



ROYAN

International Twin Congress

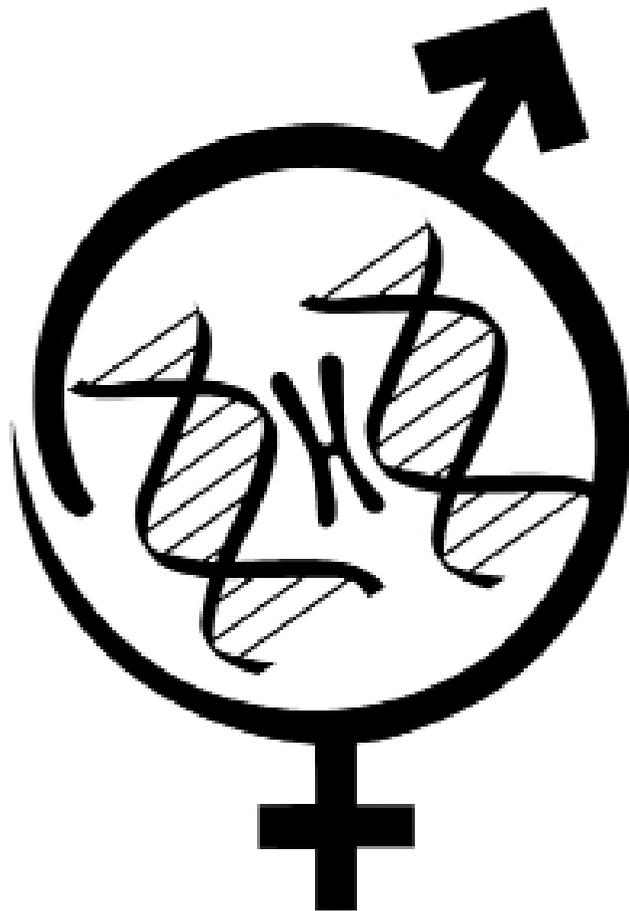
Reproductive Biomedicine & Stem Cells

20th Congress on
**Reproductive
Biomedicine**

Abstracts of
Royan International Twin Congress

20th Congress on Reproductive Biomedicine
28-30 August 2019

15th Seminar on Nursing and Midwifery
28-30 August 2019



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran



**Abstracts of the
20th Congress on Reproductive Biomedicine
15th Seminar on Nursing and Midwifery**

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Congress Chairperson



Firouzeh Ghaffari

Dear Colleagues,

It is my great pleasure to invite you to the 20th Royan Reproductive Biomedicine Congress, Which will take place on August 28-30 2019, Tehran, Iran.

This event features three days of the best in stem cells research and regenerative medicine covering different aspects of basic and translational contents.

Since 2000, annual meetings on Reproductive Biomedicine have featured latest breakthroughs in this field and hosted eminent scientists from all over the world. National and international delegates presented their latest research projects and other participants found it as a novel opportunity to exchange ideas, discover, reacquaint with colleagues and broaden their knowledge. The scope and quality of the scientific exchange in previous meetings and increasing number of participants and received articles indicated and made this event as the premier research and instructional meeting in the region. Royan Reproductive Biomedicine Congress aims to offer networking opportunities and foster debate among participants that decisively contribute towards the promotion of infertility management. This international congress intends to provide and promote modern techniques related to the field of Reproductive Biomedicine.

In the upcoming congress (20th Royan Reproductive Biomedicine), the scientific program is scheduled to the keynote speakers, plenary sessions, poster presentations and other programs such as educational and viable workshops.

So please join us for another impressive Royan Reproductive Biomedicine Congress. We will do our best to make your stay in Tehran an exciting and memorable experience.

We're looking forward to an excellent meeting with great scientists from different countries around the world and sharing new and exciting results in Reproductive Biomedicine fields.

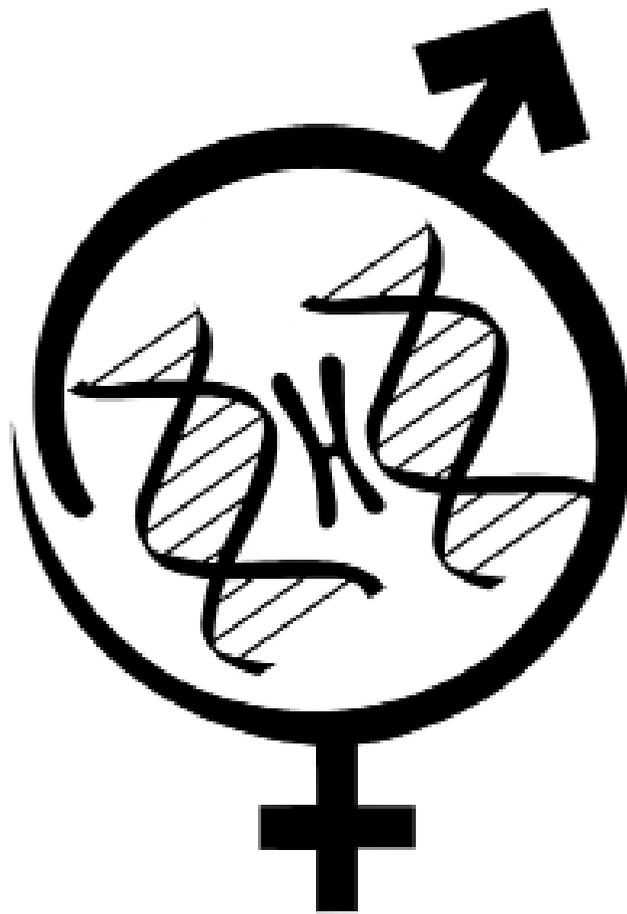
Firouzeh Ghaffari, M.D.

Congress Chairperson

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Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Invited Speakers

Andrology

I-1: How Varicocele(ctomy) Impacts on Couple Natural and Assisted Reproduction

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Background: Varicocele may affect up to 35% of men with primary infertility and up to 80% of men with secondary infertility. The better semen parameters are, the more likely is spontaneous pregnancy; so, if varicocele repair improves semen quality (in terms of sperm total count, motility, morphology and DNA integrity), it can consequently increase the pregnancy probability. The aim of this study is to discuss the effect of varicolectomy on spontaneous pregnancy but also on ART outcomes (IUI, IVF/ICSI).

