with differential culture medium for 4 weeks. After this period, the expression of non-differentiating and differentiating genes in cells of both substrates (two-dimensional and three-dimensional) were compared by Real-time PCR method.

Results: The results showed that the expression of meiotic genes in cells cultured on decellularized testicular tissue was significantly higher.

Conclusion: The results of this study showed that culturing stem cells on the substrate obtained from decellularized testicu-

lar tissue can facilitate and improve the process of differentiation of these cells *in vitro*. This study and similar studies could be a way to improve infertility in men.

Keywords: Decellularization, Differentiation, Spermatogonial Stem Cells

P-57: The Effect of Copper Nanoparticles on *In Vitro* Maturation of NMRI Mouse Oocyte

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Background: *In vitro* maturation (IVM) of oocyte is an assisted reproductive technique that avoids the side effects of gonadotropins and ovarian hyperstimulation syndrome (OHSS). Improving the culture medium is considered pivotal stage to promote oocyte maturation. Considering the importance of copper in mitochondrial oxidative phosphorylation and protection against oxidative stress and ATP production, the aim of this study was to investigate the effect of copper nanoparticles (CuNPs) as an antioxidant in IVM medium and evaluate the mitochondrial activity of immature mouse oocytes treated with examined nanoparticles.

Materials and Methods: The immature oocytes were collected from 6-8 weeks old NMRI mice and divided to four groups with different concentrations of nanoparticles (0.002%w/v, 0.004%w/v, 0.006%w/v, and 0 %w/v (as a control group) to obtain the optimal dosage. IVM rate, mitochondrial activity (JC1 staining assay) and oocyte survival (Trypan blue staining assay) were evaluated in response to the addition of CuNPs during IVM process.

Results: The nuclear maturity rate of oocytes didn't show any significant difference between the control group and the different concentrations of CuNPs. Evaluation of oocyte survival data did not show any significant reduction in survival rate between the control group and different concentrations of CuNPs. The mitochondrial activity rate was increased by elevated concentrations of CuNPs, although there was no significant difference between the control group and the maximum concentrations of CuNPs.

Conclusion: The use of CuNPs in IVM medium had no adverse effect on the survival and maturation rate of oocytes.

Keywords: Copper Nanoparticles, Cytotoxicity, *In Vitro* Maturation, Mice Oocyte, Mitochondrial Activity

P-58: Generation of Haploid Spermatids on Silk Fibroin-Alginate-Laminin-Based Porous 3D Scaffolds

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Background: *In vitro* production of sperm is one of the most important options for fertility preservation in azoospermic men and prepubertal boys with cancer. Therefore, in this study, a biocompatible porous scaffold based on silk fibroin-Alg containing laminin was developed to differentiate mouse spermatogonia stem cells (SSCs).

Materials and Methods: After extraction and characterization of silk fibroin using SDS-PAGE analysis, stable porous 3D scaffolds were successfully prepared from combined solutions through a freeze-dried method. Then, structural and biological properties, biocompatibility, water absorption, degradability and mechanical behavior of biomimic scaffolds were characterized. Neonatal mice testicular cells were seeded on 3D scaffolds after confirmation of nature and their differentiation efficiency was evaluated using Real Time-PCR, flow cytometry, immunohistochemistry techniques and H & E staining. The function of Leydig and Sertoli cells was also assessed using ELISA.

Results: Blend matrices showed uniform porous microstructure with interconnected network, which significantly maintained long-term weight and better mechanical properties than pure structures. The results of molecular analysis after 21 days of culture showed that the expression of differential markers (Acrosin, Scp3 and Prm1) in the 3D system containing laminin was significantly higher than other groups. Hormonal analysis confirmed the function of Leydig and Sertoli cells for the synthesis of testosterone and inhibin.

Conclusion: The usage of a 3D system containing laminin could lead to the differentiation of SSCs and the progression of meiosis to the stage of haploid spermatid that pave the way for new human infertility treatments in the future.

Keywords: Spermatogonia Stem Cells, Porous Scaffold, Laminin

P-59: Alginate Made Core-Shell Structure for *In Vitro* Culture of Ovine Preantral Follicles

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Background: Despite significant advances in the field of three-dimensional (3D) culture of the ovarian follicles, it is still far from the natural follicle environment in the ovary. 3D system is not successful in the translation of ovarian mechanical heterogeneity into the culture. Numerous strategies such as creating ovarian micro-tissue mimicking ovarian cortex-medulla have been applied to resemble mechanical heterogeneity. For this, we create a core-shell structure using two different concentrations of alginate for *In vitro* culture of ovine preantral follicles.

Materials and Methods: Medium size ovine preantral follicles were isolated mechanically and enzymatically and encapsulated in 1% alginate (core group) for 17 days as a control. To create a core-shell structure, 1% alginate as the core and 1.5%

alginate as the shell were applied. The follicles were loaded between core and shell. At the end of the culture, survival rate and antrum formation rate were assessed.

Results: Degenerated follicles in the core-shell group (38.3%) were higher than in the core group (14%) ($P < 0.05$). In the core-shell cultures, follicles flattened and spread between two layers. Follicles encapsulated in the core-shell structure had an antrum formation rate of 58.6% as compared to 59.8% in the core group.

Conclusion: Although developing to the antral stage was achieved in both groups, due to the higher degeneration rate, the core-shell structure made from alginate is not a desirable structure for implying mechanical heterogeneity. To improve the core-shell structure, other biomaterials especially biodegradable ones should be investigated.

Keywords: Alginate, *In Vitro* Culture, Ovine Preantral Follicle

P-60: The Improvement Effect of Thymoquinone on Oocyte Maturation and *In Vitro* Fertilization in NMRI mice Induced by Cyclophosphamide

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Background: The purpose was to assess the improvement effect of thymoquinone of the *in vitro* matured and *in vitro* fertilization of mice oocytes as well as the TGF- β superfamily genes expression in NMRI mice treated by cyclophosphamide (CPH).

Materials and Methods: In this study, female NMRI mice were divided into five groups. The groups consisted of a control group, sham group (treated with solvents), CPH group (receiving 120 mg/kg of intraperitoneally (IP), experimental groups A and B, receiving TQ (5 and 10 mg/kg/day for 4 weeks intraperitoneally). After the last injection, 7.5 international units pregnant mare serum gonadotropin (IU PMSG) and 10 IU human chorionic gonadotropin (HCG) were administered intraperitoneally for induction of ovulation. Then, all the mice were sacrificed to aspirate their oocytes for further evaluation. *In vitro* fertilization (IVF) was analyzed by using mature oocytes and embryo development was investigated up to the 2 and 4-cell levels.

Results: TQ groups showed a decrease in oocyte degeneration compared to CPH groups, dose-dependently. A lower rate of metaphase I maturation was also observed in the TQ treatment groups compared to CPH, and fewer oocytes were maturing in the phase II stage. TQ significantly increased the number of two-cell and four-cell embryos after 24 and 48 hours of fertilization compared to the CPH group. Administration of TQ to mice significantly increased the number of primary and secondary follicles compared to CPH groups. CPH administration

causes degeneration of the follicles compared to the control group. Additionally, TQ-treated groups showed significant increases in levels of BMP15 and GFDF-9 gene expression dose-dependently, compared to CPH groups.

Conclusion: TQ improved decrease of maturation, number of follicles, fertilization and embryonic growth in the 2-cell and 4-cell levels effected by the CPH administration via increasing levels of factors affecting the ovaries in mice.

Keywords: Cyclophosphamide, Fertilized Embryos, Oocyte Quality, Thymoquinone

P-61: Study of The Effect of N-Acetyl Cysteine (NAC) on Apoptotic Factors in Granulosa Cells of Women with Polycystic Ovarian Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a very common endocrine disorder and one of the causes of infertility among women. Among the factors involved in the development of PCOS and its related symptoms, Oxygen reactive species (ROS) can be mentioned, which increases the number of atretic follicles, which occurs as a result of an increase in apoptosis in granulosa cells of the follicles. In this study, NAC was used as a potent antioxidant to reduce oxidative stress and thus reduce apoptosis in granulosa cells in women with PCOS and its effect on apoptotic expression was studied.

Materials and Methods: 60 women entered the study with specific Inclusion and exclusion criteria and divided into 3 groups of 20 people. The first group consisted of 20 women with PCOS who received NAC 600 mg daily for three consecutive days for six weeks, The second group included 20 women with PCOS who received placebo three times a day for six weeks and The third group included 20 women with normal ovarian function and infertility problems due to male or mechanical factors. Follicular fluid was collected after oocyte pancreas in these patients and granulosa cells were isolated. The expression of the pro-apoptosis genes such as caspase-3 and Bax, and the anti-apoptosis genes such as Bcl-2 and XIAP were investigated in different groups using the Real Time PCR. TUNEL assay was also performed to evaluate apoptosis rate in different groups.

Results: Our results revealed a significant upregulation of BCL2 and XIAP and also downregulation of BAX and Caspase3 in NAC treated patients when compared with placebo group. TUNEL assay showed NAC can decrease the apoptosis in PCOS women.

Conclusion: Based on present study prescription of NAC can improved fertility rate and can be used for PCOS women.

Keywords: Apoptosis, Granulosa Cells, NAC, Polycystic Ovary Syndrome

P-62: Cryopreservation of Human Sperm: Comparing the Effects of Vitrification Versus Rapid Freezing on The Sperm Parameters

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Background: Sperm cryopreservation, has a key role for fertility preservation in male. Recent studies have shown that the quality of sperms is reduced after the freeze-thaw process. Therefore, choosing the optimal method of sperm freezing seems necessary to reduce cryoinjury. The aim of this study was to compare two cryopreservation methods namely vitrification and rapid freezing on human sperm parameters.

Materials and Methods: Semen samples were collected from 20 healthy men who referred to Royan Institute. After sperm processing, each sample was divided into two frozen groups: vitrification and rapid freezing. Motility parameters (the Semen Class Analysis Software, CASA), morphology (Papanicolaou staining), viability (eosin-negrosin staining) and DNA fragmentation (sperm chromatin structure assay, SCSA) of thawed sperm were evaluated for both methods.

Results: Our results showed a significant decrease in the sperm motility parameters and viability and a significant increase in DNA fragmentation index in the frozen groups compared to fresh group. Moreover, a significant reduction in sperm total motility and viability ($P < 0.01$) and a significant increase in DNA fragmentation index ($P < 0.05$) were observed in the vitrification group compared to rapid freezing.

Conclusion: In conclusion, our findings showed that rapid freezing is a more suitable method for the preservation of sperm cell quality after the freeze-thaw process compared to vitrification.

Keywords: Cellular Parameters, Human Sperm Cryopreservation, Rapid Freezing, Vitrification

P-63: Effects of Different Vitrification Solutions and Protocol on Follicular Ultrastructure and Revascularization of Autografted Mouse Ovarian Tissue

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Background: Many attempts have been made to preserve fertility by improving the cryopreservation of the ovarian tissue. This current study aimed to improve of direct cover vitrification (DCV) protocol on follicular preservation and angiogenesis in autografted ovarian tissue.

Materials and Methods: In this experimental study, sixty five female Balb/c mice (5-6 week-old) were anesthetized and their ovaries were dissected. The left ovaries were vitrified by DCV solution, thawed by descending concentrations of sucrose, and then autografted subcutaneously. The right ovaries were autografted with no vitrification procedure prior to transplantation. The animals were sacrificed under anesthesia on the 7th day after transplantation to obtain ovarian tissue. Follicular quality was assessed by histological and ultrastructure observations,

and angiogenesis was examined by immunohistochemical staining and real-time polymerase chain reaction (PCR) analysis.

Results: The histological and ultrastructure features of the follicles preserved well after vitrification of the ovarian tissue by 10% ethylene glycol (EG) and 10% dimethyl sulfoxide (DMSO). Revascularization was manifested prominently in the DCV1-vitrified/grafted ovaries by von Willebrand factor (vWF) and alpha smooth muscle actin (α -SMA) immunostaining. The ovarian tissue vitrified in DCV1 protocol had higher expression levels of angiopoietin-2 (Ang-2) and vascular endothelial growth factor (VEGF) 7 days after autotransplantation ($P < 0.01$).

Conclusion: These findings suggest that DCV with 10% of both EG and DMSO, is an effective cryopreservation solution for preservation of good quality follicles as well an upregulation of angiogenic factors after ovarian tissue transplantation.

Keywords: Angiogenesis, Cryopreservation, Graft, Mouse, Ovary

P-64: The Altered Expression of Toll-Like Receptors 2, 4, NF-KB and Trap May be Represent a Possible Molecular Explanation for Decreased Implantation in Hydrosalpinx Patients

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Background: Infertility is defined as the failure to establish a clinical pregnancy after 1-year regular unprotected sexual intercourse. Tubal disease of the fallopian tubes is one of the major causes of female infertility and hydrosalpinx is the most severe manifestation of tubal disease. Hydrosalpinx patients have lower implantation and pregnancy rates in Assisted Reproductive Technology (ART), due to a combination of mechanical and chemical factors thought to disrupt the endometrial environment. The response between the immune system and the reproductive system is very important for healthy pregnancy. Toll-like receptors (TLRs), as a member of innate immune system, activates signaling pathways that elicit effective antimicrobial and inflammatory responses.

Materials and Methods: Endometrial tissue includes case and control groups. The case group included women with hydrosalpinx ($n = 10$) and the control group included women with male factors ($n = 10$). In both groups, Q-PCR analysis was used to evaluate the relative expression of TLR 2, 4, NF κ B and TRAP genes.

Results: The mRNA expression levels of TLR 2 and 4 were significantly decreased in patients with hydrosalpinx compared with control women ($P \leq 0.05$). TRAP gene involved in TLRs signaling pathways was significantly increased; however, NF κ B was significantly decreased in endometrial samples from patients with hydrosalpinx compared with control women ($P \leq 0.05$).

Conclusion: As the role of the innate immune system in implantation has been identified, so alteration in the expression of one of its components, including the Toll-like receptors or their pathway genes, can disrupt the implantation process in these patients.

Keywords: Hydrosalpinx, Toll-Like Receptors, Inflammatory

P-65: Evaluation of Cauda Epididymal Sperm in Rat Following Administration of Acrylamide and Wheat Sprout Extract

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Background: Acrylamide (ACR) is produced in food when high carbohydrate foods prepares at high-temperature (>120 °C) processing such as cooking, frying, toasting, roasting or baking. Effects of acrylamide have been shown on the male reproductive system and sperm fertility parameters. To treat of human problems and the pains, medicinal plants have been considered. The wheat sprout (WSP) contains high amount of vitamins, minerals and phytoestrogens compounds. This study investigated the wheat sprout effects on sperm quality in rats exposed to acrylamide.

Materials and Methods: For this purpose, 20 healthy adult male rats (210 ± 20 g) were divided randomly into four groups: Control group without treatment, ACR group received 50 mg/kg/day acrylamide (orally), WSP group received 200 mg/kg/day wheat sprout extract (orally), ACR+WSP group received 200 mg/kg/day wheat sprout extract along with 50 mg/kg/day acrylamide (orally). After 21 days, the rats were sacrificed using chloroform and sperm samples were taken of the caudal of the epididymis. Sperm total count, motility, viability, and DNA fragmentation as the sperm quality parameters were examined.

Results: The results showed that sperm count, motility and viability were decreased significantly following administration of the acrylamide (P<0.05). No significant difference was seen in DNA fragmentation of sperms within the groups. On the contrary, sperm quality parameters were significantly improve by wheat sprout extract (P<0.05).

Conclusion: Results suggested that sperm quality in rats can be improved by wheat sprout extract following administration of acrylamide.

Keywords: Acrylamide, Rat, Sperm, Wheat Sprout Extract

P-66: The Effect of Iron Oxide Nanoparticles on *In Vitro* Maturation of NMRI Mouse Oocyte

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Background: Generation of mature oocytes is the main pur-

pose of *in vitro* maturation (IVM) in order to support future embryo development. Providing the optimal culture conditions including the use of some antioxidants is necessary to enhance oocyte developmental competence. Iron oxide nanoparticles (IONPs) due to their antioxidant activities were used to evaluate the IVM rate of immature oocytes of mice in present study.

Materials and Methods: Germinal vesicle (GV) oocytes were obtained from the ovaries of 6-8 weeks NMRI female mice. IVM of oocytes was performed in the presence of IONPs synthesized by co-precipitation method. To obtain the optimal concentration, different concentrations of nanoparticles (0.001% w/v, 0.002% w/v and 0.004% w/v) in the oocyte culture medium were considered and the IVM rate was compared with the control group (with 0% w/v of IONPs). In response to the addition of IONPs during IVM, the survival rate and mitochondrial activity were investigated using Trypan blue and JC1 staining methods, respectively.

Results: Using the different concentrations of IONPs showed no significant alteration in nuclear maturity rate of oocytes compared to the control group. Oocyte survival and mitochondrial activity were decreased significantly (P≤0.05) by elevated doses of IONPs compared to the control group.

Conclusion: Exposure to IONPs in IVM medium had no undesirable effects on the oocyte maturation rate. Nevertheless, the toxicity of IONPs increased in the maximum dose so that an attenuation in mitochondrial activity and oocyte survival was observed in the maximum concentration.

Keywords: *In Vitro* Maturation, Iron Oxide Nanoparticles, Oocyte, Survival Rate

P-67: The Effect of Myoinositol Antioxidant on Sperm Parameters and DNA Damage in Vitrification Compared to Conventional Freezing

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Background: Sperm freezing is well-known method to keep it for a long time but has adverse effects on sperm parameters.

Materials and Methods: 30 normal semen samples were selected and each was divided into four groups: conventional freezing (C), conventional freezing + myoinositol 2mg/ml (C+M). Vitrification (V), vitrification + myoinositol 2mg/ml (V+M). The samples were frozen and thawed at least 24 hours later. Vitrified groups were melted in hamsF10+albumin⁻ 5%. Conventional groups, were thawed in tab water. After thawing sperm motility was assessed by CASA system. Sperm vitality, morphology and DFI were assessed by eosin-nigrosin, Diff-Quick and SDFA method respectively. Data analysis were done by one-way ANOVA and Tukey posttest in GRAPHPAD prism9.

Results: V+M had the lowest DFI. DFI rate in C+M was less than C and in V+M less than V. V+M had the best morphology after thawing. Viability and motility weren't significantly different between groups.

Conclusion: V+M significantly improves sperm morphology, especially in protecting acrosomal caps and reducing vacuoles in the sperm head compared to conventional freezing and is the best group among the studied groups in terms of DFI reduction. So V+M can be a good choice for using in andrology and IVF

laboratory.

Keywords: Conventional Freezing, Myoinositol, Sperm freezing, Vitrification

P-68: The Effect of Inactivated SARS-CoV-2 Vaccination on Assisted Reproductive Technology (ART) Outcomes in Royan Institute

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Background: According to the World Health Organization advice, the only way to control COVID-19 pandemic is to get the vaccination and follow health tip. People vaccinated by the inactivated vaccine mostly in Iran. This study is about evaluating the relationship between inactivated SARS-CoV-2 vaccination and ART outcomes.

Materials and Methods: In this observational before after clinical study, all couples who undergoing consecutive ovarian stimulation for ART cycle before and after the second dose of the vaccines were approached to participate in the study (from June 2021 until February 2022). The patients with following characteristics were excluded from the study: women older than 40, men with a severe male factor reason, need for pre-implantation genetic diagnosis, an underlying disease, history of covid-19 infection, BMI>30, donor egg and embryo.

Results: 31 eligible couples have participated in this study. According to our defined inclusion criteria 29 of them were vaccinated by Sinopharm vaccine and the other 3 were vaccinated by Barekat. The approximate period between their 2 shots of vaccination was about 262 ± 27 days. The mean interval between the second vaccination time and date of OPU (Ovum Pick Up) was 75 ± 5 days. Different results of the ART cycle were not significantly different before and after vaccination, the number of antral follicle count (9.38 ± 5.83 vs. 9.25 ± 5.82), number of retrieved oocytes (5.37 ± 4.18 vs. 6.24 ± 4.57), number of immature oocytes (1.16 ± 2.07 vs. 1.45 ± 2.36), number of mature oocytes (4.35 ± 2.98 vs. 5.19 ± 4.32), number of 2PN (3.06 ± 2.46 vs. 3.87 ± 3.14), and number of embryos (2.93 ± 2.62 vs. 3.93 ± 3.20).

Conclusion: Covid-19 vaccination has no negative effect on ART cycle outcomes. More several studies with longer follow-ups are highly needed to confirm our observation in future.

Keywords: Assisted Reproductive Technology, Covid-19 Vaccination, Infertility, SARS-CoV-2

P-69: Increased Expression of Klotho Gene in Cumulus Cells of Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is the main reason for anovulatory infertility and the most common endocrinopathy affecting women of reproductive age. Due to the complicated, multifactorial, and polygenic nature of PCOS, variation in phenotypic expression among individuals has been recognized. However, little is elucidated about its genetic and molecular basis. Klotho (KI) is an antiaging gene concerned with mammals' aging process that accomplishes various functions from an obligatory co-receptor for the fibroblast growth factor family to ion channels/transporters modulation. Klotho plays a pivotal role in earthshaking signaling pathways like PI3K/Akt/mTOR, insulin/IGF-1, Wnt1, and MAP Kinase. In a recent study, Knockdown of the Klotho gene normalized signaling mentioned pathways in granulosa cells (GCs) of patients with PCOS and the ovarian tissues of PCOS rats. Moreover, the role of klotho protein in the apoptosis of GCs in patients with PCOS was identified.

Materials and Methods: Two groups of women (20 to 35 years old) referred to the Royan Institute participated after signing the consent form according to local ethical approval. Through the ART (Assisted Reproductive Technology) procedure, ovarian follicular cumulus cells (CCs) from the PCOS group (n=12) and healthy (non-PCOS) women (n=8) were collected during oocyte retrieval. After total RNA extraction and cDNA synthesis, the expression level of Klotho was quantitatively determined by Real-Time PCR.

Results: Our data indicated a higher Klotho gene expression in CCs of the PCOS group vs. the control group.

Conclusion: Current data may imply the correlation between upregulation of the klotho gene and its impact on PCOS symptoms development.

Keywords: Cumulus Cells, Expression, Klotho, PCOS

P-70: Ultrastructure of Human Ovarian Tissues Transplanted to Chick Embryo Chorioallantois Membrane (CAM) after Vitrification or Slow Freezing
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Background: Ovarian follicle depletion and premature ovarian failure are significant challenges in cancer patients subjected to radio- or chemotherapy, ovarian tissue cryopreservation (OTC) is a suitable option. The aim was to analyze the structure and ultrastructure of human ovarian tissues transplanted onto chick embryo chorioallantois membrane (CAM) after cryopreservation by vitrification or slow freezing.

Materials and Methods: Ovarian tissues from 10 cancer patients underwent cryopreservation. CAM transplantation was done on fresh and cryopreserved OTs, to assign samples to nine study groups as follows: 1) FI-FIII= fresh, 5- and 10-days post-CAM transplantation groups; 2) VI-VIII= vitrified, 5- and 10-days post-transplantation vitrified groups; 3) SFI-SFIII: slow frozen, 5- and 10-days post-transplantation slow freezing groups. Proliferation ability, folliculogenesis, structural and ultrastructure were analyzed.

Results: The density of primordial follicles did not change after both freezing methods but reduced after 5 ($P \geq 0.05$) and 10 days ($P \leq 0.05$) post-CAM transplantation. The follicular grade significantly decreased in all transplanted tissues ($P \leq 0.05$). Proliferation marker increased after cryopreservation but was reduced after transplantation ($P \leq 0.05$). The ultrastructure of ovarian follicles was more preserved in the fresh groups. Stromal ultrastructure appearing more preserved after vitrification compared with slow freezing. The results showed no any sign of infection by malignant cells after transplantation.

Conclusion: TEM evaluation showed follicular damages in both methods of freezing, with a better transplantation rate after vitrification. Also, enhanced follicular activation and depletion were observed after vitrification. Post grafting evaluation of the early events of OTs transplantation may improve the long term maintenance of OTs storage.

Keywords: Cancer Patients, Ovarian Tissue Cryopreservation, Tem Evaluation, Slow Freezing, Vitrification

P-71: Comparing The IVM Laboratory Outcomes between Stimulated IVF with Unstimulated Natural Cycles

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Background: Recently, more attention has been raised toward fertility preservation in women with cancer. One option is *in vitro* maturation (IVM) of the immature oocytes since there is not enough time for induction of ovarian stimulation protocol. The aim was to compare the IVM laboratory outcomes between stimulated and unstimulated (natural) IVF cycles.

Materials and Methods: 234 immature oocytes collected from 15 cancer patients who underwent an IVM program (natural IVM) and 23 IVF cycles with controlled ovarian hyperstimulation protocol (stimulated IVM) were analyzed. The oocyte morphology, zona pellucida (ZP), and meiotic spindle (MS) Presence were measured using PolScope technology. Also, the rates of oocyte maturation and fertilization were assessed in both groups.

Results: The IVM rate was higher in the stimulated cycle ($P < 0.05$), but the fertilization rate was insignificant in comparison to unstimulated cycles. There were no significant differences in the spindle visualization and ZP birefringence scoring

between the groups ($P > 0.05$). The oocytes normal morphology was better in the stimulated cycle compared to the natural cycle ($P < 0.05$).

Conclusion: IVM can be recommended for cancer patients as an alternative treatment when there is insufficient time for conventional IVF before chemotherapy initiation.

Keywords: Fertility Preservation, Fertilization, Maturation, Natural Cycle IVM, Stimulated Cycle IVM

P-72: The First 10 Days Events of The Human Ovarian Tissue Transplantation onto The Chick Embryo Chorioallantoic Membrane (CAM), Ischemia and Vasculogenesis Induction Following, Slow Freezing Versus Vitrification

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Background: The first-days post ovarian transplantation are critical periods, as the ischemic injury can diminish the success. In this study, the first day's events of ovarian transplantation in two dimensions of structure and ultrastructure following slow freezing and vitrification were assessed.

Materials and Methods: Ovarian tissue (OT) from 10 patients were frozen in two methods of slow freezing and vitrification. Tissues were transplanted onto the CAM, and then retrieved 5 and 10 days culture. Nine groups were assigned as follows; I-III; fresh, 5 and 10 days culture, IV-VI; vitrification, 5 and 10 days culture, and VII-IX; slow freezing, 5 and 10 days culture. Structural and ultrastructural studies were done to assess the tissue viability and integrity following CAM transplantation. Image J software was used to measure the amounts of fibrosis and necrosis.

Results: The first sign of successful transplantation was observed on day 3, post transplantation. Vitrified tissues showed higher viability and transplantation rate compared to the slow frozen group (65% vs 57.5%) ($P = 0.7$). The areas of fibrosis and necrosis and avian vessels increased significantly after 5 and 10 days culture ($P < 0.05$). A large ultra-structural follicular deformities was noticed after 10 days of CAM transplantation. CAM transplantation technique had negative effects on the integrity of follicles, independent of the freezing procedure.

Conclusion: Vitrification can be considered as a reliable alternative for slow freezing. CAM transplantation is a good technique for confirmation of tissue viability after warming, but had a negative impact on the human follicle ultrastructure, in a short period of time.

Keywords: CAM Transplantation, Hypoxia, Ischemia, Ltra-Structure Deviation, Ovarian Cryopreservation

P-73: Non-Invasive β -Thalassemia Diagnosis Using Cell Free DNA Recovered from Blastocoele Fluid and Spent Culture Medium from Pre-Implantation Human Embryo

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Background: To overcome the limitations of invasive embryo biopsy, a non-invasive alternative to preimplantation genetic testing (PGT) has been recently introduced. This study aimed to investigate the efficacy of cell-free DNA (CF-DNA) in the spent culture medium combined with blastocoel fluid (ECB) versus blastomere biopsy for β -thalassemia Diagnosis.

Materials and Methods: This study involved data from 8 couples. A total of 22 samples of ECB from fresh group who underwent PGT for β -thalassemia and 6 ECB samples from donated frozen group and 10 control media were collected and analyzed.

Results: In fresh group, the HBB mutation analysis and haplotyping results of 9 of 22 samples (40.9%) were concordant with the biopsy results. The concordance rate in the donated frozen group was higher than that in fresh group (4/6, 66.6%; $P < 0.05$). 75% of markers were semi-informative. Marker failure rate were significantly higher in fresh group (34.5% vs 4.8%; $P < 0.05$). No significant relationship between allele dropout and fail number of all markers and embryo morphology was seen ($P > 0.05$). Kappa-Agreement value between CF-DNA isolated from culture media and blastomere biopsy was 0.516 which is moderate. The specificity, sensitivity, positive predictive value, and negative predictive value of CF-DNA to detect HBB mutation were 67, 100, 100, and 67, respectively.

Conclusion: We found that ECB for detecting β -thalassemia in donated frozen group was better than ECB in fresh group. Advances in DNA extraction, amplification technique, and testing may allow for PGT as a non-invasive approach without biopsy in the future.

Keywords: Beta-Thalassemia, Blastocyst, Culture Media, Microsatellite Repeats, Preimplantation Diagnosis

Epidemiology and Helaths

P-74: The Relationship between Shift Work Disorder and Sleep Disorder with Endometriosis

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Background: Sleep is a complex process whose disorders can have negative effects on people's health and the outcome of assisted reproductive technologies. So, we assessed the relation-

ship between shift work disorder (SWD), sleep disorder, and endometriosis in fertile and infertile employed women.

Materials and Methods: In this descriptive cross-sectional study, 700 fertile and infertile women referred to Royan Institute, Arash Hospital, and Gynecological Office in Tehran were selected by sampling available method. Data were collected by questionnaires of SWD (4 Questions), Pittsburgh Sleep Quality Index (PSQI), and demographic. The level of significance was considered 0.05. Also, the data were analyzed by SPSS version 22 (Inc. Chicago, IL, USA).

Results: In this study, 700 women with a mean age of 35.20 ± 5.70 years were assessed. Eleven percent of them had endometriosis and 23.4 were at high risk for SWD. There was a significant relationship between SWD and sleep disorder ($P < 0.05$), which means that women at higher risk for SWD had been in the poor sleep group. Also, there was no significant difference between the scores of SWD in fertile and infertile women (with endometriosis, non-endometriosis, $P = 0.115$). Furthermore, women with endometriosis had been in a level of poor sleep more than women without endometriosis, but this difference was not statistically significant ($P = 0.219$).

Conclusion: The results of our study showed that SWD can impair the quality of sleep in women and seems to be effective in causing endometriosis in women.

Keywords: Endometriosis, Pittsburgh Sleep Quality Index, Women, Shift Work Disorder

P-75: The Impact of Energy Restricted Low Glycemic Index (LGI) Diet on Depression and Self-Esteem

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Background: Obesity is a growing public health problem worldwide, which is an important risk factor for inducing certain chronic diseases and psychological alterations. Some researchers suggest that weight loss is positively associated with a reduction in depressive symptoms and self-esteem, other investigators have not found these effects. The aim of this study was to evaluate the effect of energy restricted low glycemic index (LGI) diet on anthropometric variables and investigate the effect of this diet on depression symptoms and self-esteem.

Materials and Methods: 146 obese women participated in this study, and were randomized into two equal groups. The first group (A) followed a 24-week energy restricted LGI diet to cause 0.5 kg weight loss per week. The second group (B) given educational information on nutrition. Anthropometric, depression and self-esteem variables were documented at baseline and 24 weeks with intervention. All participants were requested to sustain their baseline physical activity. The short form of the International Physical Activity Questionnaire (IPAQ) was completed every 2 weeks. Depression was evaluated with the Beck depression inventory (BDI). Higher values indicating greater symptoms of depression. Self-esteem was assessed with the Rosenberg self-esteem scale (RSES). High scores indicate high global self-esteem.

Results: At baseline, there were no statistically significant differences between demographic, anthropometric, the physical activity levels and psychological characteristics of cases and controls. The mean age of all study group was 29.2 ± 5.1 years. During intervention, physical activity levels in both groups did

not differ significantly. After intervention, there was a significant decrease in body mass index (BMI) (31.9 ± 3.4 vs. 29.1 ± 2.3 kg/m²), Waist circumference (101.9 ± 5.6 vs. 95.7 ± 2.8 cm), Beck Depression Inventory (BDI) (7.45 ± 1.2 vs. 4.3 ± 1.6), and increase in Rosenberg self-esteem scale (RSES) of group (A) (19.2 ± 2.2 vs. 27.4 ± 1.3), but the changes of group (B) were not significant. Changes in the depression and self-esteem scores were significantly associated with weight loss.

Conclusion: This study demonstrates that weight loss through intervention with energy restricted LGI diet could improve depression and self-esteem in obese women.

Keywords: Low Glycemic Index (LGI) Diet, Depression, Self-Esteem

Female Infertility

P-76: Treatment of Ovarian Hyperstimulation Syndrome in A Mouse Model by Cannabidiol, An Angiogenesis Pathway Inhibitor

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Background: Prevention of ovarian hyperstimulation syndrome (OHSS) is possible with pharmacological and non-pharmacological methods. Non-pharmacological methods include reducing ovarian stimulation, reducing hCG β dose, delaying hCG β or coasting injection, canceling the cycle, freezing the fetus for replacement at a later time, follicular aspiration, and oocyte verification. However, commercially available VEGF-reducing drugs now have side effects that preclude their use as a treatment for OHSS in young women; for this reason, researchers are looking at new drugs to treat OHSS. Cannabinoids (CBD) are the active ingredient in hemp (*Cannabis sativa* L) and are the most important source of phytocannabinoids. It has no psychoactive effect and modulates the activity of CB1 and CB2 receptors, and is involved in regulating female reproductive processes such as proliferation, pain modulation and improving the path of angiogenesis. CBD affects the hypothalamic-pituitary-ovarian axis and affects the secretion of steroid hormone. In OHSS, androgens cause proliferation of the theca interna and granulosa and increase AMH, upsetting the balance of the proliferation pathway. Because CBD inhibits cell proliferation and migration. Therefore, in this study, we use CBD to improve OHSS in this study. Our aim was to investigate the role of CBD in the pathophysiology of OHSS and its use to modulate the pathway of angiogenesis and the VEGF gene.

Materials and Methods: This study was a basal genomic levels analysis in the OHSS-induced model, which used 32 NMRI mice. Thirty-two female immature NMRI rats were randomly assigned to four groups. The control group (n = 8) received saline only for four consecutive days. The remaining twenty-four rats received 10 IU of pregnant mare serum gonadotropin (PMSG) followed by 30 IU of human chorionic gonadotropin

(hCG) to induce OHSS. Group 2 (n = 8) was managed with no additional intervention after the induction of OHSS. Group 3 (n = 8) received 32nmol DMSO 2 hours before the PMSG injection for four consecutive days and 2 hours before the hCG injection on the fourth day. Group 4 (n = 8) received 30 mg/kg CBD after induction of OHSS, respectively. CBD was administered 2 hours before the PMSG injection for four consecutive days and 2 hours before the hCG injection on the fifth day. Body and ovary weight, vascular permeability (VP), and levels of VEGF in the blood serum were examined in all animals.

Results: CBD Reduced Body Weight, Ovary Weight, and VP Compared to that of the OHSS group (P<0.05). VEGF expression in ovaries and peritoneal VEGF levels were decreased in the CBD group compared to that of the OHSS group (P<0.05).

Conclusion: CBD was active in the alleviation of OHSS through suppression of VEGF and VP. Overall, we conclude that CBD is effective in the downregulation of VEGF and improvement in vascular permeability in OHSS.