Material and Methods: English Literature was searched on PubMed for the terms of: varicocele repair, male infertility, OAT, DFI (=sperm DNA fragmentation index), IUI, IVF/ICSI. We evaluated publications of past five years about these topics (meta-analyses, reviews, prospective / controlled studies) and compared Literature results with our clinical practice and experienced evidence. We have to underline the relevance of a careful selection of the patients to be addressed to varicocele repair, mainly based on an accurate diagnosis with colour-doppler ultrasound aimed to detect/confirm a basal spontaneous continuous reflux in orthostatism along the left spermatic vein - associated with at least one altered semen parameter, which we recently showed to be the most significant predictive factor of semen improvement.

Results: A growing knowledge exists on the pathogenic mechanisms of varicocele and their consequences on spermatogenesis (hypoxia, hyperthermia, adrenal catabolites reflux to testis, ROS and DFI increase, and abnormal oxido-reduction potential of semen...). The most recent studies in the Literature confirm the positive role of varicolectomy on spontaneous pregnancy rate in infertile couples without a major female factor.

Insufficient evidence exists to determine if correcting varicocele improves IUI outcomes, although some positive reports are available. Moreover, varicocele repair can often allow couples to use the less invasive form of ART.

Prospective controlled studies concerning IVF/ICSI outcome - in terms of fertilization rate, pregnancy rate, live-birth rate and miscarriage rate - generally report better results after varicolectomy in all the aforementioned rates: some conflicting results seem mainly due to bias regarding couples that achieve spontaneous pregnancy or with IUI after varicolectomy, without undergoing IVF/ICSI as previously established. One of the most important effects of varicocele repair seems to be the seminal ROS and, consequently, sperm DFI decrease, a well known factor negatively affecting fertilization process and embryo development.

Conclusions: Repair of clinical varicocele should be offered as a treatment option to affected infertile males, in order to improve semen parameters, to achieve more likely spontaneous pregnancies, to reduce the level of ART and also to obtain better IVF/ICSI outcome.

I-2: Some Options to Optimize Male Gametes for ICSI

Colpi GM

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Introduction: The aim of the study was to discuss the andrologic approach to azoospermia, cryptozoospermia and oligoasthenoatozoospermia (OAT) in the era of the assisted reproductive technology (ART), and the importance of predictive tests, like the DNA fragmentation index (DFI), to decide the sperm source (from testis or from ejaculate) for ART in male factor infertility. Also we summarize the effect of freezing sperm on the DFI and the ICSI outcome according to the current Literature.

Material and Methods: The English literature was searched on PUBMED by the terms of: azoospermia, cryptozoospermia, OAT, DFI, ejaculated vs testicular sperm for cryptozoospermic or OAT patients, and predictive tests for ICSI. The only publications in the most recent years about this subjects were evaluated. We will make a comment about every topics in the light of the Literature and compare it with our daily practice in this area.

Results: In NOA, Micro-TESE is the sperm retrieval procedure of choice and testicular histology influences the success, with better results in cases of hypospermatogenesis.

Testicular sperm are more advantageous for ICSI patients with cryptozoospermia for take-home baby rate.

DFI rate is lower in testicular sperm than in ejaculated sperm in the severe OAT patients.

Testicular sperm are superior to ejaculated sperm for ICSI to achieve pregnancy in infertile males with high DFI.

Cryopreservation of the fresh ejaculated sperm from normozoospermic patients can increase DFI, and negatively affect sperm progressive motility, viability and normal morphology.

However in NOA, it seems there is no statistically difference between fresh and frozen-thawed testicular sperm with regards to fertilization rate, but no reliable data were found about implantation, miscarriage and live birth rates.

Conclusion: Using DFI test before programming ICSI can help to decide the better sperm source selection (ejaculate, testicle), especially in cryptozoospermic and severe OAT patients.

I-3: Chronic Epididymitis and Male Infertility

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Chronic inflammatory conditions of the genital tract are frequently encountered in male fertility problems, but are still unsatisfactorily recognized due to inappropriate definitions and inconsistent diagnostic criteria. The most popular term used for description of inflammation and infection of the genital tract is MAGI (male accessory gland infection). In asymptomatic patients the diagnosis is primarily based on leukocytospermia (i.e., more than 1 million peroxidase-positive leukocytes per ml ejaculate). However, in many cases of chronic epididymitis, the number of leukocytes in the ejaculate is below the threshold of 106 per ml; therefore, consideration of additional markers of inflammation such as granulocyte elastase, pro-inflammatory cytokines (e.g. interleukin-6 or 8), or reactive oxygen species,

is helpful for establishing the diagnosis. With regard to the impact on male reproductive function, epididymitis seems to be more relevant than inflammation/infection of the prostate and/or seminal vesicles. Chronic epididymitis may result in reduced sperm number and motility. In addition, further sperm functions, in particular DNA-integrity, may be affected.

Keywords: Chronic Epididymitis, Leukocytospermia, Male Accessory Gland Infection, Male Infertility

I-4: Current Options for Medical Treatments of Male Infertility

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Apart from assisted fertilization and surgery male infertility/subfertility can be treated with a variety of drugs. As up to 50% of male fertility disturbances are classified as “idiopathic” no clear selection criteria for this kind of treatment are available in these cases. That is why only empiric treatment approaches can be tried. Drugs used comprise antiestrogens such as tamoxifen and clomiphene, recombinant or highly purified FSH as well as aromatase inhibitors and antioxidants. Recent studies show the best results after FSH therapy. Effective treatment regimens are available for retrograde ejaculation by administration of α -sympathomimetics, imipramine or brompheniramine.

When male fertility disturbances are associated with inflammation/infection of the genital tract treatment with nonsteroidal anti-inflammatory drugs and/or antibiotics is possible. However, there is only low evidence due to a limited number of only small studies. Follow-up therapies with mastcell blockers or glucocorticosteroids as well as antioxidants have partly revealed promising results, whereas glucocorticosteroids are not recommended for immunological infertility.