Keywords: Animal Model, Cannabidiol, Ovarian hyperstimulation Syndrome, VEGF

P-77: The Effect of Simvastatin on Uterine Fibroids Development and ART Outcome in Infertile Women: A Pilot Study

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Background: Uterine fibroids are the most common form of benign gynecologic tumors with a lifetime risk of around 70%. Statins are the most commonly prescribed lipid-lowering agents that have been reported to inhibit proliferation of fibroid cells in animal model. In this study, an attempt was made to compare sizes of uterine fibroids in women received simvastatin orally or vaginally and those received placebo undergoing ART outcome.

Materials and Methods: This randomized controlled trial study was conducted on 30 infertile women undergoing ART cycles. Patients with presence of at least one intramural fibroid with size >4 cm or two intramural fibroids with size >3 cm confirmed by ultrasound included the study. Patients with age > 40 years old, body mass index (BMI) <25 Kg/m², number of fibroids ≥ 5 , any hormonal therapy in the last 3 months, alcohol consumption, allergic reactions to simvastatin, diagnosis of cancer, Hb ≤ 7 g/dl, severe male factor infertility and severe endometriosis, spontaneous pregnancy and patients with signs or symptoms of medical conditions and use of medications that interfere with simvastatin excluded from the study. Patients were randomly assigned to one of the three groups of women (group A: received simvastatin 40 mg orally & placebo vaginally; group B: simvastatin 40 mg vaginally & placebo orally

and group C: placebo vaginally & placebo orally) for 3 months (10 women in each group). The primary outcome measure was uterine fibroid size and secondary outcome measures were uterine size and reproductive outcome after ART. For data analysis, StATA software was used. In all tests, the significance level was considered less than 0.05.

Results: All groups were comparable in regards to mean age, BMI, infertility duration, type and causes of infertility. There were no statistical differences between groups in respect to sizes and volume of fibroids and dimensions and volume of uterus before and after treatment. Although the rate of clinical pregnancy was higher in women who received simvastatin 40 mg orally than two other groups, this increase was not statistically significant ($P=0.139$).

Conclusion: These data suggest to design a further clinical trial study with larger sample size using different oral doses of Simvastatin.

Keywords: Assisted Reproductive Technology, Infertile, Outcome, Uterine Fibroids, Simvastatin

P-78: Association of Increased Body Mass Index with Lipid Peroxidation in Patients with Polycystic Ovary Syndrome (PCOS): A Review, and Meta-Analysis

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Background: Polycystic ovary syndrome PCOS is the most common endocrine-metabolic disorder and the most common cause of anovulatory infertility in women of reproductive age. Oxidative stress (OS) plays a critical role in the pathogenesis of PCOS. Several studies have demonstrated increased OS in PCOS patients. One of the important biomarkers of oxidative stress is lipid peroxidation. Malondialdehyde (MDA) is a lipid peroxidation product. MDA is an indicator of persistent OS, resulting from lipid peroxidation of polyunsaturated fatty acids. Its concentration increases when the formation of Reactive oxygen species (ROS) increases.

Materials and Methods: The keywords "oxidative stress," "polycystic ovary syndrome," and Malondialdehyde (MDA) were searched in PubMed, EMBASE, MEDLINE, Scopus, and Cochrane Library databases (CENTRAL). 9 articles with the desired parameters were extracted, and the accumulated data were analyzed using Comprehensive Meta-Analysis V3 software for meta-analysis.

Results: 9 observational studies from 1995 to 2022 with a sample size of 17913 women were reviewed and showed that change in MDA levels in women with PCOS. Respectively, Result showed a significantly higher MDA level (SMD= 1.16; 95% CI, 0.76, 1.55; $P=0.001$; $I^2>75\%$), in the PCOS group compared with the healthy control group. In addition, after performing a meta-regression, a relationship was found between body mass index (BMI) and MDA level (P value= 0.01; 95% CI; Tau-sq: 0.17, R^2 : 0.45); Which shows an increase in lipid peroxidation with weight gain.

Conclusion: Monitoring of specific MDA biomarkers might be beneficial to the diagnosis and prognosis of PCOS. Also, the

increase in lipid peroxidation in PCOS obese women showed us the importance of setting a proper diet for these patients.

Keywords: Biomarkers, Malondialdehyde (MDA), Meta-analysis, Oxidative Stress, Polycystic Ovary Syndrome (PCOS)

P-79: The Protective Effect of Co-Administration of Salvia Officinalis Extract and Vitamin E on The Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. The pathophysiology of PCOS is complex, therefore, in the present study, we simultaneously investigated Salvia officinalis extract as an antioxidant and vitamin E in PCOS mice.

Materials and Methods: In this experimental study, PCO model was developed in adult female mice during 8 weeks. The 30 mice were divided in five groups include control, PCO model (using estradiol valorite or EV at 40 mg/kg), Salvia officinalis extract (400 mg/kg day), vitamin E (60 mg/kg/day, orally), and the Salvia officinalis extract + vitamin E model. After mice sacrificing, their ovarian tissue was removed and histological and morphometric studies were performed. After tissue lysis, total antioxidant capacity was measured by FRAP method and oxidative stress was measured by flow cytometry.

Results: The results of tissue sections showed that EV administration caused cyst formation in the study groups. Cyst formation and the percentage of preantral and antral follicles in the group that received Salvia officinalis extract and vitamin E together were significantly lower ($P\leq 0.001$) than the groups that received each alone. In the group that received a combination of both antioxidants and vitamin, the level of oxidative stress and total antioxidant capacity were the lowest and highest, respectively, compared to the other groups.

Conclusion: The results showed that co-administration of Salvia officinalis extract and vitamin E in PCO mice, in addition to reducing the amount of oxidative stress by increasing the antioxidant properties, but also by reducing the number of cysts and improved the symptoms of this syndrome.

Keywords: Estradiol Valorite, Polycystic Ovary, Salvia Officinalis Extract, Vitamin E

P-80: Nutrigenomics: Future Prospect of Personalized Assisted Reproductive Technology Strategies

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Background: Following birth of Louise Brown in 1978, health world observed a great success of assisted human reproductive technology (ART) progresses. Although, what was discovered, is the tip of the iceberg Infertility is a major reproductive health problem, with 40–50% of cases having a male factor component. Current data provide that male obesity negatively influences male fertility potential. There is now a provided proof

that obesity in men impairs children's metabolic and reproductive health. At the first glance, these are seemed bidirectional terms that are interrelated as two sides of a coin.

Materials and Methods: Metformin therapy considered as the first line of treatment of obesity. Recently, it has been reported some contradictory findings on that this drug can act in different organs and tissues including the male reproductive system. Furthermore, it is well established that spermatogenesis is a dynamic and multistep process of male germ cell proliferation and differentiation.

Results: Today, there are increased evidence of a direct link between genome/ epigenome and diet that makes one of the most important emerging areas of medical science. Nutrigenomics represents a constellation of understanding of genetics, omic technologies and nutrition. It has the potential to shift towards individual genetic makeup. The world has tasted its sweetness in the illnesses like phenylketonuria and lactose intolerance. A strong step to pass selecting individual dietary components.

Conclusion: There are great expectations for nutrigenetics, especially in regard to effect of diet on the epigenetic modulation of gene expression. The specific field that is proposed on the premise a more useful approach. This exciting area of interaction between genotype and diet that may illustrate different strategies of reproductive health. Today, we must pass "one size fits all" model and enter to "personalized nutrition" way and do not forget "caution". Also, we must not ignore pharmacogenetics approaches in the ART clinical practice.

Keywords: Male infertility, Nutrigenetics, Metformin therapy, Obesity, Pharmacogenetics

P-81: Expression of 3-BHSD Protein in Abdominal Subcutaneous Adipose Tissue of Pregnant Women with Polycystic Ovary Syndrome (PCOS)

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Background: Numerous studies have shown that altering the expression of steroid genes and thus the expression of their proteins can be one of the main causes of PCO syndrome. Most recently, it has been suggested that adipose tissue may contain the abnormal conversion the Steroid proteinogenesis. The 3-BHSD protein, which is an isomerase, is essential for the synthesis of

all types of steroids.

Materials and Methods: In this case-control study, 3-BHSD protein expression were compared in subcutaneous adipose tissue of 11 pregnant women with and 11 without PCOS who were matched with each other in terms of weight characteristics before pregnancy and age. A subcutaneous adipose tissue samples (3-4 grams) was obtained during cesarean section. The expression of 3-BHSD protein in adipose tissue was assessed using Western blotting method. All obtained data were statistically analyzed using SAS software.

Results: According to the analysis and quantification of the results, it was shown that the expression of 3-BHSD protein in the subcutaneous adipose tissue of the abdomen of the group with PCOS increased significantly compared to the non-PCOS group (P=0.006).

Conclusion: Our results showed a link between s genes and proteins involved in steroid metabolism and fatty acids in AT of pregnant women which warrants further studies.

Keywords: Adipose Tissue, PCOS, Pregnancy, Steroids Protein

P-82: Evaluation of Toll-Like Receptors 3, 9 and Their Signaling Pathway (Il-6, Myd88 and Irf3) Genes to Understanding How Hydrosalpinx Affect Endometrial Receptivity

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Background: Although a physiological level of inflammation is required for successful implantation, pathological inflammation suppresses the normal function of genes and molecules preparing endometrium to be receptive. Hydrosalpinx is an inflammatory condition that reduces the pregnancy and implantation rates due to several mechanisms including leakage of the hydrosalpinx fluid to the uterus, mechanical barrier, or chemical factors. TLRs are expressed in both immune cells and non-immune cells at the maternal-fetal interface. While the relevance of TLRs in pathogen defense has been acknowledged, the role of these receptors and their signaling pathway in hydrosalpinx are unknown.

Materials and Methods: Ten infertile patients with uni or bilateral hydrosalpinx who refer to the Royan infertility clinic for salpingectomy were selected. Endometrial biopsy during the window of implantation (days 21-24 of the menstrual cycle) was prepared prior to surgery. TLR3, TLR 9, IL6, MYD88 and IRF3 mRNA genes expression of were evaluated by Real-time PCR.

Results: The mRNA expression levels of TLR 3 and 9 were significantly decreased in patients with hydrosalpinx compared with control women. IL6 was significantly increased; however,

IRF3 and MYD88 were significantly decreased in endometrial samples from patients with hydrosalpinx compared with control women ($p < 0.05$).

Conclusion: The altered expression pattern of TLR 3 and TLR 9 in hydrosalpinx condition leads to inaccurate immunological responses and consequence disturbing in physiological endometrial inflammation. The results show the importance of immune responses to active hydrosalpinx infection but also a putative contribution to immunopathology in the receptivity status of the endometrium.

Keywords: Hydrosalpinx, Implantation, Toll-Like Receptors,

P-83: Vitamin D and Calcium Independently Mediate the Female Reproductive Performance

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Background: Vitamin D deficiency (VDD) reduces the chances of successful fertilization; however, it remains to be validated whether this effect is dependent or not on calcium. To answer this, mouse models were used and placed in different situations of vitamin D (Vit D) or calcium deficiency to dissociate the calcium from the Vit D effect.

Materials and Methods: The models consisted of: of: 1/ control; 2/ sham groups; 3/ Vitamin D overdose and normal Calcium; 4/ Vitamin D and Calcium deficient /Vitamin D deficient and normal calcium; 5/ Vitamin D normal and hypocalcemia. After the measurement of serum levels of Vit D, calcium and phosphorus, an analysis was carried out in terms of activation and maturation of oocytes as well as reproductive performance.

Results: Vit D overdose, despite increasing number of mature oocytes, it reduced maturation, developmental competence and offspring survival. VDD reduced the number and percentage of mature oocytes and blastocyst rates, as well as fertility rate and offspring survival. Calcium deficiency, when Vit D levels were normal, had a similar effect. The effects of VDD were all edited by a diet that corrected calcium level.

Conclusion: Both Vit D overdose and deficiency have a negative effect on female reproductive performance. Calcium deficiency in a normal Vit D context also has a negative effect on female reproductive performance. In conclusion, although closely related, Vit D and calcium act in part independently of each other in defining the "optimum" for female reproductive performance.

Keywords: Calcium Deficiency, Oocyte Number and Maturity, Vitamin D Deficiency

P-84: Early Onset and Atypical Severe Preeclampsia in A Woman Undergone *In Vitro* Fertilization

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Background: Assisted reproduction technology (ART) is used worldwide, at increasing rates, and data show that some adverse outcomes occur more frequently than following spontaneous conception.

Materials and Methods: To report a rare case of severe and atypical preeclampsia in gestational age of 18 weeks in a woman undergone IVF due to sex selection not infertility. A 35-year-old woman G3P2L2 with gestational age of 18 weeks referred to our hospital with blood pressure (BP) of 220/120 mm Hg, headache, and 3100 g protein in 24-hours urine collection. Her pregnancy had achieved via IVF due to sex selection (male selection) by frozen embryo, so she did not have infertility. She had a history of hypothyroidism. She did not have any chronic hypertension (HTN) or other diseases. Sonography of her pregnancy showed normal nuchal translucency (NT) in 13 weeks. The risk of down syndrome was 1/75 in double test, so she had done cell-free DNA and it was low risk for aneuploidies. Lab data including CBC, LFT, and serum creatinine were normal. Antiphospholipid, lupus, anti-GBM, and complement tests were within normal ranges.

Results: Due to severe preeclampsia and uncontrolled blood pressure regardless of 300 mg labetalol prescription, termination of pregnancy was scheduled for her by vaginal route. The male fetus with 300 g weight was born. The placenta was normal. Following delivery, she had BP of 160/100 for 1 week that controlled by valsartan after that, BP became normal without any medication. Four weeks later, quantitation of proteinuria was 80 g.

Conclusion: Although, infertility may contribute to the adverse outcomes of pregnancy such as pregnancy induced HTN (PIH) in IVF, but factors related to hormone stimulation and or IVF methods per se also as a part and a woman should be informed about these adverse maternal outcomes before undergoing IVF, even without history of infertility.

Keywords: *In Vitro* Fertilization, Preeclampsia, Pregnancy

P-85: Ectopic Pregnancy Rates in Frozen Versus Fresh in IVF/ICSI Cycles: A Retrospective Analysis of Embryo Transfer Cycles at Royan Institute

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Background: The incidence of ectopic pregnancy (EP) after *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) is about 2.5-5 times that of natural conceptions; however, the etiology of this increased risk remains uncertain. A suggested practice change to reduce the incidence of EP is the transfer of frozen ET (Embryo Transfer) rather than the transfer of fresh ET. There is also variation in the reported incidence of EP following frozen ET. A retrospective study was conducted to compare the risk of ectopic pregnancy after frozen ET with fresh ET in women who underwent IVF/ ICSI and became pregnant between 2015 and 2020.

Materials and Methods: Research conducted at Royan research institute during the period October 2015 to March 2020 examined women who underwent IVF and ICSI treatments. SPSS software version 25 is used to analyze the data. The odds ratio was calculated using MedClac software with a 95% confidence interval.

Results: Among 21203 ET cycles, 7658 (36.11%) pregnancies were clinical, of which 3189 (32.7%) were fresh ET and 4469 (39.02%) were frozen ET. Of all the pregnancies, 131 (1.71%) were ectopic. Among 131 ectopic pregnancies, there were 67 (2.1%) EPs from fresh ETs and 64 (1.43%) EPs from frozen ETs. The odd ratio of EP was 1.096 more in fresh cycles than freeze cycles (CI95%: 0.68 to 1.78, P= 0.711).

Conclusion: Based on our study, frozen ET reduces the number of ectopic pregnancies compared to fresh ET. In patients with a history of ectopic pregnancy or those who have risk factors for ectopic pregnancy, a frozen embryo transfer is generally recommended.

Keywords: Ectopic Pregnancy, Embryo Transfer, Fresh Embryo Transfer, Frozen Embryo Transfer

P-86: PRKACG as A Potential Diagnostic Molecular Biomarker for Uterine Corpus Endometrial Carcinoma

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Background: Uterine corpus endometrial carcinoma (UCEC) is one of the most common gynecological malignant tumors and remains a major public health problem. Although important efforts have been made in explaining the progression of UCEC, it is still warranted that molecular pathways and mechanisms underlying the pathogenesis of UCEC are to be elucidated. Here, we aimed to investigate the relationship between Protein Kinase CAMP-Activated Catalytic Subunit Gamma (PRKACG), and UCEC for the first time.

Materials and Methods: The bioinformatics tools, UALCAN and gene expression profiling interactive analysis 2 (GEPIA2) were used to analyze differential expression and the relationship of gene expression with tumor stage in UCEC patients. Besides, to find microRNAs and metabolites associated with DEGs, TargetScan and HMDB were used, respectively. Additionally, the functional enrichment analysis was performed for PRKACG co-expressed genes in UCEC using the Enrichr database.

Results: We found that the mRNA expression of the PRKACG was down-regulated in UCEC (P value=0.0015). This low expression was also correlated with tumor stage. MiRNAs analysis showed that miR-3064-5p, miR-326, miR-1298-5p, miR-381-3p, miR-504-5p.1, miR-149-5p could target PRKACG significantly. The co-expressed gene analysis using HMDB

showed that PRKACG was linked to different metabolites such as, 1H-Indole-3-acetaldehyde, 5-hydroxy, Serotonin, and S-Adenosylhomocysteine. The functional enrichment analysis revealed the top related biological processes (cerebellar granule cell differentiation, cerebral neuron cell differentiation, and regulation of vascular smooth muscle contraction) in GO enrichment analysis, and also KEGG pathway analysis showed several pathways associated with UCEC.

Conclusion: Taken together, PRKACG, miRNAs, and metabolites might be used as novel biomarkers for UCEC, as well as for diagnosis and guiding therapeutic strategies. However, further studies are required to confirm these results.