Keywords: Male Fertility Disturbances, Drug Treatment, FSH, Antiestrogens, Genital Tract Inflammation, Antiphlogistics Antioxidants

I-5: Challenges in Pre-Pubertal Boys Surgical Sperm Retrieval

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In recent years advances in cancer treatment result in a more survival rate in the patients with various cancers. In the other hand one of the well-known side effect of cancer therapy is the gonadotoxic effects and so infertility in the future. One group of cancer victims is young men or pre-pubertal boys who wish to be a father in the future. One of the challengeable issues in these boys is the preservation of fertility.

Cryopreservation is a successful method for fertility preservation in men that may be used in boys by cryopreservation of testicular tissue. The most challengeable issue in pre-pubertal boy may be the preservation of fertility by sperm preservation. In older boy who are able to produce semen through masturbation, sperm cryopreservation is a successful method that is

non-invasive, non-expensive and can be performed without any delay. Boys who are unable to produce semen by masturbation may undergo vibratory stimulation or electrostimulation with acceptable results. Sperm may be collected via testicular biopsy if spermatogenesis has been occurred. Immature sperms that obtained by testicles biopsy or aspiration have been freeze for sperm banking. For younger boys, the only options available are experimental and require further development through basic science research. Cryopreservation and subsequent *in vitro* maturation or transplantation of spermatogonial stem cells and testicular tissue has theoretical potential but cannot currently be used in a clinical setting.

I-6: What Is The Role of PFD in Male Unexplained Infertility and its Treatments?

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Introduction of sperm function test changes the approach to male infertility and subfertility. Semen analysis, once considered as a fundamental test in male fertility assessment, has been supplanted with sperm function test in special cases (varicocele, recurrent pregnancy loss or assisted reproduction technique (ART) failure, etc.). Since increased sperm DNA injury can reduce the success rate of assisted reproduction techniques (ART) outcome (Panner Selvam and Agarwal, 2018), strategies for obtaining sperm with better DNA integrity seem to be necessary. In our center, such strategies have been categorized into three subgroups based on the day of ejaculation.

Pre-sampling methods include treatment of underlying causes which may disturb sperm DNA integrity (varicocele, infection, etc) or empirical antioxidant medical therapy. Sampling methods include changing the way to obtain the samples such as short abstinence and testicular sperm. Post-ejaculation methods include advanced and sometimes expensive technologies for sperm processing, which may result in sperm with better DNA integrity. These technologies can be categorized as 1-complex-expensive techniques such as MACS magnetic activated cell sorting), physiologic intracytoplasmic sperm injection (PICSI), intracytoplasmic morphology selected sperm injection (IMSI), etc. which may not be available in the majority of andrology clinics. 2- cost-effective simpler methods such as Zeta potential, micro fluid, etc., which can be set up easily in most infertility laboratory and ART wards. Besides the complexity and cost of these newer methods, there is also controversy about their efficacy on sperm DNA integrity and ART outcomes. In this session we will talk about these strategies as a step wise approach in male factor infertility management.

I-7: Sperm Retrieval in Sertoli Cell only Syndrome (SCO) Patients

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I-8: Male Sexual Dysfunction Management

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While previously considered the domain of younger generations, recent large cohort studies have demonstrated that many aging men and women engage in sex, with close to 50 % indicating that sex is important in their lives and with 26–90 % of men and women aged 70–90 years engaging in some form of sexual activity. Male sexual dysfunction can be categorized in to: loss of libido, erectile dysfunction, ejaculatory dysfunction, orgasmic dysfunction

Penile erection is a complex phenomenon which implies a delicate and coordinated equilibrium among the neurological, vascular and the tissue compartments. ED is defined as the persistent inability to attain and maintain an erection sufficient to permit satisfactory sexual performance. ED may affect physical and psychosocial health and may have a significant impact on the quality of life (QoL) of sufferers and their partners. There is increasing evidence that ED can be an early manifestation of coronary artery and peripheral vascular disease.

Although premature ejaculation (PE) is a common male sexual dysfunction, it is poorly understood. Patients are often unwilling to discuss their symptoms and many physicians do not know about effective treatments. As a result, patients may be misdiagnosed or mistreated.

In this lecture we will talk about different aspects of male sexual dysfunction (Epidemiology, etiology, pathophysiology, Risk factors, Diagnostic evaluation and Treatment options).

Animal Biotechnology

I-9: Reproductive Technologies in Small Ruminants: Environmental and Economic Effects in Iran

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Sheep and goat breeding have been a source of supply for meat, milk, wool, and other human-required products. Many breeds of small ruminants can graze in inferior rangelands considering important for their survival in Iran with its semi-arid climate. However, during recent years, drought and inappropriate use of rangelands made it very difficult to provide enough food to breeding sheep with traditional methods. Assisted reproductive technologies (ART) could increase the efficiency of reproduction in livestock, optimize the use of food resources and pastures and reduce production costs. Estrus synchronization protocols provide out of season reproduction and also apply other reproductive technologies such as artificial insemination and embryo transfer. Different methods of artificial insemination can increase the selection intensity in male and proliferate desirable traits. In embryo transfer technology, it is possible to select a superior mother, in addition to the father, and also is possible to provide several fetuses from the mother with the

desired genes by superovulation methods. Diagnosis of pregnancy can improve herd health management and inhibit costs of feeding and maintaining barren sheep for several months. Cloning and producing transgenic animals are technologies that can help us face future challenges by accelerating genetic progress, helping to produce drugs, and preventing extinction of endangered species.

I-10: Epigenetic Reprogramming in Mammalian Development

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Epigenetic modifications established during gametogenesis regulate transcription and other nuclear processes in gametes, but also have influences in the zygote, embryo and postnatal life. The best-studied epigenetic modification is DNA methylation, which established at discrete regions of the oocyte and sperm genomes and governs genomic imprinting. Genomic imprinting is referred to an allele-specific methylation marking process that causes a sex-dependent monoallelic expression. These methylation marks remain on the paternal and maternal alleles into the next generation as a lifelong memory of parental origin. DNA methylation undergoes dramatic reprogramming after fertilization. The impairment of DNA methylation reprogramming behind assisted reproductive technologies (ART) and the recognition of most vulnerable sequence elements are new debating topics. Some studies showed the effect of culture media, ovarian stimulation or embryo transfer on the methylation pattern of embryos emphasizing the need to face ART-associated defects and search for strategies to mitigate adverse effects on the health of ART-derived children.

I-11: Folic Acid Deprivation and siRNA-Inhibition of SUV39h1/2 in Fibroblast Donor Cells Improves Reprogramming of Bovine SCNT Embryos

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Efficiency of SCNT has remained low due to a strong resistance of somatic donor cells to epigenetic reprogramming. Many epigenetic drugs, targeting epigenetic status of donor cells or reconstructed oocytes to improve development of SCNT embryos. In our recent studies, we examined the effect of siRNA inhibition and chemically inhibition of H3K9me3 and also induced DNA hypo-methylation following in vitro folate deficiency in fibroblast donor cells on in vitro development of bovine SCNT embryos. Chaetocin was supplemented during the culture of donor cells for 3 days. In addition siRNA knock-down of SUV39H1/H2 was done in donor cells. Both chaetocin and siSUV39H1/H2 significantly reduced the relative intensity level of H3K9me3 in fibroblast cells. siSUV39H1/H2 transfection but not chaetocin treatment improved in vitro development of SCNT embryos. In addition, siSUV39H1/H2 altered the expression profile of the selected genes in the derived blastocysts

similar to those derived from IVF. DNA methylation in cells cultured in folate deficient (folate -) medium in presence of 0.5% serum was decreased. Bisulfite sequencing analysis indicated a decrease in DNA methylation of POU5F1 promoter and gene expression analysis revealed an increase in expression of POU5F1 gene. Blastocyst rate in folate - group was significantly higher than folate + group. The DNA methylation level in POU5F1 promoter and ICR of H19 and IGF2 of SCNT derived embryos in folate - group was similar to IVF derived blastocysts. In conclusion, our results may provide two new and novel approaches for improving mammalian SCNT efficiency for agricultural and biomedical purposes.

I-12: The Effect of Vitamin D in Male Infertility

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Despite numerous research on the role of vitamin D in the field of reproduction, based on 2019 publications, the conclusions remain controversial. There are publications suggesting that vitamin D deficiency in the female is associated with low embryo quality. In contrast, there are publications which suggest oocytes derive from individuals with vitamin D deficiency have higher quality. Similarly, research on the effect of vitamin D deficiency on male infertility remains also controversial. These controversies are mainly related to direct and the indirect effect of vitamin D and how vitamins D act in combination with minerals and metabolites such as folic acid and vitamin B. Therefore, the aims of this presentation are to reveal light on these ambiguities in the hope of providing clinical information of significant value for clinicians working in the field of assisted reproduction and to open room for future research.

I-13: Sequential Regulatory of *In Vitro* Meiotic Maturation and Developmental Competence of Oocytes

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Mimicking the intrafollicular environment during *in vitro* maturation (IVM) of oocyte may have beneficial effects on developmental competence of oocytes. In follicle structure, oocyte, cumulus cells (CCs), granulosa cells (GCs) and circulating factors act as meiotic inhibitory and regulatory elements toward achieving fully grown oocyte. This study aimed to assess the effects of exogenous granulosa secreted factors on development competence of sheep oocytes. Immature ovine cumulus oocyte complexes (COCs) were cultured in presence of combination of exogenous granulosa secreted factors. Cultured COCs were used for assessment of (1) cumulus expansion; (2) maturation rate (3) expression of cumulus-related transcripts and (4) yield and quality of derived embryos. Ligands and receptors related to granulosa secreted signaling had lower expression in cumulus cells derived from small follicles in compare to larger follicles. The best meiotic regulatory, gap junction communica-

tion and developmental rate achieved by sequential exposure of COCs to exogenous granulosa secreted factors. These results may provide an alternative IVM method to optimize oocyte competency and *in vitro* embryo production in sheep.

I-14: New Area for Testosterone and GDNF-Induced Roles in Varicocele-Related Infertility; Correlation with Spermatogonial Stem Cell Self-Renewal

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Based on previous clinical and experimental trials, the testosterone withdrawal in association with severe oxidative stress remarkably suppresses spermatogenesis as well as sperm count and quality in both animal models and infertile individuals. Indeed, testosterone through the classical (androgen receptor) and the non-classical (testosterone-mediated $[Ca^{2+}]$ influx) pathways boosts/maintains the spermatogenesis process. On the other hand, Sertoli cells are involved in maintaining/progressing spermatogenesis and spermiogenesis processes through several pathways. Among these pathways, the Glial cell-derived neurotrophic factor (GDNF) in association with its special receptors Gfra1 and C-RET are actively participated in spermatogonial stem cells (SSCs) self-renewal process. However, this question remains that, does testosterone affect GDNF expression/synthesis in Sertoli cells or not? To respond this question, a two-step study (*in vitro* and *in vivo*) was designed. In the *in-vitro* part; the mouse TM3 (Leydig) line cells were cultured, and the TM3-produced testosterone was included in TM4 (Sertoli) cells culture media, in a concentration (0.1 ng/ml, 0.2ng/ml and 0.4 ng/ml)-dependent manner. As a positive control, the same concentrations (0.1 ng/ml, 0.2 ng/ml and 0.4 ng/ml) of exogenous (commercial) testosterone was included in additional four Sertoli cell groups. Following 12 hours, the GDNF expression was evaluated by ELISA, qRT-PCR and ICC techniques. Observations showed that the TM3-produced testosterone (at 0.1 ng/ml and 0.2 ng/ml concentrations) and exogenous testosterone (at three concentrations) could significantly ($P<0.05$) up-regulate the GDNF expression compared to those testosterone non-treated Sertoli cells. This finding clearly illustrates that the testosterone is able to directly boost GDNF expression in Sertoli cells. This pathway was investigated parallelly in *in-vivo* condition by inducing experimental varicocele in rats. Observations revealed that simultaneous with Leydig cell elimination and testosterone suppression in varicocele rats, the GDNF expression (assessed by western blot, IHC and qRT-PCR) was diminished. Therefore, we can suggest that the varicocele-suppressed testosterone production is able to negatively affect the GDNF in Sertoli cells (in *in-vivo*) similar to those in *in vitro* condition. Next, minding that GDNF interacts with its receptors, this hypothesis came out, does GDNF act as transcriptional/boosting factor for its receptors or not? To respond this Delima different concentrations (0.1 ng/ml, 0.2 ng/ml and 0.4 ng/ml) of TM4-produced and exogenous GDNF were included in SSC culture medium. Observations showed that TM4-produced GDNF (0.1 ng/ml and 0.4 ng/ml) and the exogenous GDNF (at all concentration levels) could amplify Gfra1 and C-RET expression in SSCs. Thus, we came close to this fact that GDNF not only interacts with its receptors to initiate SSC self-renewal but also boosts Gfra1 and C-RET expres-