Keywords: Bioinformatics, Diagnosis, Tumor Differentiation, Uterine Corpus Endometrial Carcinoma

P-87: Correlation between Meiosis and Homologous Recombination Genes Expression with Diminished Ovarian Reserve

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Background: Diminished ovarian reserve (DOR) is a common disease that is identified by a decrease in ovarian reserve from normal range. DOR is a multi-factorial disease that affects 10% of women seeking fertility treatment. Although DOR is common, its etiology is less well known. Studies show that genes involved in the meiotic recombination pathway are associated with ovarian reserve; among them, we selected StAG3, MCM8, DMC1, FANCM, RBBP8 and MSH5 genes which only their mutations had previously been reported in ovarian reserve diseases. The goal of this study was to evaluate the expression of genes involved in meiosis in granulosa cells of women with DOR.

Materials and Methods: Follicular fluid was collected from 10 women with DOR and 10 healthy women as control group, referred for male infertility factors, and isolating of granulosa cells with minimum epithelial cells was processed. RNA extraction, cDNA synthesis and RT-qPCR were performed. Gene expression was compared between DOR and control groups and P value<0.05 was considered as a significant difference.

Results: Expression of StAG3, MCM8, DMC1 and FANCM

genes greatly decreased ($P < 0.001$) and RBBP8 gene downregulated ($P < 0.007$) in women with DOR. Although MSH5 gene expression in DOR was lower than controls, it was not statistically significant ($P < 0.078$).

Conclusion: According to downregulation of StAG3, MCM8, DMC1, FANCM and RBBP8 genes, the relationship between meiotic genes alteration with ovarian reserve seemed to be crucial. These data are useful to better understand DOR etiology for providing more appropriate medical services.

Keywords: Homologous Recombination, Meiosis, Ovarian Reserve

P-88: Evaluation of TSH Level in The First Trimester of Pregnancy in Women with No History of Thyroid Disorder referred to Imam Khomeini and Razi Hospitals of Ahvaz

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Background: Pregnancy causes physiological changes in maternal thyroid function tests and changes are sometimes confused with thyroid abnormalities therefore the aim of this study was to determine the TSH levels in the first trimester in pregnant women without a history of thyroid disease. And to determine the importance of screening of thyroid diseases in early pregnancy.

Materials and Methods: This is a descriptive analytic study on 1200 women in the first trimester of the pregnancy that referred to prenatal clinic for routine prenatal care, for each case a checklist was completed containing demographic information (age, weight and height), as well as the variables required for research (history of previous pregnancy, history of medical diseases and family history of thyroid disease). In the quantitative variables, the mean of the data center and the standard deviation was used to describe the scattering of data. Prevalence and percentage were used to describe the data in qualitative variables. t test and Chi-square test were used to analyze the data. The normalization of the Kolmogorov-Smirnov test was studied. The significance level was considered to be 0.05 All analyzes were performed using SPSS software version 22.

Results: Seventy percent of our cases were aged between 25 and 35 years old. The mean age of pregnant women was 29.44 ± 0.13 and the mean of TSH level in the first trimester of pregnancy was 2.40 ± 0.49 . There was no significant relations between different age groups, abortion, number of pregnancies and diabetes in our cases and TSH level ($P > 0.05$). However, there was a significant correlation between BMI, family history of thyroid diseases and hypertension in pregnant women without past medical history of thyroid diseases and TSH level in the first trimester of pregnancy ($P < 0.05$).

Conclusion: The results of this study showed that TSH level in majority of our cases ranging from 2.5 to 3.9 mIU/ml. 310 (25%) cases more than 2.5 mIU/ml and 188 (15.66%) cases more than 3.9 mIU/ml. According to this approximately 16% of our cases need assessment for hypothyroidism and required treatment. If screening of TSH in first trimester was not done 188 cases of hypothyroidism would not have diagnosed. It is reasonable to perform this test in first visit of pregnancy routinely.

Keywords: First Trimester, Hypothyroidism, Thyroid Function, TSH

P-89: The Effect of Administration Licorice Root Extract on The Improvement of Polycystic Ovary Syndrome

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Background: Polycystic ovary model is considered as a factor in induction of oxidative stress within ovarian tissue. Chemical and herbal extract antioxidants can be used to counter with effects of these agents. In this study, the preventive effects of licorice root extract treatment as antioxidant on a mouse model of polycystic ovary was assessed.

Materials and Methods: 5 adult NMRI female 6 weeks' mice, after 2 months model induction with a single injection of Estradiol Valerate (40 mg/kg), were assessed by measurement of oxidative stress and histopathology method. Then 60 mice were divided to 4 groups: control, sham, experimental groups 1 and 2. Experimental group 1 received a single injection of Estradiol Valerate (40 mg/kg). Experimental group 2 received a single injection of Estradiol Valerate following by 450 mg/kg licorice root gavage for 21 consecutive days. In this study, oxidants and antioxidants levels in the ovarian tissue were assessed by flow-cytometry and FRAP and histological study by morphometric method, embryo development with IVF. These results were analyzed by ANOVA and Chi square tests.

Results: The obtained results showed that Estradiol Valerate (40 mg/kg) are able to increase oxidative stress within ovarian tissue and causes ovarian cysts after two months. In addition licorice root extract treatment (450 mg/kg) can significantly ($P \leq 0.05$) increase antioxidants concentration and percentage of *In Vitro* fertilization compared to the other groups. The correlation between antioxidants concentration and ROS levels showed that their relationship in polycystic ovary syndrome (PCO) group and licorice root group was strong inverse, while that in two other groups were moderate inverse. Cyst formation in licorice root group decreases compared to PCO group.

Conclusion: Licorice root extract treatment (450 mg/kg) could prevent cyst formation in PCOs group, improvement of *in vitro* fertilization and made balance between oxidants and antioxidants inside ovarian tissue. The medicinal properties of plants could be due to the antioxidant compounds of them.

Keywords: Antioxidant, Estradiol Valerate, Licorice Root, Polycystic Ovary, *In Vitro* Fertilization

P-90: In Silico Study of Investigation of Overexpression Genes in Umbilical Cord Blood of Pregnant Women Exposed to Covid-19 and Vertical Transmission to The Fetus Talebizadeh F^{1*}, Dabbagh MR¹, Kazemi H²

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Background: One of the challenges associated with the COVID-19 pandemic was the impact of the SARS-CoV2 virus on pregnant mothers and, subsequently on their infants. While there have been reports of neonatal infections, no direct evidence of

vertical intrauterine transmission has been found. This bioinformatics study aimed to investigate the overexpression of genes involved in the SARS-CoV2 signaling pathway in the umbilical cord blood of pregnant women exposed to COVID-19. These findings lead to a better understanding of the effects of exposure to COVID-19 in the early stages of life, vertical transmission from mother to fetus, and therapeutic targets.

Materials and Methods: The gene expression profile of GSE195938, selected from the Gene Expression Omnibus database, was analyzed for differential gene expression between exposed to COVID-19 and control term neonates by Transcriptome Analysis Console software.

Results: Overexpression of 14 essential genes involved in the SARS-CoV-2 signaling pathway, such as RAPIGDS1, SMAD5, TRIM59, etc., was observed in cord blood samples of pregnant women exposed to COVID-19. (P value<0.05, Fold-change > 1).

Conclusion: In studies to date, no positive RT-PCR results were observed in amniotic fluid, placenta, or umbilical cord blood, and the probability of vertical transmission was not reported. Despite the overexpression of 709 genes in samples exposed to COVID-19 compared to normal, we see no difference in ace2 gene expression. Nevertheless, 14 overexpressed genes are involved in the SARS-CoV-2 signaling pathway, which may indicate indirect effects of coronavirus on the fetus and vertical transmission.

Keywords: COVID-19, Neonatal, SARS-CoV-2 Virus, Vertical Transmission, Pregnancy

P-91: Metformin Reduces Cyclophosphamide-Induced Ovarian Damage by Maintaining Ovarian Reserve

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Background: Activation of primordial follicles appears to be a common side effect of cyclophosphamide for patients receiving chemotherapy through over-activation of the AKT/mTOR pathway. This has a detrimental effect on fertility since the density and proportion of intact primordial follicles are important factors in the reproductive health of women. In this study, we used metformin, a drug used to treat type 2 diabetes as well as polycystic ovary syndrome, as an AKT/mTOR pathway inhibitor.

Materials and Methods: Prepubertal NMRI female mice were divided into three groups: Control, cyclophosphamide (cyc), and cyclophosphamide treated with metformin (cyc-met). In the cyc group, in every three days, one dose of 65mg/kg cyclophosphamide was injected intraperitoneally for three times. In cyc-met group, two days before starting cyclophosphamide injection, metformin was injected in a dose of 150 mg/kg for 11 consecutive days. The mice were sacrificed 24 hours after the last metformin injection, and the ovaries were gathered for histological evaluation.

Results: Degeneration of primordial and growing follicles was similar between all groups. The counting of the primordial follicle number showed that the ovarian reserve in the cyc-met group was similar to that of the control group, and it had a significant increase compared to the cyclophosphamide group (P=0.0005). On the other hand, the number of growing follicles in the cyc-met group significantly reduced compared to the cyc group (P=0.0002).

Conclusion: The presented data show that metformin may prevent cyclophosphamide-induced ovarian damage, which over-activates the AKT/mTOR pathway.

Keywords: Cyclophosphamide, Metformin, Ovarian Reserve

Genetics

P-92: Association of Galt Gene Variant Rs2070075 with Iranian Primary Ovarian Insufficiency Patients

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Background: Primary ovarian insufficiency (POI) is the deficiency of ovarian follicles with interruption of menstruation before the age of 40. GALT is one of the genes whose defect can lead to POI besides galactosemia. Due to association of GALT's rs75391579 variant with POI in galactosemia and its presence in the Iranian general population, we aimed to determine the association of rs75391579 and its neighbor variants with POI in Iranian patients.

Materials and Methods: According to ESHRE guidelines 2015, DNA samples were obtained from 94 primary POI patients' blood. Iranome, the Iranian ethnical genome variation database which includes 100 healthy individuals from each of the eight great ethnic groups, was used as the control. Polymerase Chain Reaction (PCR) and Sanger sequencing for the region of interest were done using a 655 base-pair PCR product from intron 5 to exon 7 of GALT. Sanger sequencing data analysis was conducted by FinchTV. Chi-square and Fisher's exact test were used to compare the genotype and allele frequencies between the POI and control groups.

Results: The exonic C>T rs2070075 and the intronic G>A variants (rs2277202, rs41274867) were detected in the studied patients. The genotype frequencies of rs2070075 were CC (91.4%), CT (4.3%), TT (4.3%) in the POI group and CC (84.7%), CT (14%), TT (1.3%) in the control group. The allele frequencies in the patients and controls were 93.6% and 91.8% for C and 6.4% and 8.3% for T alleles, respectively. There was a remarkable significance between the genotype distribution of rs2070075 between groups (P=0.002) but not in allele frequencies (P=0.374). No significant differences were observed for

rs2277202 and rs41274867 between the groups. rs75391579 was normal in the studied patients.

Conclusion: Since four of the eight patients in which C>T rs2070075 was found were homozygous TT, this form of rs2070075 might be correlated with Iranian POI patients. Further investigation is recommended.

Keywords: GALT, Genetic Variants, Primary Ovarian Insufficiency

P-93: Paternal Balanced Reciprocal Translocation, 46 XY, t (6:13) (p22.2; q11.1) Resulted in Blighted Ovum following Assisted Reproductive Technique

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Background: Infertility is an important problem involving a lot of couples in the world. One of causes of infertility can be chromosomal rearrangements such as translocations. In this study, we explained the outcome of intra-cytoplasmic sperm injection (ICSI) cycle of an infertile couple with translocation.

Materials and Methods: A couple with history of 3 years' infertility referred to our infertility clinic. Semen quality of the husband was normal according to WHO 2021. The couple had a history of one failed intra uterine insemination (IUI) and also one failed ICSI cycle. After evaluation of laboratories tests and physical examination, the couple referred to genetic center for karyotyping. The wife showed normal karyotype, although the husband presented balanced reciprocal translocation between short arm of chromosome 6 and long arm of chromosome 13 [46 XY, t (6:13) (p22.2; q11.1)]. While couple was consulted to do PGD/ICSI cycle, they persist on ICSI cycle only. Following hormonal stimulation, 6 oocytes retrieved following flushing. After sperm injection, fertilized oocytes were cultured for 3 days. 4 good quality embryos were frozen. 2 days before embryo transfer, thawing was performed and embryos were cultured for 2 other days.

Results: 2 embryos arrived to blastocyst stage (1 expanded and 1 early) and transferred. After 14 days, β -hCG was evaluated and showed positive result. Sonographic evaluation in sixth week following embryo transfer showed an empty gestational sac.

Conclusion: Paternal balanced reciprocal translocation, 46 XY, t (6:13) (p22.2; q11.1) resulted in blighted ovum following ICSI cycle.

Keywords: Blighted Ovum, Chromosomal Rearrangements, Infertility, Karyotype, Translocation

P-94: Increased Expression of BARX1 and PITX2 Genes in Plasma of Patients with Endometriosis

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Background: Endometriosis is an inflammatory and estrogen-dependent disease that causes infertility-affects 15-20% of women worldwide- and pain. Since endometriosis is a multifactorial disease, a definitive and non-invasive diagnostic method is not available for diagnosis of this disease. Genetic factors play an important role in the pathogenesis of endometriosis. Previous royan study on expression of HOX cofactor genes showed higher levels of two homeobox transcription factors named BARX1 and PITX2, in ectopic tissues of women with endometriosis vs. normal endometrium. We hypothesized that the mRNA of these genes may be found in the circulation of endometriosis patients. The aim of this study was to investigate the expression levels of BARX1 and PITX2 genes in plasma samples instead of endometrial lesions, as potential biomarkers for noninvasive and early detection of endometriosis.

Materials and Methods: We collected peripheral blood samples from non-endometriosis and endometriosis women (3 cc per person, n=4, each group), after obtaining written consent according to the local ethical approval. The plasma fraction was separated by centrifuge. Then RNA was extracted from plasma samples and the expression levels of BARX1 and PITX2 genes were evaluated by qRT-PCR technique.

Results: Our data showed the levels of BARX1 and PITX2 mRNA are increased in plasma of women with endometriosis compared to normal women.

Conclusion: Current study implies the potential role of BARX1 and PITX2 genes as suggested biomarkers for investigation and developing of non-invasive diagnosis methods of endometriosis pathology.

Keywords: BARX1, Endometriosis, Gene Expression, Plasma, PITX2

P-95: Evaluated miRNA Gene Expression in Human Granulosa Cells Derived from PCOS Patients and Controls: In Silico Study

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Background: Polycystic ovary syndrome (PCOS) is an endocrine disorder in women of childbearing age and the most common cause of infertility without ovulation. MicroRNAs (miRNAs) are short, non-coding RNAs involved in regulating gene expression at the post-transcriptional level. In recent years, there has been increasing evidence that abnormal expression of miRNAs in granulosa, leukocytes, adipose tissue, serum, and peripheral blood leukocytes is diagnosed in women with polycystic ovary syndrome and plays a crucial role in the development and progression of PCOS. This study investigated the expression of miRNAs in granulosa cells and their role in PCOS.

Materials and Methods: First, the keywords PCOS and granulosa cells were searched in the GEO DataSets database, and the data of GSE84376 were selected from the GEO DataSets database. Their results were also reviewed and analyzed in the Pubmed database.

Results: Considering the P value<0.05 and Log₂ FC>3, miRNAs has-miR-3188 and has-miR-1231 showed the greatest change in expression in patients with PCOS compared with controls.

Conclusion: The results of this study indicate that increased expression of some miRNAs may play a role in the development and progression of PCOS and that miRNAs can be used as biomarkers for the diagnosis of PCOS patients in healthy women. However, further *in vivo* and *in vitro* studies on their role in PCOS are needed. This may also lead to a new understanding of the mechanism of PCOS and may be helpful in identifying new miRNA therapeutic targets.

Keywords: Granulosa Cell, miRNA, miRNA Expression, Polycystic Ovary Syndrome

P-96: APOE Gene Polymorphism as A Risk Factor for Implantation Failure: A Comparison between Fertile Women, ART Success Cases and RIF Patients

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Background: Multiple genetic as well as uterine factors may affect endometrial receptivity during window of implantation (WOI). APOE is one of the genes that has been associated with increased endometrial expression during WOI. APOE is a polymorphic gene with 3 major allelic forms (E2, E3 and E4) and main precursor of cholesterol and steroid hormones. In this study we compared frequency of APOE variants in fertile

women, ART success cases and RIF patients.

Materials and Methods: 100 women with successful ART who became pregnant (FHR+) in their first or second cycle of IVF or ICSI, 100 infertile RIF cases and 100 normal fertile controls were included. Genotyping was done by using PCR+RFLP with HhaI enzyme. Statistical analysis performed by chi-square and Fisher Exact test in SPSS (p-value<0.05).

Results: Genotype frequencies in RIF patients, ART success group and fertile women were: E2/E3: 5, 5, and 8%, E3/E3: 66, 82, and 77%, E3/E4: 29, 13, and 15%, respectively. Based on statistical analysis RIF group had significantly higher frequency for E3/E4 genotype compared to the other two control groups (P value=0.007). The allele frequencies in RIF, ART+ and fertile groups were: E2: 2.5, 2.5, and 4%, E3: 83, 91% and 88.5%, E4: 14.5, 6.5, and 7.5%, respectively. There was significant difference for E4 allele frequency in RIF group (P value=0.007) compared to other groups.

Conclusion: According to our results we suggest that APOE4 polymorphism can be considered as a potential risk factor for human embryonic implantation.

Keywords: Assisted Reproductive Technology, APOE Polymorphism, Endometrial Receptivity, Recurrent Implantation Failure

P-97: Association between The C161T Polymorphism of The PPARγ Gene and Asthenozoospermia Infertile Men Referred to Royan Institute

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Background: Peroxisome proliferator activator receptor gamma (PPARγ) is a nuclear transcription factor, which mainly regulate the expression of target genes involved in lipid and energy metabolism. Energy from glucose and fat metabolism mediated by PPARγ signaling is required for sperm motility, affecting male fertility. In the testis, PPARγ protein is detected at Sertoli cells & spermatocytes. One of the most important single nucleotide polymorphisms of this gene is C161T, which is associated with decreased transcription of PPARγ. This polymorphism is in linkage disequilibrium with other mutations of the gene that regulate the activity of PPARγ and its association with asthenospermia has not been studied so far.