sion, at the same time. As an *in vivo* part; we demonstrated that when testosterone is administrated to varicocele-induced animals, the GDNF and at the same time Gfra1 and C-RET expressions were increased. Thus, we can conclude that varicocele by suppressing testosterone production is able to negatively affect the SSCs self-renewal process leading to severe germ cell loss through spermatogenesis.

Keywords: Varicocele, GDNF, Testosterone, Spermatogonial Stem Cells, Self-Renewal

Embryology

I-15: Application of Cryopreserved Ovarian Tissues

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Transplantation of frozen/thawed ovarian tissue for fertility restoration and regaining of fertility and menstrual cycles is rapidly gaining ground as a valid method for fertility preservation alongside cryopreservation of embryos and oocytes. More than 130 healthy children have been born worldwide as a result of this procedure after an estimated more than 500 women have had tissue transplanted. The procedure is most often carried out by excising one ovary or part of an ovary and leaving the remaining ovary in situ in case the treatment does not destroy all follicles or alternatively to be used as a suitable site for transplantation.

The application of frozen/thawed ovarian tissue was originally developed towards cancer patients, but it is now starting to be used in a number of other situations, where it is not only fertility but also the ovarian endocrine function which may be the desired effect upon grafting.

Thus, the indications for performing ovarian tissue cryopreservation is now being expanded – the feasibility of using this technique in connection with following conditions will be covered during my presentation.

Fertility preservation for cancer patients; Rare genetic diseases – confirmed potential in children; Known causes of POI; Endometriosis; Postponing menopause; Social freezing – alternative to mature oocytes; In combination with *in vitro* follicle activation; Anovulatory PCOS women – modern wedge resection

Further, our laboratory has now successfully retrieved ovarian tissue from a transplanted woman and re-frozen it for a second time. This provides additional options for specific patient categories. In addition, it has now been documented that ovarian tissue from truly pre-pubertal girls possess the capacity to sustain pregnancy when transplanted into an adult woman.

I-16: Fertility Preservation in Pre-Pubertal Boys

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Introduction: One fertility preserving strategy in you pre-pubertal boys is collection of small testicular biopsies to cryopreserve spermatogonial stem cells (SSCs), which is now per-

formed in many centers world-wide. From the biopsy SSCs may be propagated with intention of transplantation back into the testis to initiate spermatogenesis in adult life or to produce *in vitro* haploid germ cells to be used for procreation. This freezing method has mostly been used in boys with cancer, in which the appropriate treatment posed a risk of rendering them devoid of spermatogonial stem cells with no possibility of fathering their own biological children. Another category of patients that runs the risk of being infertile in adulthood is boys with cryptorchidism, which is the most common defect in newborn boys' affecting up to around 3% of all newborns. Despite that orchidopexy is nowadays performed between 6 and 12 months of age, men with a history of cryptorchidism, especially bilateral cryptorchidism, is exposed to a considerable risk of being infertile in adult life. We have now started to collect testicular biopsies from cryptorchid boys being operated in order to save fertility in adult life and have attempted to grow and propagate SSCs from such biopsies.

We used small testicular biopsies cryopreserved from five boys with an average age of 1.5 years operated for cryptorchidism. SSC colonies were obtained by 2-step enzymatic digestion and single cells were cultured in supplemented Stempro-34 medium. SSC-like cell clusters were propagated by passage at least 5 times and SSCs identified by immunohistochemical and immunofluorescence staining plus q-PCR reaction analysis including markers like LIN-28 homolog A (LIN28A), G antigen 1 (GAGE1), promyelocytic leukemia zinc finger protein (PLZF), integrin alpha 6 (ITGA6), ubiquitin carboxy-terminal hydrolase L1 (UCHL1) and integrin beta 1 (ITGB1). We have now shown that that SSCs from infant boys potentially successfully can be *in vitro* propagated and may represent a fertility preservation strategy for young boys, who adversely may lose their fertility. The presentation with focus on our current results and the potential feasibility of this technique.

I-17: Artificial Testis, Current Research and Future Direction

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In recent decades, epidemiological studies have shown an increase in the prevalence of disorders in both female and male reproductive system, which can lead to sexual dysfunction, subfertility or infertility. In the field of male infertility, although intracytoplasmic sperm injection (ICSI) using frozen sperms has revolutionized infertility treatment, but it is not applicable for the patients who undergo some invasive treatment like as chemotherapy before puberty since spermatogenesis has not begun, or those with idiopathic non obstructive azoospermia, and those with Klinefelter syndrome (KS).

Reproductive Tissue Engineering (REPROTEN) is new branch of tissue engineering and regenerative medicine. The goal of this new scientific approach is to apply tissue engineering strategies to restore fertility and/or improve the quality of life of patients affected by reproductive dysfunction through creation, replacement or regeneration of cells, tissues or organs of the reproductive system. The largest area of interest to emerge in the field of REPROTEN has been fertility restoration in male patients.

Testis as a banking of progenitor cells for producing mature gamete cells, have elaborate communication network between

several cell types. For this reason REPROTEN in the male is not as easy as other organs.

Some strategies around regenerative of testis include: cell therapy into clinical male infertility treatment, use of three-dimensional (3D) culture systems thorough demonstrated different scaffolds materials like fibrin, alginate, human serum albumin, poly (vinyl alcohol), and collagen etc., Decellularization of human testis tissues and extracellular matrix extraction, producing male gamete cells by differentiation of other cell source like as primordial germ cells, apply coculture method for stimulating testicular cells to produce in vitro haploid cell. In conclusion, REPROTEN of testis is progressing, and is a promising area of research if achieved, will be a revolution in the field of male infertility treatment.

I-18: Safety by Design Interaction of Nanoparticles and Cells

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Two reasons herald Nanotechnology as a revolution in science: i. the way in which chemicals and elements behave compared to traditional scientific understanding of their properties; ii. the impact of these new discoveries can transform the daily life of consumers. Miraculous developments, (just thinking to Nanomedicine that offers the promise of diagnosis and treatment at the molecular level to detect and treat presymptomatic disease), sound like science fiction and combined with the emerging commercial impact of nanotechnology applications to consumer products will reshape civil society - permanently. The point that everyone is an end-user of nanotechnology, even without realising that nanotechnology has touched daily life, raises big safety concerns. Scientists and governments agree that unknown risks to human health and the environment exist for the application of nanotechnology. Particularly unexplored is the nanotechnology impact on the environment and non-human species, consequently, the impact on human health. In response to these emerging issues, a plethora of drafts about nanotechnology are floating around the web and in legislatures around the world to create a science policy.

The recent progresses of biomedical nanotechnology increased the development of drug delivery systems (DDSs) for diverse therapies. To improve clinical responses and tolerability, conventional nanocarriers (NCs) have been developed and evolved into smart DDSs with stimuli-responsive characteristics. Several clinical trials have shown that these DDSs have better clinical effects in the treatment of many pathologies than those of free drugs. Furthermore, the use of molecules increased the specific targeting of therapeutic DDSs against pathological cells and tissues without affecting health tissues and limiting their toxicity against unspecific body compartments.

A significant issue to reduce possible dangers for human and environment health or improve the efficacy of NCs, relies on the thorough knowledge of the biological interactions and subsequent effects. In this context, many factors must be considered, including size, shape, surface charge, and several physico-chemical characteristics of NCs, because of, ideally, nanomaterial development should incorporate a safety by-design approach.

Here we discuss on opportunities emerging from the development of multifunctional NCs. Challenges related to biocompatibility of nanomaterials in relation to their characteristics (size,

aggregation, shape, surface charge, reactivity, dissolution, etc.) are also discussed.

I-19: Nutrition Coaching Versus Nutrition Counseling in Management of PCO and Infertile Obese Couples

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Adherence to healthy dietary pattern is associated with fertility and infertility treatment outcome mainly in polycystic ovarian syndrome and obese cases. Large body of literature not only emphasis on the critical role of dietary intervention and nutrient environment on semen quality, ovary function and endothelium responsiveness in infertility treatment outcome, but also the recognition of fetal origins of adult disease has highlighted the importance of comprehensive nutrition and dietary counseling during preconception and prenatal care.

As intrauterine environment has a broad and long-lasting impact, influencing fetal and childhood growth and development as well as future risk of non-communicable disease including infertility, providing the most appropriate intervention strategies to improve dietary intervention compliance before conception is undoubtedly appreciable. The greatest challenge in compliance to dietary counseling is to engage couple and to sustain their engagement.

A new clinical approach is providing personalized nutrition coaching versus nutrition counseling by focusing on non-dietary aspects of a person's life which influence hormonal responses and efficacy of nutrition interventions. Nutrition coaching focuses on empowering people with skills which mobilize internal strengths and external resources for enhanced pregnancy chance. Therefore, such approach is beyond the scope of energy calculation and diet prescription. Nutrition coaching is based on initially monitoring and analyzing personal nutritional behavior under stress and pressure. This approach is followed by practical mindfulness eating trainings, equipping couples with the skills which improve their nutritional decision and reduce their common struggles about whether or not their food choices are influencing their pregnancy outcome and feeling confident about cooking and eating healthy, whether at home or on-the-go.

Recent trend of studies are even evaluating effectiveness of applying web based, m-health and coaching applications in improving dietary intake in comparison to standard advices offered by national health services and associations worldwide.

I-20: The Effect of Vitamin D in Female Infertility

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Vitamin D is a group of fat-soluble steroids responsible for enhancing intestinal absorption of calcium and phosphate, which is directly related to the maintenance of the normal structure and function of the skeletal system. Observational studies have described associations between low circulating levels of vita-

min D and a large number of diseases, including cardiovascular diseases, malignancies, diabetes, obesity, and infections, neuropsychiatric and autoimmune diseases.

Accumulating evidence from animal and human studies suggests that vitamin D is involved in many functions of the reproductive system in both genders.

Vitamin D has a biologically plausible role in female reproduction; it has been associated with features of polycystic ovary syndrome (PCOS), endometriosis and in vitro fertilization (IVF) outcome.

Increasing evidence suggests that vitamin D might have a regulatory role in PCOS-related symptoms, including ovulatory dysfunction, insulin resistance and hyperandrogenism. Although several data converge towards a beneficial effect of vitamin D supplementation in metabolic disturbances in women with PCOS, a significant knowledge gap precludes the establishment of a clear cause-effect relationship.

Vitamin D deficiency also has been reported to contribute to the pathogenesis of endometriosis due to its immunomodulatory and anti-inflammatory properties. Although most of the studies supported a role of vitamin D in the onset of these diseases, randomized controlled trials to assess the efficacy of vitamin D supplementation have never been performed.

Studies investigating the association of vitamin D status with IVF outcome revealed inconsistent results. Some studies report positive effect; however other found that vitamin D deficiency did not play an important role in the outcome of assisted reproductive technology (ART). Given such contradictory results, there is a need for further researches with better design.

I-21: The Impact of Chemotherapy and Radiotherapy on Pre-Pubertal Gonad

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Gonadal toxicity account as one of the most common complications through chemotherapy and radiotherapy of patients with malignancy. In this regard in spite of effective treatments and improving the survival rate of patients with cancer, decreasing the outcome of hypogonadism and gonadal toxicity is inevitable. Modern multidisciplinary treatments and new generations of chemotherapy agents are making gonadal toxic effects less than before. It is important to point that some chemotherapy agents may be use in other non-malignant diseases. In this way all of the patients should inform about the possibility of gonadal toxicity prior their treatment.