Materials and Methods: In this case-control study, genomic DNA was extracted from blood samples of 60 infertile men with asthenospermia and 70 fertile controls. PCR-RFLP (Restriction Fragment Length Polymorphism) with PmlI enzyme

was performed to screen the aforementioned polymorphism and the results were confirmed by Sanger sequencing.

Results: Results showed that among 60 infertile men, 73.33% had CC genotype, 25.00% had CT genotype and 1.66% had TT genotype. Of the 70 fertile controls, 72.85% had CC genotype, 27.14% had CT genotype and 0% had TT genotype. The obtained data showed no significant association between two groups ($P=0.748$).

Conclusion: It is concluded that C161T polymorphism cannot be a risk factor for asthenozoospermia infertile men in the current sample size of Iranian populations.

Keywords: Asthenozoospermia, C161T, Polymorphism, PPAR γ

P-98: A Comparison of The Efficiency of RNA Extraction from Extracellular Vesicles of Endometriosis Patients Using Two Different Methods

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Background: Extracellular vesicles (EVs) involved in multiple physiological processes and are major contributors through progression of disease. As EVs contain lipoproteins, DNA, mRNA and are present in body fluids, are good candidate for studying diseases by minimally invasive methods. Their role in a variety of diseases; has been widely studied but little is known about their role in endometriosis. Most gene expression studies of endometriosis are performed on endometrial tissue, which has an invasive sampling process. Our main goal is to study of gene expression in endometriosis using EVs which are present in the circulation instead of endometriotic tissues.

Materials and Methods: So, we optimized EV isolation from plasma of endometriosis patients and compared two methods of RNA extraction from EVs. We conducted a pilot study based on ethical approval on blood samples of 4 endometriosis women referred to Royan Institute after consent was obtained from them. Plasma was divided into two parts. First part was used to separate EVs using ultracentrifuge followed by characterization by morphology, DSL and western blot (CD81, CD9, TSG101).

Characterized EVs and second part of plasma (without further manipulation) were used to RNA extraction using Plasma/serum circulating and exosomal RNA purification kit. After cDNA synthesis the expression of EN gene (which is highly expressed in endometriosis) evaluated quantitatively by real-time PCR.

Results: The results showed that direct extraction of EV RNAs from plasma is more efficient than RNA extraction from isolated EVs using ultracentrifuge, and yielded lower CTs through real-time PCR.

Conclusion: These data imply the importance of selectin of the appropriate technique in the study of EVs as small and delicate samples that eliminate the need for invasive methods.

Keywords: Comparison of RNA Extraction, Endometriosis, Extracellular Vesicles, Gene Expression

P-99: The Subfamily of DEAD-Box RNA Helicases Expression between Human Testis of Patients with Non-Obstructive Azoospermia and Normal Cells

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Background: DEAD-box RNA helicases are essential regulators of RNA metabolism and have been implicated in the development of man reproduction. These helicases constitute a major recurring family of RNA-binding proteins vital for protecting the genome.

Materials and Methods: We used microarray to examine the 54 DEAD-box RNA helicases subfamily expression genes in the five human testis sections of the normal case, azoospermic patients and utilized in-silico databases to predict pathways functional and molecular enrichment. Current studies have provided insight into the link between genomic stability and several DEAD-box RNA helicase families, including DDX17, DDX11, DDX49, and DDX31.

Results: The microarray analysis of human cases with different levels of non-obstructive Azoospermia revealed that expression of DDX17 was up-regulated and expression of DDX49, DDX11, and DDX31 was down-regulated with the normal case. For this purpose, Enrichr Shiny GO databases were used for pathway enrichment analysis and gene ontology. StRING was applied to predict proteins' functional and molecular interactions and then performed to recognize the master pathways. Functional enrichment analysis showed that the biological process (BP) term "Ribosome biogenesis" and "rRNA modification in the nucleus and cytosol" was significantly overexpressed in up-regulated differentially expressed genes (DEGs). BP investigation of down-regulated DEGs highlighted "RNA binding", and "Spliceosome". Overrepresented molecular function (MF) terms in up-regulated DEGs included "double-stranded RNA binding" and "ubiquitin-protein protease activity". In addition, MF analysis of down-regulated DEGs showed overexpression in "acetylation-dependent protein binding" and "Adenosine riboprotein binding".

Conclusion: Our findings suggest that the DEAD-box RNA helicases subfamily can help determine the pathophysiology of non-obstructive Azoospermia.

Keywords: Azoospermia, DEAD-Box RNA Helicases, Infertility

P-100: The Study of Common Breakpoints in Balanced Chromosomal Abnormalities Related to Male Infertility in Patients Referred to Royan Institute: 2009-2021

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Background: Finding common chromosomal breakpoints in infertile men carrying balanced chromosomal translocations is significant since these patients usually possess normal clinical features. Numerous studies have been performed on the translocations of various chromosomes. However, there has not been any report of the study of common breakpoints in balanced chromosomal abnormalities related to male infertility in Iran.

Materials and Methods: 2,500 sample files of patients referred to the Royan Research Institute from 2009 to 2021 were reviewed, of which, 391 cases met the input criteria (samples with balanced translocations). The affected regions were obtained using the breakpoints in their karyotype.

Results: Out of 391 patients' files, inversion accounted for the largest share, followed by reciprocal translocation, Robertsonian translocation, and insertion. The prevalence of balanced translocations showed that the highest balanced translocations were related to three chromosomes 9, 3, and 1, in which 9q12, 1p36, and 3p11 were the most frequent breakpoints. Chromosomes 9, 22, and 14 had the largest proportion in inversions, reciprocal translocations, and Robertsonian translocations, respectively. The frequency of normal chromosomal variations and breakpoints in the relevant chromosomes showed a possible relationship between normal variations and infertility. This important finding suggests that, despite existing hypotheses, normal variation is likely associated with male infertility.

Conclusion: Chromosomes 9, 1, and 3 showed the highest incidence of breakpoints among chromosomes, respectively. 9q12, 1p36, and 3p11 were the most common breakpoints in these prevalent chromosomes. These data can be used to suggest appropriate treatment methods and to prevent carrying of abnormal chromosome to the next generation.

Keywords: Chromosomal Abnormalities, Common Breakpoints, Male Infertility

P-101: Differential Expression of ARX and OTX2 Genes in Blood Samples of Women with Endometriosis versus Healthy Control

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Background: Endometriosis is a multifactorial disease with an unknown origin, in which endometrial-like tissue forms outside the uterine cavity. Because of the invasiveness of current diagnostic methods, the lack of specific biomarkers, and also a long diagnostic delay after onset of symptoms, specific biomarker for non-invasive diagnosis of the disease is necessary. Previous study at Royan Institute demonstrated that the mRNA expression of ARX and OTX2 (homeodomain-containing genes) were significantly upregulated in ectopic tissues of endometrium compared to normal endometrium. The purpose of this study is to answer this question: Is the expression profile of ARX and OTX2 genes different in the plasma of women with endometriosis versus healthy control?

Materials and Methods: Peripheral blood samples were collected from endometriosis women in III and IV disease stages, as well as healthy women (male factor) referred to Royan Institute (n=4, in each group), after obtaining written constant according to the local ethical approval. The plasma fractions were separated by centrifuge. RNA extraction was performed on the plasma samples of both groups, and the expression levels of candidate genes were evaluated by qRT-PCR technique.

Results: ARX and OTX2 genes showed significant increased expressions in plasma of the endometriosis group compared to the control group.

Conclusion: Our results suggest ARX and OTX2 genes as potential biomarkers for the non-invasive diagnosis of endometriosis in the future.

Keywords: ARX, Endometriosis, Gene Expression, OTX2, Plasma

P-102: Are Variants of NOBOX's Exon 4 Associated with Premature Ovarian Insufficiency?

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Background: Premature ovarian insufficiency (POI) is a disease that disrupts the normal function of the ovaries in women before the age of 40 and Several factors have effects on its occurrence. The newborn ovary homeobox gene (NOBOX) is an oocyte-specific gene that plays an important role in folliculogenesis and oocyte development. NOBOX is one of the genes related to POI. The aim of this study was to investigate the association between NOBOX and POI in Iranian patients.

Materials and Methods: Through an in silico preliminary approach, the reported NOBOX single nucleotide variants (SNVs) in ClinVar as a valid database were investigated for classification of the genetic variations considering their clinical importance. Next, the ClinVar SNVs was checked in Iranome, an exome sequencing-based database for frequency of SNVs in Iranian general population; Then, the SNVs that had been reported in both ClinVar and Iranome excluding benign and likely benign SNVs were selected. The wet lab study was performed on 100 Iranian women with POI. The inclusion and exclusion criteria were based on the ESHRE 2015 guideline. The polymerase chain reaction (PCR) and sanger sequencing were performed on blood DNA. Sanger sequencing analysis was conducted by finch tv.

Results: rs187273709, which is located in exon 4 of NOBOX, was targeted because it has been previously reported in the Iranian general population according to the Iranome database and its uncertain clinical significance in POI. A 709 base pairs PCR product was used for Sanger sequencing. NOBOX's exon 4 in addition to some of its upstream and downstream intronic bases were investigated. The rs187273709 was normal in our patients and no other variants were found.

Conclusion: Based on the Iranome we expected to find rs187273709 in the studies patients, however no one carried this variant. Although this variant does not seem to be frequent in Iranian POI patients, further investigation with larger sample size could be helpful for prioritization of the NOBOX variants testing in Iranian patients with POI.

Keywords: NOBOX, Premature Ovarian Insufficiency, Sanger Sequencing

P-103: Crucial Role of Meiosis Recombination and DNA Repair Genes in Diminished Ovarian Reserve (DOR)

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Background: Diminished ovarian reserve (DOR) refers to women under the age of 38 years, whose number and quality of the oocyte pool in their ovaries have decreased. DOR does not have an obvious phenotype whilst early detection of DOR is very useful to increase the chances of successful fertility. Recent studies have shown that the pathogenesis of DOR is likely to be multifactorial. Little is known about the molecular and genetic factors associated with ovarian reserve. DNA double-stranded breaks and failure to repair them seemed to be one of the main factors for decrease in ovarian reserve according to reported mutations in pedigree analyses with ovarian reserve diseases. The relationship between alteration of genes associated with meiosis and homologous recombination with DOR has not been studied so far. The aim of this study was to evaluate the expression level of MSH4, PSMC3IP, HFM1, SYCE1 and MCM9 genes, involved in meiosis and homologous recombination, in granulosa cells of women with DOR.

Materials and Methods: Granulosa cells were isolated from follicular fluid of 10 women with DOR and 10 healthy control women, referred for male factor infertility, with minimum number of epithelial cells. RNA extraction and CDNA synthesis were performed. The expression level of the genes was assessed by using q-RT PCR. p value <0.05 was considered as statistically significant differences.

Results: The expression level of MSH4, PSMC3IP and SYCE1 genes in women with DOR compared to controls greatly decreased (P<0.001), additionally MCM9 gene expression in DOR significantly decreased (P=0.009). Although HFM1 gene also decreased in the DOR group but it was not statistically significant (P=0.112).

Conclusion: Due to significant reduction in the expression level of genes involved in meiosis recombination and DNA repair including MSH4, PSMC3IP, SYCE1 and MCM9, these pathways seemed to have a crucial role in quantity and quality of oocyte pool certainly in DOR.

Keywords: Homologous Recombination, Meiosis, Ovarian Reserve

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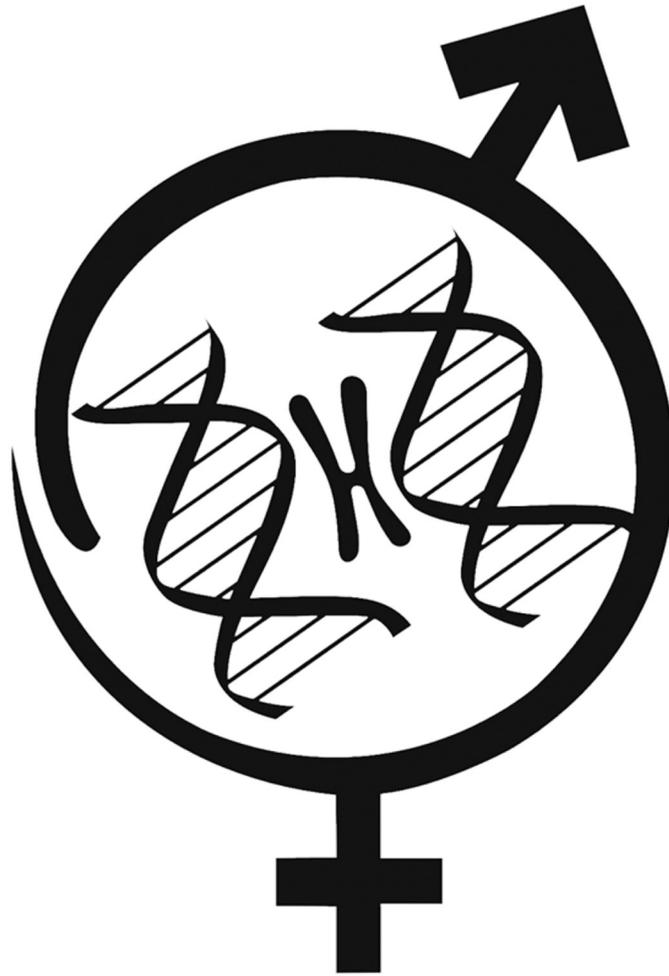
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Abstracts of
Royan International Hybrid Twin Congress

17th Seminar on Nursing and Midwifery
7-8 September 2022



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Invited Speaker

Inm-1: The Genetic of Premature Ovarian Insufficiency

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Premature ovarian insufficiency (POI) is the cessation of menstruation before the age of 40 (1% of normal population) and characterized by amenorrhea, hypogonadism and elevated gonadotropin level.

POI is a heterogeneous disease caused by a variety of mechanisms, including genetic, autoimmune, Iatrogenic and Idiopathic. Though the underlying cause remains unexplained in the majority of cases (70%), various data indicate that POI has a strong genetic component.

The most common genetic cause of POI is the X-Chromosome abnormalities (eg, turner syndrome or X-Structural abnormalities). Autosomal and X-linked mutations on the genesis of POI has also been well described. Yet in most cases, the genetic origin remains unknown and there are multiple candidate genes.

In this review, I am going to introduce some of genetic abnormalities and genes associated with syndromic and non-syndromic POI.

The identification of several causative genes with GWAS and NGS studies may allow for early detection and would provide better opportunity for early intervention, and will help direct potential targets for future treatment.

Inm-2: The Role of Midwives and Nurses in Psychological and Emotional Counseling and Support in Patients with Decreased Ovarian Reserve

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Infertility is an event that alters life and produces experiences that cause anxiety. Recent research has noted that patients have insisted that ongoing emotional support and stress counseling should be part of IVF treatment. This presentation discusses how Infertile women with a low ovarian reserve who are undergoing infertility assessments and treatments might experience anxiety and sadness, as well as what midwives and nurses can do to help them manage this anxiety.

Studies have shown there is a need for midwives and nurses to offer special care for infertile women who suffer from low ovarian reserve. The main themes identified are the need to ensure continuity of care and support at all stages of treatment, particularly prior to pregnancy tests, during pregnancy as well as Prenatally, and especially after unsuccessful treatment.

Keywords: Infertility, IVF Clinic, Quality of Health Car

Inm-3: Fertility Preservation in Poor Ovarian Reserve Patients

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Poor ovarian reserve (POR) patients have a limited reproductive lifespan to conceive with their own eggs so clinicians should use controlled ovarian stimulation (COS) protocols which maximize the number of achieved oocytes. Majority of these women need to undergo IVF and accept lower oocytes number, lower pregnancy rate and higher pregnancy loss compared to age-matched women with normal ovarian reserve. Because of oocytes quality and quantity reduction, shortage of reproductive lifespan, decision to delay conception and other reasons; infertility risk increases and patients should be counselled about fertility preservation options. Embryo, oocyte and ovarian tissue cryopreservation can be performed as fertility preservation strategies. First choice is embryo cryopreservation that is even more widely available and long-established part of assisted reproduction. But if it is not applicable at the time of puncture, oocyte cryopreservation can be performed. Ovarian tissue cryopreservation is the last and important choice especially when there is no time for ovarian stimulation but according to ESHRE guideline it should probably not be offered to patients with low ovarian reserve.

Inm-4: Epidemiology of Diminished Ovarian Reserve

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Diminished ovarian reserve (DOR), as a common reason for referral to fertility centers, is a condition characterized by a decline in the quality and/or quantity of oocytes in the ovaries. DOR with advanced age is a normal physiologic occurrence but about 10% of all women are at risk of DOR earlier in their reproductive lifespan. In infertile women, the incidence of DOR ranges from 6 to 64% of different ages. Repeated ovulation failure, ovulation cancellation, unavailability of embryos, and failure of implantation influences these women both physiologically and psychologically. Estimates from the US based national Society for Assisted Reproductive Technology (SART) system show 32% diagnosis of DOR among *in vitro* fertilization (IVF) cycles. However, a research using two years of SART data concluded that DOR is likely to be over-diagnosed as the definition of DOR is not standardized nor specific. This talk is about the reports on epidemiology and outcome of DOR in recent studies.

Inm-5: Postoperative Management of Reconstructive Surgeries

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Sometimes, in the course of treating infertility, it is necessary to perform reconstructive surgeries to correct some of the causes of infertility. Hydrosalpinx and ismosele can be mentioned among these disorders.

In patients with identified hydrosalpinx, implantation and pregnancy rates are significantly lower than in patients suffering from other types of tubal injuries. Hydrosalpinx is used for

any obstruction of the distal tubules. Regardless of the cause, it means that a non-tubular infection such as adjacent appendicitis can also cause hydrosalpinx. Obviously, laparoscopy is the ultimate method for diagnosing hydrosalpinx and pelvic adhesion-related pathology, however, this method is very invasive and should be used when necessary. Therefore, investigation and treatment of hydrosalpinx helps to successfully treat infertility and increase the chance of pregnancy.

Ismocele is a diverticulum secondary to cesarean scar and can be seen in the form of a sac on the anterior walls of the uterus in the cervical canal. The first method of examining transvaginal ultrasound is at the end of the proliferative phase. Hysterosonography (SIS) is the best examination method, which has more quality and accuracy than transvaginal ultrasound. If the thickness of the myometrium in front of the defect is less than or equal to 2.2 in TVS ultrasound and less than or equal to 2.5 in SIS, the defect (ismocele) is considered large. Isoselele can be asymptomatic or accompanied by symptoms such as pelvic pain, long-term spotting, secondary infertility and affects the quality of life. Among the problems caused by ismocele, we can mention implantation in the scar site of previous cesarean section, abnormal replacement of the placenta, accreta, damage to the bladder, bleeding and premature delivery. Therefore, investigation and treatment of this issue is necessary. The choice of surgical method depends on the sonographic characteristics of the lesion, symptoms, and the patient's desire to become pregnant in the future. Improvement of pain and bleeding symptoms of patients after hysteroscopic and laparoscopic surgery has been reported up to 85%. In this lecture, we will discuss the postoperative management of reconstructive surgeries.

Inm-6: Social and Ethical Dilemmas Faced by Muslim Infertile Couples Worldwide with Respect to Gamete Donation

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Abstract

Inm-7: The Application of Imaging in Detecting Cesarean Scar Defects

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Assisted reproductive technology produced the world's first IVF baby, Louise Brown, in the UK in 1978; since then, many infertile couples have achieved a family through IVF. For the minority of couples where gamete donation is required, this remains a controversial and taboo topic within Islam. Whilst Sunni Islam absolutely forbids the use of third-party reproductive gametes/assistance, Shi'a Islam is somewhat more lenient towards this practice. Reproductive tourism has therefore become prevalent in Shi'a-majority Middle Eastern countries (Iran) that permit the use of donor gametes in assisted reproductive technologies; however, this continues to evoke a strong bioethical debate both within the Shi'a and the majority (85%) Sunni Muslim groups.

The social and ethical dilemmas faced by Muslim couples, both those living in Muslim majority countries, as well as in Western

countries, remain very similar and this paper will explore the progress made by scholars, legislators, physicians and patient groups to understand the pain these couples face from being childless and options that some of them take despite religious rulings on third party donation.

Inm-8: The Predictive Value of Serum Human Chorionic Gonadotropin (β -hCG) in Detection of Ectopic Pregnancy Following Assisted Reproductive Technology (ART)

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Ectopic pregnancy is a well-known risk of IVF. The rate of ectopic pregnancy is higher in pregnancies resulting from assisted reproduction technologies (ARTs) than in spontaneous pregnancies. Ectopic pregnancy is the most common reason for maternal mortality in early pregnancy and 5 to 10% of all maternal deaths.

The incidence of ectopic pregnancy after IVF generally ranges from 2.1 to 8.6% of all clinical pregnancies. Several factors were associated with increased risk of ectopic pregnancy after ART and include tubal factor infertility, use of assisted hatching, and intracytoplasmic sperm injection, fresh compared with frozen embryo transfers, day of embryo transfer, and the hormonal milieu specific to ovarian stimulation. Delay in detection of EP following ART has occurred in 12.9% of cases. EP rupture which occurred due to delay in its early diagnosis is responsible for more than a third of maternal mortality in the first trimester.

Early diagnosis of ectopic pregnancy is done by β -hCG titers and Transvaginal sonography. But ultrasound is not completely effective before 5-6 weeks of pregnancy.

Human chorionic gonadotrophin (HCG) is one of the prime physiological candidates for embryo-endometrial signal transduction, which can be detected in maternal blood as early as 6-8 days after fertilization. As a key pregnancy hormone secreted by trophoblast cells during initial implantation and subsequent pregnancy, maternal serum HCG is widely used in pregnancy tests. Moreover, HCG is regarded as the earliest reliable factor for predicting clinical outcomes after embryo transfer in IVF and intracytoplasmic sperm injection (ICSI) cycles. Higher initial maternal serum HCG levels are associated with better outcomes on ongoing pregnancies and live births, whereas lower HCG levels indicated poor prognosis such as biochemical pregnancy, miscarriage, ectopic pregnancy. Different studies have determined the cutoff value of HCG that can discriminate viable from non-viable pregnancies. However and ideally, each ART center should analyze its own data to determine HCG cutoff value based on its experience.

Inm-9: The Poor Responders, Definition, Etiology, Management

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Different authors have used different criteria to define POR.

At least two of the following three features must be present: I. Advanced maternal age (≥ 40 years), II. A previous POR (≤ 3 oocytes with a conventional stimulation protocol); III. An abnormal ovarian reserve test (i.e., AFC ,5–7 follicles or AMH, 0.5–1.1 ng/ml).

Prevalence: 10--15% of women undergoing IVF have a poor response.

Etiology:

Advanced maternal age, premature ovarian failure, Decreased ovarian reserve due to: Genetics, Surgery, Endometriosis, Radiation, Chemotherapy.

Diagnosis:

The diagnosis of Poor responders is based on:

Female age, clinical evaluation: no of follicles: 1-4, no of oocytes: 1-4, Peak Estradiol levels: < 500 pg/ml, excessive requirements of gonadotropins: > 450 IU.

Tests: Basal day 3 FSH, CC challenge test, inhibin B, AMH, ovarian and volume, antral follicle count.

In the poor responder there are: large doses of stimulation needed, less than optimal number of egg, low oocyte quality, high cancellation rate, low fertilization rate, low embryo number, low embryo quality, low pregnancy rate, low birth rate.

The management of poor responders:

Conventional treatment:

Modified stimulation with: Agonist protocol, antagonist, mini dose protocol, stop² protocol, short/ultra-short/flare protocol, micro dose flare protocol, natural cycle,

Adjuvant therapy: Growth hormone, androgens (Testosterone & DHEAS), soft protocols, r-LH, l-arginine, steroids, aspirin.

New treatment: Dostim protocol, natural and modified natural protocol, luteal estradiol, donor oocyte, PRP and stem cell therapy.

Inm-10: Acquired and Congenital Anomalies of The Uterus in Infertility

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Prevalence in infertile women and fertile women with normal reproductive outcomes is similar approximately 2-4%.

Congenital uterine anomalies: Septate uterus: poor septal blood supply, resulting in poor implantation efficiency and embryo growth.

Uterine myoma: 70% of all reproductive-aged women 5–10% of infertile women. There is a clear consensus that submucous myomas have significant adverse effect on clinical pregnancy rates and delivery rates.

Acquired uterine anomalies: Asherman Syndrome: Intrauterine adhesions develop as a result of trauma. Any insult severe enough to remove or destroy the endometrium can cause adhesions.

Endometrial polyps are hyperplastic endometrial growths with a vascular center and a sessile or pedunculated shape extending into the uterine cavity.

Chronic endometritis has been regarded traditionally as a distinct but uncommon cause of reproductive failure, but its true prevalence in infertile women is unknown.

Inm-11: Ethical and Legal Issues in Female Fertility Preservation

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Fertility preservation (FP) is a term referring to cryopreservation of oocytes, embryos or in special cases, female ovary tissue to achieve pregnancy in the future. FP is offered for two different situations: a. medical and b. non-medical. Medical situation or FP with Medical Reasons is mainly for patients undergoing radiotherapy or chemotherapy for cancer or some other diseases (e.g. invasive mole). As we know chemo and radiotherapy can destroy gametes and make the cancer patient infertile. So, FP is offered to make the future pregnancy possible.

As for every woman, menopause happens in their life and from that time no menstrual cycle happens, so no ovulation will be performed by body. Recently, age of marriage has been increased and there is a fear for woman to become infertile because of age. This is the situation of Non-medical FP in which eggs of the woman are cryopreserved for the future use.

Each type of FP (cryopreservation of embryos, eggs or tissue) and also FP for medical and non-medical reasons have their own ethical issues. Here we discuss the ethical issues in every situation separately.

FP for medical reason: It seems that overall, FP for medical reasons is ethically acceptable, but there are some ethical considerations in this procedure.

First, the procedure can be cryopreservation of embryo, oocyte or ovarian tissue. Embryo freezing is the oldest and the most promising procedure for FP, but is performed in two conditions: first producing embryos is normally for couples not for single person. Ethically, doctors must offer both embryo and egg freezing to married people, so, in case of any uncertainty in the couples' relationship, woman can freeze their eggs and decide for the usage in the future. For, mature woman, egg freezing is more promising than cryopreservation of ovarian tissue. Cryopreservation of ovarian tissue is used for immature girls or very invasive cancer in which, time is very important and there is no time for ovarian stimulation and oocyte pick-up. As said, it is not promising and cannot guarantee the future pregnancy at all. Second, as the prognosis of each cancer in different stages are different, it is very important for oncologist to explain the situation and FP possibilities to the patient and guide them to the FP centers. There are two problems in this regard: it is the physician's responsibility to inform the patient about FP procedure otherwise patient cannot know about such procedure and may lose their fertility after chemo/radiotherapy and normally in Iran, cancer patients are not well informed about the cancer situation and prognosis, so, they cannot decide about performing FP or not. A person can decide to do a procedure for being pregnant in the future only if they know about the prognosis of the disease and the survival rate which, very few physicians have adequate information about FP methods and believe that they must inform their patients about it. Also, cancer patients are seldom informed about their survival time because physician believe that it makes the patient hopeless and fragile, so they never give enough information about the prognosis and survival rate of the cancer. In this situation, as the patient does not know their life expectancy, they cannot make a good decision about choosing FP or not. Although it seems worthless to do FP for low life expectancy, but ethically it is the patient's choice.

One of the most important points is the possibility of transmission of cancer cells to the offspring. If this possibility is high, for example in ovarian cancers, it will put the offspring in danger. Although it is just a possibility, but must be considered.

There are some other considerations like breast cancer with estrogen and progesterone receptors which are sensitive to hormone therapy. In these cases, special ovarian stimulation (using letrozole) is needed for oocyte retrieval.

Some ethicists believe that it is unjustified that medical resources are used for poor prognosis or cancers with possibility of transmission of cancer to the offspring. Resource allocation to FP is not always justified and must not be offered to all. On the other hand, prevention of future infertility for women with reasonable life expectancy is a good reason that makes FP ethically acceptable.

It seems that informing the patient about the cancer situation and FP procedure is the most important point about FP and decision about the methods and procedures should be done by a triangle including oncologist, gynecologist and embryologist for best results. Detail counseling with the clients are necessary along with informed consent that for minors must be gotten from their parents.

FP for non-medical reason: As the age of marriage is increasing worldwide and people postpone their marriage or even childbearing because of education, social or economic states and etc... , infertility due to menopause became common worldwide. As the chromosomal abnormality in the oocyte is more common at higher ages, the risk of miscarriage and children with abnormalities is increasing too. Possibility to freezing the oocyte brings the hope for healthy child for women, by freezing their oocytes in a good age and then postpone the pregnancy to later.

The main ethical issue here is public information about this procedure is good or bad. First we shall say that: "people have the right to know". Meanwhile there are two opposite arguments: if we inform people about FP, this may change the trend of the society and increase the age of marriage or childbearing more and more, but if we don't inform, single or childless people realize the possibility of being infertile in their advanced age in which physicians can do nothing for them. So, it seems that we are responsible for public information about FP.

Detail counseling the clients about their situation, success rate of pregnancy in the future, possible complications of IVF, procedure phases and length and etc... is necessary.

Virginity is an issue in Islamic and Middle Eastern countries. It is obvious that FP procedure injures hymen. According to Islamic law, when a procedure is medically necessary, there is no need to think about virginity. An informed consent in which there is complete information about the procedure and possible injury to the hymen is enough and if some girls needed virginity confirmation certificate before operation, it is the duty of forensic medicine department where they can be referred.

So, it seems that FP both for medical and non-medical reasons is ethically acceptable with special consideration.

Keywords: Fertility preservation, Virginity, Islamic, Cancer, Menopause

Inm-12: Age, Disease and Its Effect on Male Fertility

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Aging and male fertility

Spermatogenesis never stops in male and testes produces sperm lifelong.

In the developed world, the age of first-time parenthood has increased steadily during the last decades (Schmidt et al., 2012). The effect of increased maternal age on reproduction has been widely studied, as it influences negatively pregnancy rate and oocyte quality while increasing the likelihood of aneuploidies in developing embryos (Hendershot, 1984; Munne et al., 1995; Benadiva et al., 1996).

However, the effect of paternal age on both sperm characteristics and reproductive outcomes has been comparatively less studied in the general population, and there is no common consensus on its role in reproductive success.

Multiple reports show a decline in seminal volume, motility and morphology with increasing paternal age (Dondero et al., 1985; Centola and Eberly, 1999; Kidd et al., 2001; Eskenazi et al., 2003; Hellstrom et al., 2006; Stone et al., 2013).

Discrepancies, however, remain when considering sperm concentration; while some studies describe a decline with age (Auger et al., 1995; Centola and Eberly, 1999; Aboulghar et al., 2007; Luna et al., 2009), others reported no association (Carlsen et al., 1992; Whitcomb et al., 2011) or even an increase in concentration (Irvine et al., 1996; Andolz et al., 1999).

the effect of paternal age in ART

As older men tend to have older partners, it is difficult to control for the effect of maternal age on oocyte quality

Paternal age was found to have a detrimental effect on pregnancy rates in cycles of conventional IVF adjusted for maternal age (Klonoff-Cohen and Natarajan, 2004),

Other studies only considered ICSI when investigating the effect of male age.

Unfortunately, the findings are still inconclusive, with some reports describing a significant decrease in both blastocyst and live birth rates in men over 50 years (Frattarelli et al., 2008), or a decline in implantation rates in men over 60 years (Luna et al., 2009), while others indicate a lack of effect of paternal age altogether (Gallardo et al., 1996; Luna et al., 2009; Whitcomb et al., 2011).

There was no significant association between male age and the average morphological score of the embryos generated.

Regarding the fertilization rate and mean embryo quality, our results show no differences among male age groups.

We found a positive relation between the morphological score and the fertilization success rate.

we found no correlation between male age and any of the reproductive outcomes

it is undeniable that natural conception and pregnancy rates after minor manipulations, such as intrauterine insemination, decrease with increasing male age, even after controlling for maternal age

Oral Speaker

Onm-1: Relationship between Ovarian reserve markers with Congenital Anomalies

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Background: Ovarian reserve is a measure of a woman's fertility life. Studies have shown that congenital uterine abnormalities can affect ovarian reserve. This study survey Relationship between Ovarian reserve markers with Congenital Anomalies.

Materials and Methods: The present case-control study was performed on 224 women in both case and control groups from 2016-to 2017 in Royan Center. The case group consisted of 81 women with infertility that had intramural congenital uterine anomalies. The control group consisted of 143 women who had infertility and did not have these lesions. The sampling method was continuous and available. The data collection tool was a questionnaire in two parts of individual variables. The second part was related to both groups' ultrasound results (number of antral follicles and ovarian volume) and laboratory (FSH and AMH) done on the third day of the cycle. Data analysis was performed using SPSS software and t-test and a chi-square test.

Results: The result showed that there was no statistically significant difference between the two groups in FSH ($P > 0.05$) but in AMH there was a statistically significant difference between the two groups ($P < 0.05$). Also, the results showed that the number of antral follicles in right ovarian, and the number of antral follicles in the left ovarian were not statistically significant between the two groups ($P > 0.05$). But the variables of the size of uterine, echo uterine, and intramural fibroma was statistically significant ($P < 0.05$).

Conclusion: The results showed that uterine abnormalities with different mechanisms such as changes in ovarian blood flow lead to a decrease in ovarian reserve and infertility. Therefore, treatment and surgery can reduce these effects and improve the fertility of the affected women.

Keywords: Infertility, Ovarian Reserve, Uterus Congenital Anomaly

Onm-2: Designing and Psychometric Properties of Endometriosis Reproductive Health Questionnaire (ERHQ)

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Background: Endometriosis reproductive health questionnaire (ERHQ): A self-administered questionnaire to measure the reproductive health in women with endometriosis

Materials and Methods: This study used an exploratory mixed-methods design and included three phases. In the first phase, a sample of 20 women with endometriosis participated in semi-structured interviews. In the second phase, item pools were generated. In the third phase, face validity, content validity, and construct validity were assessed. To estimate the reliability of the tool internal consistency and test-retest methods were used.

Results: Sixty items were included in the psychometric evaluation stage of the scale. After the assessment of the content validity ratio (CVR), content validity index (CVI), and exploratory factor analysis, 15 items were omitted, leaving 35 items in the final scale. The exploratory factor analysis revealed four factors: physical problems, psychological problems, counteracting strategies, and instability of marital life. The reliability, according to Cronbach's alpha was 0.809, and the external reliability, as evaluated by the test-retest method and the intraclass correlation, was 0.825.

Conclusion: ERHQ is a new, valid, and reliable patient-generated instrument to measure the reproductive health in women with endometriosis. It can be used by researchers and health providers to provide a better understanding of the impact of endometriosis on different aspects of reproductive health over time and to meet the needs of patients living with this condition.

Keywords: Endometriosis, Exploratory Mixed Method, Psychometrics, Questionnaire, Reproductive Health

Poster Presentation

Pnm-1: The General Health Quality among Fertile and Infertile Women in Babol, Iran

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Background: Women's health especially maternal health is a basic concept of development and socioeconomic welfare. The aim of the present study was to compare the general health quality (GHQ) among infertile and fertile women in Babol (Iran).