Sperm banking could be effective way for patients who are going to confer with chemotherapy and radiotherapy. Because spermatogenesis is so sensitive to treatment modalities of malignancies. Testosterone deficiency in males could be easily improve with testosterone replacement.

Ovary is also sensitive to chemotherapy and radiotherapy modalities. Storage of ovarian tissue could be feasible but the possibility of pregnancy in future should be noted. Also uterine function could be damage by radiotherapy and chemotherapy. Cancer survival rates is increasing recent years. Although treatment modalities have outcomes and late effects, the most

important of those effects is gonadal toxicity. Increasing the knowledge of patients about this effect and suggesting future plans for dealing with this challenge is of necessities.

Keywords: Chemotherapy, Gonadal Toxicity, Radiotherapy

I-22: Techniques of Vitamin D Measurement and Cut-Off Point Interpretations

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I-23: Application of Microfluid Systems in ART Laboratory

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In the past few decades, an extensive efforts have been made to improve the outcomes of human assisted reproductive technologies (ART). Culture medium, laboratory technologies and equipments have changed over time and have positively influenced ART results. Recent progress in microfluidic technology has turned the research's attention to the human ART procedure. Studies on microfluidic system in ART labs have been focused on sperm cell (selection and cryopreservation), oocyte (selection, denudation and cryopreservation) as well as In vitro fertilization (IVF) and embryo culture. In this regard, selection of sperm by using microfluidic technologies gained considerable attention. Microfluidic devices have been designed based on sperm characteristics such as motility, rheotaxis, chemotaxis, thermotaxis and boundary flowing property. Microfluidic sorted sperm samples showed lower DNA fragmentation compared to conventional selection methods. Moreover, cryopreservation of low number human spermatozoa by using microfluidic chip has advantage over the micro-quantity straw. Microfluidic based oocyte selection and denudation improved fertilization of human and animal oocyte. Finally, application of dynamic microfluidic system resulted in improved embryo development compared to conventional culture system. In conclusion, microfluidic technologies could provide a simple and user-friendly system for all of the procedure of human ART.

Keywords: Microfluidic, Sperm, Oocyte, Embryo culture and ART

Female Infertility

I-24: Pregnancy Over 40: Risks & Opportunities

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Nowadays, motherhood at advanced ages is progressively increased due to socio-economic status. Moreover, developments in assisted reproductive technologies (ART), such as oocyte and embryo donation, offer pregnancy options for peri-menopausal women. So that live births for women aged ≥ 45 years reported approximately 0.05–0.2% of all births.

On the other hand, pregnancy among women of advanced maternal age (AMA) is highly related to adverse maternal and fetal outcomes. Maternal complications such as hypertension and diabetes, post-partum hemorrhage, placental abruption, as well as increased rates of abortion, still birth, fetal anomalies and malformations were stated. Furthermore, some long-term effects in post-menopausal period were mentioned among AMA women. As, it has been found that women, who have pregnancy in advanced ages, were 50% more likely to be involved in hemorrhagic stroke in later stages.

However, it is ethically argued that IVF itself is a principled method to carry out in women of AMA, it is further debated that this is unfair to get women's right of maternity in advanced age. Therefore, it is preferred that AMA women be completely informed about the success rates and hazards such as multiple gestations as a minimum risk related to ART.

In conclusion, there are significant ethical concerns and medical (maternal and fetal) complications related to pregnancy in AMA women. It is recommended that physical, psychological, financial, and social resources should be implemented for early marriage and natural childbearing.

I-25: Surgical Intervention in Submucosal Myoma Grade II and III

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Endometriosis is present in approximately 5 to 15% of infertile couples. However, the best treatment approach and the place of surgery for these patients are still in dispute. Detecting infertile women who are at risk for endometriosis and optimal imaging evaluation are now replacing the use of laparoscopic surgery to diagnose pelvic endometriosis.

Laparoscopic surgery for patients with minimal and mild peritoneal superficial endometriosis (SUP) improves the chance of spontaneous conception and live birth rate. This treatment effect before IVF is relatively small, and thus, it may not be a cost-effective intervention.

The ovaries with endometrioma (OMA) ovulate spontaneously and do respond to stimulation with gonadotropin. The size of OMAs not requiring surgical intervention tends to rise, especially if the infertility is present. Removing the OMAs does not improve the ART outcome; furthermore, the procedure may significantly decrease the ovarian reserve and previous surgery for OMA is a significant prognostic factor for a reduced ART success rate. It is of great importance to maintain the ovarian reserve and to start ART before ovarian reserve is compromised. Surgery for deep infiltrating endometriosis (DIE) is complex and carries certain risks and does not improve fertility and ART outcome. Data does not support the use of surgery before ART for infertile patients with DIE. IVF is the first line treatment for these patients.

Today, for infertile patients with endometriosis, ART is the first line and surgery is the final treatment. Surgery prior to ART

must be carefully considered regarding factors such as patient's age, duration of infertility, other infertility factors such as the presence of hydrosalpinx, severity of pain, previous surgery for OMAs, and most importantly ovarian reserve. Young patients with short infertility duration and pelvic pain might be good candidates for primary surgery. If surgery is used to treat endometriosis, the conception should be attempted shortly after surgery.

The main goals are to do surgery -if required- only once in a life time for the patients with endometriosis, to postpone surgery to the closest time to pregnancy attempt or ART, and to maintain a good ovarian reserve for future spontaneous conception or ART.

I-26: Surgical Intervention Isthmuscele

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Cesarean section as a major surgery has many short and long term complications which can be responsible for maternal and fetal mortality or morbidity. By increasing number of cesarean sections worldwide, the rates of these complications are growing up. A lately recognized complication is impaired uterine wound healing, which could lead to a diverticulum formation known as isthmuscele or niche.

Association between isthmuscele with various gynecological and obstetric problems, such as uterine rupture, cesarean scar pregnancy, bleeding disorders, and infertility, has been reported by many authors. The number of cesarean sections is the main risk factor. Isthmuscele is a growing surgical concern that needs attention in identifying, diagnosing, managing, and treating this problem. Currently, treatments include medical and surgical approaches. Hysteroscopy as well as laparoscopy are used in the treatment. At this article we presented its symptoms, risk factors, effects on future fertility, diagnostic modalities, and choice of medical or surgical treatments.