Materials and Methods: In this case-control study, we compared the general GHQ of 250 infertile women with the GHQ of 250 fertile women with at least one child. The case group was based on the Reproductive Health and Infertility Research center data from a cohort study that is collecting data and the control group included fertile women referring to Babol prenatal clinics. The General Health Questionnaire – 28 (GHQ-28) was used for measure the psychological aspect of the participants.

Results: The mean of GHQ score ($P=0.019$), scales of somatic complains ($P<0.0001$) and anxiety ($P=0.009$) in the fertile group were significantly higher than infertile group. The distribution of general health morbid symptoms was higher in fertile women than in infertile women ($P=0.041$). While the mean score of social dysfunctions ($P=0.018$) and morbid symptoms of social functioning in infertile women was significantly higher than fertile women ($P=0.035$). Also, after controlling a confounding variable, the chance of general health morbid symptoms was 3.1 times higher than infertile women (AOR = 3.19, CI 95%: 1.44, 7.10).

Conclusion: Based on the results, in order to improve the general health of fertile women as well as infertile women, 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: Female, Fertility, Infertility, Mental Health, Women's Health

Pnm-2: The Effectiveness of Cognitive-Behavioral Stress Management Therapy on Quality of Life and Sexual Function of Women of Reproductive Age in Malekshahi

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Background: Women are one of the most important pillars of society and family and community health depends on ensuring their physical and mental health. Was a royal city.

Materials and Methods: The present study is a quasi-experimental study with pre-test-post-test and control group. The statistical population was 102 women of childbearing age who had referred to Health Center No. In Malekshahi in 1400, of which 40 females were selected as the sample size using available sampling method and observing the inclusion criteria

and were randomly divided into two experimental groups ($n = 20$) and the control group ($n = 20$). The experimental group received stress management cognitive-behavioral therapy sessions for 10 weekly sessions. Data collection was performed using a general quality measurement questionnaire. The life of the World Health Organization (short form) and the Sexual Function Questionnaire of (FSFI) were performed. Data analysis was performed using SPSS software version 26 in two levels of summer statistics and analysis of covariance.

Results: The results showed that the mean scores of quality of life and sexual function in the experimental group had a significant increase in the post-test stage compared to the control group ($P=000/.$)

Conclusion: The results of this study indicate the effectiveness of cognitive-behavioral stress management therapy in improving the quality of life and sexual function of women of child-bearing age.

Keywords: Cognitive-Behavioral Therapy for Stress Management, Fertile Women, Quality of Life, Sexual Function

Pnm-3: Is Vitamin D Related to Hormones of Infertile Men with Impaired Spermogram? A Cross Sectional Study

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Background: The prevalence of infertility is increasing worldwide, and vitamin D deficiency can be a major factor. The aim of this study was to investigate the Association between serum vitamin D levels and sex hormones in infertile men with impaired spermograms.

Materials and Methods: This retrospective cross-sectional study was carried out on 220 infertile men who were referring to a selected urology clinic in Tehran. The relation of serum levels of vitamin D with men sex hormones including FSH, LH, FT and TT was evaluated by appropriate tests. $P<0.05$ was considered as significant level.

Results: None of hormones did not show significant relation with serum vitamin D levels. Comparison of hormone levels of FSH, TT, FT in the two groups of vitamin D did not show statistically significant difference when participants allocated in two groups based on vitamin D3 level (<20 ng / ml and ≥ 20 ng / ml). While LH hormone levels were statistically significant higher in men with vitamin D3 ≥ 20 ng /m than other group ($P = 0.02$).

Conclusion: Vitamin D in infertile men is not associated with serum FSH, LH, FT and TT, but there is a significant difference in serum LH between men with and without normal serum levels of vitamin D.

Keywords: Infertility, Men, Sex Hormone, Vitamin D

Pnm-4: Frequency of The Phenotypes of Polycystic Ovarian Syndrome in Iranian Adolescents (Mashhad) Based on Rotterdam Criteria in 2019

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Background: Polycystic ovary syndrome (PCOs) is the most common endocrine disorder in women. Due to the fact that PCOS starts from adolescence, this study was conducted with aim to investigate the prevalence of this syndrome and its phenotypes based on Rotterdam criteria in adolescent girls in Mashhad.

Materials and Methods: This cross-sectional study was performed on 650 girls aged 14 to 19 years in public schools of Mashhad in 2018-2019. Sampling was performed as a multi-stage classified cluster. After completing the Individual and Demographic Information Questionnaire, if they had criteria for entering the study, a clinical examination was performed to investigate the clinical symptoms of hyperandrogenism, including hirsutism, acne, and hair loss with the male pattern, and if any of the above symptoms were positive, they were referred for hormonal tests and sonography. The diagnosis of polycystic ovary syndrome and its phenotypes was made based on Rotterdam criteria. Data were analyzed by SPSS software (version 22) and Chi-square and Fisher tests. $P < 0.05$ was considered statistically significant.

Results: Frequency of quadruple phenotypes of polycystic ovary syndrome based on Rotterdam criteria included phenotype A (clinical hyperandrogenism / biochemistry + polycystic ovary) was found in 3 people (7.7%), phenotype B (menstrual disorders + clinical hyperandrogenism / biochemistry) in 11 (28.2%), phenotype C (menstrual disorders + polycystic ovaries) in 14 (35.9%) and phenotype D (menstrual disorders + clinical hyperandrogenism / biochemistry + polycystic ovaries) in 11 (28.2%). The overall prevalence of PCOs was 6.8% based on Rotterdam criteria.

Conclusion: Due to the prevalence of four phenotypes of polycystic ovary syndrome and the similarity of symptoms with clinical manifestations of adolescence, it is better to start the diagnosis and treatment of this syndrome from adolescence.

Keywords: Adolescents, Infertility, Iran, Polycystic Ovary Syndrome

Pnm-5: The Effect of Narrative Writing on Stress, Depression, Sexual Satisfaction and Fatigue in Infertile Couples Undergoing Assisted Reproductive Technology (ART) 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 ($z = 6.528, P < .001$) and sexual satisfaction ($z = 3.148, P = .003$) and significant reductions in depression ($z = -4.850, P < .001$) and fatigue ($z = -4.597, P < .001$) 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: Assisted Reproductive Technology (ART), Infertile Couples, Narrative Writing, Psychological Disorders, Sexual Satisfaction

Pnm-6: A Comparison between Laboratory Fertilization and Egg Donation: Assisted Reproductive Techniques in Women with Endometriosis

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Background: Endometriosis distorts pelvic function and anatomy through adhesions which could be associated with disorders in ovulation, fertilization and implantation in women with this problem. Recent advances in assisted reproductive techniques including artificial insemination and egg donation have increased the likelihood of childbearing for women with endometriosis who wish to have children. Objective: The present study was conducted to compare between laboratory fertilization and egg donation in women with endometriosis.

Materials and Methods: This systematic review was performed in Medline, EMBASE, Cochrane library, Science direct and Springer databases to find relevant articles. Search terms included laboratory insemination, egg donation and endometriosis. Cohort studies (prospective and retrospective, case-control and case report studies assessing the results of egg donation and laboratory fertilization techniques in infertility among women with endometriosis) were included. Out of 52 papers identified through initial search, 40 relevant studies were selected from which, 19 papers were included in this systematic review.

Results: As compared to egg donation, rate of fertility and implantation were lower in laboratory fertilization per each cycle of using this technique in women with endometriosis (after 3 course of treatment, 48 v 58%). A direct association was observed between age of egg donor, egg quality, number of transferred fetuses to the uterus with implantation and live birth pregnancies ($P < 0.05$).

Conclusion: It seems that egg donation method could be among acceptable approaches in order for infertility treatment among women with endometriosis.

Keywords: Assisted Reproductive Techniques, Egg Donation, Endometriosis, Laboratory Fertilization

Pnm-7: Can Nutrition Help Infertility Treatment Outcomes

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Background: 15 to 17% of couples in the world are infertile. Nutrition plays an important role in fertility in both men and women. The global epidemic of obesity and declining sperm counts have concurrently become health concerns. Maternal nutrition has a significant effect on the metabolic activity of oocytes, oocyte quality and embryonic growth.

Materials and Methods: This narrative essay searches all English articles indexed in google scholar and pubmed databases, from 2018 until now, with the keyword “nutrition, infertility, reproduction, assisted reproductive technique”.

Results: Evidence shows that more consumption of omega-3 polyunsaturated fatty acids and lower intake of trans fats are associated with a shorter time to pregnancy and better ART outcomes. Obesity is directly linked to lower sperm count and testosterone levels. Chronic oxidative stress due to obesity can also cause systemic inflammation by affecting the testicles and seminal vesicles. The use of nutritional antioxidants such as vitamins C, E, beta carotene and micronutrients such as folate and zinc are essential for normal semen quality and reproductive function. A "healthy" diet includes a higher intake of whole grains, grain, nuts, fruits, vegetables and fish and reducing the consumption of trans fatty acids and red meat. There is a relationship between this nutritional approach and the reduction of infertility due to ovulation in women and the improvement of semen quality parameters in men.

Conclusion: It shows that nutritional status can affect the results of infertility treatment outcomes. Therefore, nutritional interventions before infertility treatments improve results, reduce costs and increase mental health and fertility of couples.

Keywords: Assisted Reproductive Technique (ART), Infertility, Nutrition, Reproduction

Pnm-8: The Role of Complementary and Alternative Medicine in Managing Endometriosis

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Background: Endometriosis is a female reproductive system disease characterized by the presence of tissue similar to those in the endometrium outside the uterus. The use of Complementary and Alternative Medicine (CAM) in endometriosis treatments has grown rapidly in recent years. CAM is considered as a type of non-traditional medicinal therapy such as acupuncture, herbal and physical therapies. The purpose of this paper is to search the existing scientific literature on the use of CAM to determine which methods are useful and safe.

Materials and Methods: A literature search was conducted on PubMed.

Results: Women with endometriosis may suffer from debilitating painful symptoms such as chronic pelvic pain especially

dysmenorrhea, dyspareunia, and infertility which all may negatively affects patients' quality of life. These women desire to find the best possible ways of dealing with this disease and prefer to use noninvasive and confidently alternative therapies with fewer side effects. In addition to medical treatments, there are a number of strategies for women diagnosed with endometriosis. Stress management is one the most serious parts of these strategies which advance the feeling of being in control in respects of treatment decision-making, emotions and behavior. Good diet as another essential part of a comprehensive treatment plan is an effective way to overcome some of the debilitating nature symptoms of this disease. There are various herbs that are suggested for women suffer from endometriosis in different ways of teas, necessary oils for massage, oral extracts and salves. Exercise, acupuncture, acupressure and massage therapy are another helpful programs in reducing stress, improvement of self-esteem, relieving dysmenorrhea and improving pregnancy. **Conclusion:** An integrated approach encompasses stress management, appropriate nutrition, herbal and physical therapies in combination with conventional medical or surgical treatment will be expected yield the most beneficial treatment for patients. **Keywords:** Complementary and Alternative Medicine, Endometriosis, Infertility

Pnm-9: Psychological Status of Infertile Couples in The COVID-19 Pandemic: Systematic Review

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Background: With the onset of the COVID-19 pandemic at the beginning of 2020, all non-essential medical treatments were suspended, including fertility treatments. Infertility is already a stressful experience. Indeed, it has been widely documented that the experience of infertility has a significant impact on the psychological well-being of both partners. Even though various studies have demonstrated the importance of the mind-body connection and fertility, the psychosocial aspects of infertility have not been adequately addressed. Aims of research: With the present study, we evaluated the psychological status of infertile couples in the COVID-19 Pandemic.

Materials and Methods: Electronic databases (PubMed, Scopus, and ISI Web of Science) were searched to find published articles on sexual health and Coronavirus until May 2022. All relevant studies were screened by inclusion and exclusion criteria and compatible studies were chosen.

Results: The occurrence of anxiety and/or depression feelings was increased with psychological disorders in infertile couples. There was a significant decline in self-reported quality of life. Also, treatment suspensions had harmed psychological status.

Conclusion: Fertility treatment suspensions have had a considerable negative impact on women's psychological status and quality of life. Psychological counseling should always be offered especially during this difficult period.

Keywords: COVID-19, Infertile, Psychological Status, Review

Pnm-10: Effect of Counseling and Education on Infertile

Couples

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Background: Infertility has the most stressful experience with various psychological damages to couples. Infertile couples regard the iterative and long treatment periods as a recurring crisis. Therefore, paying attention to the educational and counseling needs of infertile patients has an important role in their treatment course.

Materials and Methods: The study reviewed several significant articles in the field between 2010 and 2022. Reviews in the journal and PubMed, Science Direct, Elsevier, ProQuest, Willy, springer, and google scholar databases with keywords infertility, Counseling, Education. The results were analyzed and ranked in a schematic way. For statistical analysis, statistical tests were used.

Results: The result shows that counseling education program in treatment course for infertile couples controls their stress and fear levels. Patients are also more cooperative and hopeful with the knowledge of their treatment process and perform the treatment steps correctly. They also do not despair when treatments fail and seek other treatments. In case of sexual and psychological problems, consult a counselor.

Conclusion: According to the present results, the use of such an infertility counseling education program is recommended when patients face infertility.

Keywords: Counseling, Education, Infertility

Pnm-11: Effect of Environmental Factors on ART Result

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Background: Many environmental factors affect embryo development and the subsequent outcomes of human ART. Studies show that oocytes, sperm embryos, and eggs can be affected by events and conditions before the oocytes and sperm are collected and brought together in the ART laboratory.

Materials and Methods: The study reviewed several significant articles in the field between 2010 and 2022. Reviews in the journal and PubMed, ScienceDirect, Elsevier, ProQuest, willy, springer, and google scholar databases with keywords oocyte, sperm, ART, environmental temperature, air pollution. The results were analyzed and ranked in a schematic way. For statistical analysis, statistical tests were used.

Results: The result shows that these factors include metabolic disorders, general health, and disease; physical and psychological stress, exposure to environmental estrogens and other toxins, pharmaceuticals, alcohol, smoking, and drug abuse can affect oocytes and sperm before ART treatment. Also, results recommend that patients under ART treatment avoid high environmental temperature and air pollution.

Conclusion: According to the present results, it is important that health workers are aware of adverse outcomes that may result from such exposures, and to incorporate this into the analysis of clinic data for the purposes of quality management.

Keywords: ART, Environmental Factors, Oocyte, Sperm

Pnm-12: Impact of Infertility on Sexuality

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Background: Many studies show that infertility and its treatment methods can affect sexual behaviors. Infertility often has stress, psychological demands, and physically intrusive procedures. These can affect a person's sexual self-image, desire, and performance.

Materials and Methods: The study reviewed several significant articles in the field between 2010 and 2022. Reviews in the journal and PubMed, Science Direct, Elsevier, ProQuest, Willy, springer, and google scholar databases with keywords infertility, sexuality, sexual behavior, ART. The results were analyzed and ranked in a schematic way. For statistical analysis, statistical tests were used.

Results: The result shows that infertility and its treatment approaches could lead to changes in sexual self-esteem, sexual relationship, and sexual function. Also, it can be associated with failure, frustration, anger, and resentment. The pressure to perform, and to have sex due to infertility treatment plans can push couples even further apart. Fertility treatments can make sex less spontaneous and less enjoyable.

Conclusion: According to the present results, many infertile subjects experienced trouble in various aspects of sexuality. Therefore, it should also be addressed whether these changes affect the partners of the infertile couples.

Keywords: ART, Infertility, Sexual Behavior, Sexuality

Pnm-13: Epilepsy During Pregnancy and Fetal Development Outcomes

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Background: Epilepsy is one of the most common neurological diseases during pregnancy. Women with epilepsy are at risk of pregnancy complications, however, the influence of epilepsy on fetal growth is not understood. This review has been conducted to assess the adverse fetal growth outcomes in women with epilepsy.

Materials and Methods: MEDLINE, Cochrane and Google scholar have been searched between Jan 1, 2012, and Jan 1, 2022, with imposing English language restrictions, for observational studies of pregnant women with epilepsy, which assessed the risk of epilepsy and pregnancy outcomes including fetal growth parameters.

Results: Estimated risk of fetal growth restriction (FGR) was 1.28-fold higher in epileptic pregnant women than in non-epileptic women ($P < 0.05$) in previous literature, however, the odds of early preterm birth, gestational diabetes, fetal death or stillbirth, perinatal death, or admission to neonatal intensive care unit did not differ between women with epilepsy and those without the disorder. Given the course of previous studies results show that FGR rate is significantly increased even if antiepileptic drug (AED) were taken.

Conclusion: Although modest bias cannot be avoided, our review analysis indicated that epilepsy participates in fetal development as an unfavorable factor, and AEDs seemed to be useless in decreasing the occurrence rate of FGR. This increased risk of FGR should be taken into account when counselling women with epilepsy.

Keywords: Antiepileptic Drug, Epilepsy, Fetal Growth Restriction, Pregnancy

Pnm-14: Determining The Relationship between Spiritual Intelligence and Resilience in Infertile Couples in Iran

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Background: Infertility is a global problem. It's a psychological crisis for a couple, spouse and their families. Due to the positive effects of spiritual intelligence on positive adaptation and growth of the individual under difficult circumstances, the aim of this study was determining the relationship between spiritual intelligence and resilience in infertile couples in Iran.

Materials and Methods: This cross-sectional study was performed in 2020 on 162 infertile couples in Isfahan, Iran. Data were collected by convenience sampling method from infertile couples who referred to Moshtagh infertility center and Shahid Beheshti Hospital Infertility Center in Isfahan by questionnaire includes: demographic and fertility characteristics, spiritual intelligence and resilience as self-reported. King's Spiritual Intelligence Self-Report Inventory (SISRI) and Connor-Davidson Resilience Scale (CD-RISC) was used to collect research data. Analyze the data was performed using descriptive statistical methods. Also, in order to investigate the relationship between spiritual intelligence and resilience score, inferential statistical methods of Pearson correlation coefficient and linear regression were used by SPSS software. A P-value of less than 0.05 was considered significant.

Results: Pearson correlation coefficient showed that there was a significant Positive direct relationship between resilience score with total spiritual intelligence score ($r=0.36$), critical existential thinking ($r=0.60$), Personal Meaning Production ($r=0.38$), transcendental awareness. ($r=0.43$) and conscious state expansion ($r=0.53$) ($P<0.001$). The personal meaning production ($t=6.38$) and the Conscious State Expansion ($t=2.15$) were significant predictors of resilience scores.

Conclusion: It seems that couples with a higher level of spiritual intelligence have better understanding of the problems and harms of primary infertility and will cope with it more efficiently. So, developing spiritual intelligence is effective for better and more efficient adaptation to the consequences of infertility and continued treatment.

Keywords: Infertility, Intelligence, Resilience, Spirituality, Iran

Pnm-15: The Importance of Health Education in Iranian Pregnant Women About Toxoplasmosis Prevention

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Background: Toxoplasmosis is a disease that is caused by *Toxoplasma gondii*, an intracellular obligatory parasite. *T. gondii* is broad universal, as a minimum 30% of the world's people is contaminated. Toxoplasmosis can cause critical situation in high-risk groups as pregnant mother, if occur during pregnancy. Contamination with *T. gondii* before pregnancy cause small or without risk to the fetus but in mothers who contaminated up to three months before beginning pregnancy.

Materials and Methods: In our study, we evaluated 330 women between 18 to 42 years in preconception consultation about seropositive toxoplasma IgG test. All of them were referred to one lab with unique method of measurement.

Results: After testing from 330 women, 132 (40%) cases were seronegative for toxoplasmosis before pregnancy. The finding of IgG antibodies and lack of IgM antibodies shows a previous infection. And 198 (60%), of cases were seropositive for IgG toxoplasmosis.

Conclusion: In our opinion health education and giving information about toxoplasmosis is necessary. Prevalence of maternal contamination and pattern of diet and consumption of complete cooked food should be explain to parents. Prenatal counseling should include education regarding prevention of toxoplasmosis. As a base, prevention is better and more useful than treatment.

Keywords: Health Education, Prevention, Toxoplasmosis

Pnm-16: Investigation of the Relationship between The Incidence of Preterm Delivery and Uterocervical Angle in Cases of Intermediate Cervical Length

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Background: Studies show that in cases where the length of cervix is less than 15 to 20 mm, there is a possibility of preterm delivery. The length of the cervix, between 25 and 30 mm is discussed and challenged, and in some cases causes premature labor and, in some cases, leads to term pregnancy. Uterocervical angle may be effective in determining the prognosis of pregnancy in these cases.

Materials and Methods: In this small study, which evaluated prospective, mothers who referred for trans vaginal sonographic examinations at 16 to 18 weeks, all of these mothers were single pregnancy. In these mothers, uterocervical angle was also measured and classified into values less than and greater than 90 degrees. Then the prognosis of pregnancy and delivery time of mothers were compared.

Results: 70 mothers had an angle of less than 90 degrees. Fifty mothers had a uterocervical angle greater than 90 degrees. In fact, mothers with angles greater than 90 degrees had straight and direct cervix (The cervix was not anterior or posterior). Among 50 mothers with an angle of more than 90 degrees, 7 cases (14%) had preterm delivery between 34 and 37 weeks, which was due to beginning of labor pains or rupture of membrane. Of the other 70 mothers who had an angle of less than 90 degrees, none had preterm labor due to rupture of the amniotic sac or beginning of labor.

Conclusion: The evaluation of the angle between the uterus and the cervix can be helpful in predicting the onset of preterm labor in cases of intermediate Uterocervical angle.

Keywords: Cervical Length, Premature Labor, Uterocervical Angle

Pnm-17: Endometriosis Through the Lens of Iranian women**Namazi M^{1*}, Behboodi Moghadam Z²****1. Department of Midwifery and Reproductive Health, Tehran University of Medical Sciences, Tehran, Iran****2. Department of Midwifery and Reproductive Health, Tehran University of Medical Sciences, Tehran, Iran***Email: masnamazi@yahoo.com***Background:** Considering the wide impact of endometriosis on various aspects of health, this study aimed to explore the impact of endometriosis on Iranian women's lives.**Materials and Methods:** The present study used a qualitative approach and conventional content analysis. Twenty patients suffering from endometriosis referring to Imam Khomeini Hospital in Tehran took part in this study. The sampling was done purposefully until data saturation. After deep semi-structured interviews, the content analysis of the interviews was done according to the steps proposed by Zhang and Wildemuth.**Results:** The mean age of the participants was 34.53 (SD: 5.81) years (range: 23-43) with duration of illness of 6.30 ± 5.86 years. Their educational level varied from high school to university, and most of them were employed. Analysis of the data from participants' experiences led to the formation of 5 categories under the titles "Physical suffering, instability of marital life, mental disorder, disruption in social life and self-care" and 11 subcategories.**Conclusion:** The findings of the present study showed that endometriosis can adversely affect women's lives including physical, sexual, psychological and social problems. Although in some cases patients adopt different strategies for self-care, the need for more support is felt.**Keywords:** Endometriosis, Health, Iranian Women, life Experience, Qualitative Study**Pnm-18: Predicting Health-Related Quality of Life in Women with Infertility Based on Alexithymia, Spiritual Health, and Sexual Satisfaction Due to The Mediating Role of Perceived Stigma Infertility and Loneliness****Namvar H^{*}, Amini Masouleh M****Department of Psychology, Humanities Islamic Azad University, Saveh, Iran***Email: hooman.namvar@gmail.com***Background:** This study aimed to identify the role of alexithymia, spiritual health, and sexual satisfaction due to the mediating role of perceived infertility stigma and feeling loneliness in predicting health-related quality of life in women with infertility.**Materials and Methods:** The statistical population included all infertile women in Tehran in 2020. Among them, 250 people were selected by the convenience sampling method. The research instruments included standard questionnaires including standard questionnaires of health-related quality of life (Weber, 1992), Toronto alexithymia (1992), Gaur and Sharma's spiritual health (2014), Larson sexual satisfaction (1988), Fu's perceived stigma infertility (2014) and Ditommaso social and emotional loneliness scale for adults (2004). The data were analyzed using SPSS software version 23 and Smart-PLS.**Results:** The results showed that 8.5% of changes in health-related quality of life are due to changes in variables of alexithymia (mood dysphoria), spiritual health and sexual satisfaction; that all of these variables have a significant effect on health-related quality of life ($P < 0.001$). The results showed that the role of perceived infertility stigma and feeling loneliness are minor mediating, which means that alexithymia, spiritual health and sexual satisfaction can affect both, directly and indirectly, health-related quality of life (through perceived infertility stigma and feeling loneliness) ($P < 0.001$).**Conclusion:** Based on finding perceived infertility stigma and feeling loneliness as a mediator, it can, directly and indirectly, be involved in the relation between alexithymia, spiritual health and sexual satisfaction with health-related quality of life in women with infertility.**Keywords:** Alexithymia, Health-Related Quality of Life, Perceived Stigma Infertility, Sexual Satisfaction, Spiritual Health**Pnm-19: The Effect of Air Pollution on Assisted Reproductive Technology****Norouzi M^{1*}, Rezghi M^{2,3}****1. Midwifery and Reproductive Department, Tehran, Iran****2. Department of Persian Medicine, Babol, Iran****3. Traditional Medicine and History of Medical Sciences Research Center, Babol, Iran***Email: norouzi7378@gmail.com***Background:** Ambient air pollution has negative effects on human health including reproductive system. The aim of the current study is to review the impact of air pollution on assisted reproductive technology (ART).**Materials and Methods:** Eligible studies were selected from an electronic literature search from the PubMed, Scopus, and Google Scholar databases to April 2022 and associated references in published studies. We have included the human studies corresponding to the search terms. The results were restricted to publications in English. We have excluded articles whose results did not concern fertility. Totally 11 studies included in this review.**Results:** Air pollution has a negative effect on the quantity of gametes, a reduction in the number of retrieved oocytes, frozen embryo transfer cycles, embryo development, fetal development, and an increase in miscarriages. The severity of these effects depends on several factors, including the duration of exposure to air pollution and the level of ambient air pollution. Hormonal disturbances, oxidative stress induction, inflammation, cell DNA, and epigenetic alterations are mechanisms to explain this negative impact.**Conclusion:** These findings suggest that air pollution may adversely affect and highlight the need to reduce ambient air pollution exposure for successful ART.**Keywords:** Ambient Air Pollution, Assisted Reproductive Technology, Air Quality, Pregnancy**Pnm-20: Genital Microbiota and Assisted Reproductive Technique****Norouzi M^{1*}, Khoshnam Rad M²****1. Department of Midwifery and Reproductive Health, Tehran, Iran****2. Midwifery and Reproductive Department, School of Nursing and Midwifery, Tehran University of Medical Sciences, Tehran, Iran***Email: norouzi7378@gmail.com***Background:** The vaginal microbiome has a pivotal role in women's health. Lactobacillus genus are beneficial organisms and act as a defense against pathogens. The aim of this study is to assess the vaginal microbiome on the assisted reproductive

technique (ART) outcome.

Materials and Methods: We searched a range of electronic databases for appropriate studies in English, including PubMed, Google Scholar, and Scopus up to April 2022. Identified articles screened using inclusion and exclusion criteria. A manual search of the references within the resulting studies was performed. Finally, 11 articles were included in this review.

Results: The vaginal microbiome can affect the results of ART. Lactobacillus abundance and stability of the vaginal microbiome are associated with better reproductive and ART outcomes and the achievement of pregnancy.

Conclusion: The microbiome of the genital tract plays an important role in the case of ARTs. Dysbiosis and the lack of lactobacilli can explain implantation failures in ARTs.

Keywords: Assisted Reproductive Technique, Microbiome, Infertility, Microbiota

Pnm-21: Oxidative Stress and Pathogenesis of Male Infertility

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Background: A relationship between infertility and the generation of reactive oxygen species (ROS) has been reported previously. A small amount of ROS is necessary for the physiological function of sperm including the capacitation, hyperactivation and acrosomal reaction. However, high levels of ROS can cause infertility through different pathways. The aim of this study is reviewing the association between oxidative stress and male infertility.

Materials and Methods: A review literature search of eligible studies was conducted in PubMed database from 2015 to 2022 for studies evaluating the association between oxidative stress and male infertility using the following search strategy: ("oxidative stress" OR "ROS") AND ("male infertility") as keywords. Studies (original papers, systematic review and meta-analysis) were included in this review.

Results: Mechanisms responsible for the role of oxidative stress in male infertility include oxidative destruction of sperm lipid membranes, damage to gamete DNA both by gene mutation and by direct breakdown of the DNA backbone, mitochondrial dysfunction and apoptotic cell death. The intrinsic reactivity of these metabolites in peroxidative damage induced by ROS, particularly H₂O₂ and the superoxide anion, has been proposed as a major cause of defective sperm function in cases of male infertility.

Conclusion: Accurate assessment of ROS levels and, subsequently, OS is vital, as this will help clinicians both elucidate the fertility status and identify the subgroups of patients that respond or do not respond to therapeutic strategies.

Keywords: Infertility, Oxidative Stress, Reactive Oxygen Species

Pnm-22: The Effectiveness of Mindfulness-Based Intervention on Psychological Wellbeing in Infertile Women (A Review Study)

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Background: Infertility is a stressful experience for couples and is considered as a serious problem in public health. Studies have shown that due to prevailing culture in society, infertility is often considered a female problem and therefore women face more emotional problems than men. Stress due to infertility leads to a decrease in psychological well-being by creating tension and confusion. Psychological well-being is one of the positive areas of psychology and is a multi-component concept that includes self-acceptance, positive relationships with others, autonomy, rapport with an environment. One of the positive psychological interventions is mindfulness that means focusing on the present situation Without any judgment. This kind of attention increases the clarity, and awareness of the current reality.

Materials and Methods: Databases used were PubMed, CINAHL, Google Scholar, EMBASE, and Scopus. Articles published in English from 2010-2021.

Results: Mindfulness leads to positive psychological effects, including increasing peace of mind and awareness of positive emotions and helping people by modifying negative behaviors. Such a measure for infertile women that usually take more responsibility for infertility and have experience more negative emotions, is beneficial and improve their level of psychological well-being.

Conclusion: Mindfulness causes people to deal with negative emotions in life events with a positive attitude and by reducing psychological disturbances, a person experiences higher levels of competence and life satisfaction. Which ultimately leads to the promotion of mental health and psychological well-being.

Keywords: Infertile Women, Mindfulness, Psychological Well-being

Pnm-23: An Overview of The Outcomes of Uterine Transplantation Procedures Performed Worldwide

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Background: Uterine transplantation (UTx) is a major surgical initiative for the treatment of absolute uterine factor infertility (AUI) and includes the use of both assisted reproduction and reproductive surgery. This article aims to review the outcomes of uterus transplantation procedures performed in the world.

Materials and Methods: In this review study, the PubMed database was searched, with no time, language, or location restriction until March 1, 2022, using the words "uterine transplantation" or "uterus transplantation". Only peer reviewed original articles describing human UTx procedures were included in the study.

Results: From 720 identified articles, 32 papers were entered the study for qualitative review. Based on the results, 63 UTx procedures were performed worldwide (82.5% using living donors (LD) and 17.5% using deceased donors (DD)). Of the 15 uterine grafts that were failed, 73.3% were LD UTx and 26.7% were DD UTx and 31 live births were recorded (84% were the result of LD UTx and 16% were the result of DD UTx).

Conclusion: Uterine transplantation can give women with AUI the opportunity to give birth to their biological child. Due to the small number of UTx procedures performed, especially using a deceased donor, determining the safety and effectiveness of UTx requires more data.

Keywords: Absolute Uterine Factor Infertility, Deceased Donor, Living Donor, Uterus Transplantation

Pnm-24: Risks of Uterine Transplantation

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Background: Uterine transplantation (UTx) is a complex procedure involving extensive abdominal surgery on the donor and recipient. This procedure has not yet been recognized as the standard treatment for absolute uterine factor infertility (AUI). The present study aimed to determine the risks of UTx.

Materials and Methods: In this review study, the PubMed database was searched, with no time, language, or location restriction until March 1, 2022, using the words "uterine transplantation" or "uterus transplantation". Only articles describing the risks of UTx in real procedures performed on humans were included in the study.

Results: From 720 identified articles, 40 papers were entered the study for qualitative review. Based on the results, removal of the uterus from a living donor is a very invasive surgery. Surgical and postoperative risks, including bleeding, infection, ureteral injury, and psychological risks such as depression, anxiety, and decreased quality of life can occur for the donor. The recipient is also exposed to the risks associated with *in vitro* fertilization (IVF), the risks associated with transplantation surgery, cesarean section, hysterectomy and psychological risks. Exposure to immunosuppressive drugs and a high risk of developing complications during pregnancy such as preterm delivery, preeclampsia and gestational diabetes are among the factors that threaten the health of the fetus and neonate.

Conclusion: UTx is associated with risks for the living donor, recipient, fetus and neonate. Increasing the number of UTx procedures increases our understanding of its risks. The development of less invasive methods, such as robotic surgery, reduces the complications of surgery in a living donor.

Keywords: Absolute Uterine Factor Infertility, Living Donor, Uterus Transplantation,

Pnm-25: Assisted Reproductive Technique Outcome and Oxidative Stress

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Background: Infertility may occur due to problems with the female, male or both partners. Assisted reproductive technique (ART) includes a range of methods used to circumvent human fertility. Some studies reported that oxidative stress may appear as a possible reason for poor ART outcome. Our aim was to review the association between oxidative stress and assisted reproduction.

Materials and Methods: This review has been conducted based on analysis of available literature indexed in MEDLINE, Cochrane and PubMed databases between 2015 and 2022. Specific keywords including "oxidative stress", "infertility" and "assisted reproductive technique" have been used. Epidemiological and experimental studies, review articles and systematic articles on the mentioned theme were included.

Results: High seminal reactive oxygen species (ROS) level is associated with impaired sperm fertilizing ability and lower pregnancy rates after *in vitro* fertilization. Also, negative as-

sociation has been observed with embryo development to the blastocyst stage after intracytoplasmic sperm injection. Oxidative DNA damage may impair embryo development, cause miscarriage and birth defects in the offspring. Three recent meta-analyses revealed a correlation between elevated sperm DNA fragmentation and reduced pregnancy rates following intrauterine insemination.

Conclusion: High grade oxidative stress may negatively affect the maintenance and outcome of pregnancy. Applying the detection of oxidative stress biomarkers in ART patients may select patients with higher success rate and/or those who require antioxidant therapy. This would lead to improvement of ART outcome as well as natural fertility.

Keywords: Assisted Reproductive Technique, Infertility Treatment, Oxidative Stress

Pnm-26: Vitamin D and Female Infertility: New Evidence

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Background: Vitamin D plays an important role in fertility and infertility concepts. Several pathogenetic mechanisms may be proposed as a linkage between vitamin D deficiency and infertility. The purpose of this review is to provide up-to-date evidence regarding the impact of vitamin D on female infertility.

Materials and Methods: "Vitamin D, fertility and infertility" terms was searched on Pubmed for English language publications from 2015 up to May 2021. There are a large number of *in vitro*, animal as well as human observational studies which are looking at an association between vitamin D and female fertility. Just 20 most recent studies were included in this review.

Results: Most of the studies suggest that vitamin D might have beneficial effects on metabolic/hormonal parameters of PCOS and endometriosis, however the cause-effect relationship has not yet been established. Also, it has been shown that vitamin D is involved in the modulation of the reproductive process in women due to the expression of vitamin D receptor (VDR) and 1 α -hydroxylase in reproductive tissues such as ovary, uterus, placenta, pituitary and hypothalamus.

Conclusion: These findings strongly show that Vitamin D is a key role in female fertility and therapeutic approach to use it in combination with other infertility treatments can be beneficial. To determine the exact role of vitamin D in female infertility, Large-scale, high quality studies with longer follow-up are needed.

Keywords: Fertility, Infertility, Vitamin D Receptor

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