I-27: Adenomyosis and Infertility

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Adenomyosis is a very difficult disease to have when hoping for fertility. Many patients will consult with their gynecologist for heavy bleeding, clots and significant monthly pain during and around periods.

This presentation is timely and relevant for several reasons. The incidence of adenomyosis begins to rise from the mid-thirties. Moreover, more women are delaying their first pregnancy until later in their thirties or forties, and consequently adenomyosis is encountered more frequently in the fertility clinic during diagnostic work-up. Furthermore, it is difficult to diagnose adenomyosis before surgery, because there are no pathognomonic signs, symptoms or physical findings. Finally, reference data are very limited.

Adenomyosis remains a fairly frequent and debilitating disease that will be encountered with increasing incidence in the infertile female population. While spectacular advances have been made in recent years in the non-invasive diagnosis of the condition, non-surgical treatment options for infertile patients with

adenomyosis arise but need to be confirmed in larger series.

To date, there is no consensus for the most appropriate treatment of adenomyosis with infertility. Conservative surgery could be effective option to treat adenomyosis, especially in women who seek fertility preservation and, in women with previous history of IVF failure. However, the latter finding could be partly attributed to the higher rate of early miscarriage. In this presentation, reported data regarding treatment of adenomyosis were summarized and then the most appropriate approach to treat infertility associated with adenomyosis will be discussed.

I-28: Evidence Based Management of Recurrent Pregnancy Loss

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Recurrent pregnancy loss (RPL), either early or late in the gestational period is a serious problem and has both psychological and social impacts on the women who suffer from it. In some cases, it may lead to divorce or other social problems. Recurrent Pregnancy Loss (RPL), defined as two or more consecutive pregnancy losses, affecting 1–5 % of reproductive-age woman. There is a strong belief that RPL is a multifactorial condition that many factors affect such as chromosomal abnormalities, uterine anatomic malformation, endocrine dysfunction, thrombophilia, immunologic factors, infections, and environmental factors. However, the etiology of RPL remains unknown in ~50 % of cases.

This review presentation highlights the current understanding of the various etiologies implicated in RPL, including factors known to be causative, as well as those implicated as possible causative agents. The appropriate diagnostic evaluation, therapy, and prognosis are also addressed.

Keywords: Recurrent Pregnancy Loss, Spontaneous Abortion, Habitual Abortion

I-29: Inherited Thrombophilia and Recurrent Pregnancy Loss

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Recurrent pregnancy loss (RPL) affects 1–3 % of women of reproductive age, which is a stressful condition for married couples. Unfortunately, in many cases the exact etiology remains undetermined. Thrombophilic gene polymorphism is a known risk factor for recurrent pregnancy loss (RPL). There are a lot of studies on the association between thrombophilia gene polymorphism. Hereditary thrombophilia comprise a number of conditions, such as antithrombin (AT) III deficiency, protein S (PS) and protein C (PC) deficiencies, factor V Leiden, prothrombin 20210A mutation, elevated factor VIII level, and mutation of gene encoding the enzyme methylenetetrahydrofolate reductase (MTHFR). These mutation increases prothrombin levels, which in turn raises the risk for thromboembolism. Two types of polymorphism identified due to low efficiency of the MTHFR enzyme. In homozygous individuals, the efficiency level decreases by 35% from normal. Although not at the same level, the efficiency of the enzyme is also reduced in heterozy-

gote individuals. Therefore, the homocysteine level increases. Also, it has been reported that, during trophoblast invasion, urokinase plasminogen activator receptor and plasminogen activator inhibitor-1 (PAI-1) control proteolysis of structures and remodeling of maternal tissues. RPL could also include pregnancy losses up to gestational week 28. So, vascular placental pathologies, retarded intrauterine development, such as in preeclampsia, late fetal loss and abruptio placenta can be observed in carriers of these mutations. Subsequent management of these patients in pregnancy by adequate antithrombotic therapy can be initiated from very early days of pregnancy to prevent placental vasculopathy and coagulation defects, and thereby improve maternal and perinatal outcomes of these pregnancies to a great extent.

Keywords: Recurrent Pregnancy Loss, Thrombophilia, Polymorphism, Mutation

I-30: The Negative Impact of Obesity Associated Advanced Glycation End Products on Female Fertility

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Background: The incidence of overweight and obesity is growing worldwide. Around 60% of reproductive aged women in developed countries are overweight/obese before conception and these women experience a higher incidence of infertility and placental associated pregnancy complications. The pre-conception uterine environment is key in achieving embryo implantation and a healthy pregnancy. Therefore, we aimed to quantify levels of advanced glycation end products (known to be elevated systemically in obese subjects) within the obese infertile versus lean fertile uterine environment and determine if obese levels of advanced glycation end products (AGEs) within the uterine cavity detrimentally alter tissue function in embryo implantation and placental development.

Materials and Methods: Levels of AGEs examined within uterine lavage assessed by ELISA to determine differences between lean and obese women. Expression and localization of AGEs, receptor for AGEs (RAGE) and NFκB within endometrial tissues obtained from lean and obese women determined by immunohistochemistry. Endometrial epithelial cells (ECC-1), primary human stromal cells and trophoblast cells (HTR8-SVneo) treated with lean (2000nmol/mol lysine) or obese (8000nmol/mol lysine) uterine levels of AGEs and p65-NFκB (western immunoblot), real time adhesion, proliferation migration and invasion (xCelligence real time cell function analysis), decidualization (cell morphology and prolactin release), endoplasmic reticulum stress (western immunoblot for p-PERK) determined. Co-cultures of endometrial epithelial cells and blastocyst mimics (trophectoderm spheroids) similarly treated with lean or obese uterine levels of AGEs to determine their impact on embryo implantation.

Results: AGEs were significantly elevated (P=0.004) within the obese (6503.59 μmol/mol lysine) versus lean (2165.88 μmol/mol lysine) uterine cavity (uterine lavage) with increased immunostaining for AGEs, RAGE and NFκB within obese endometrial tissues during the proliferative phase of the menstrual cycle. Obese uterine levels of AGEs demonstrated a trend to activate NFκB signaling within endometrial epithelial (ECC-1) cells and inhibited their adhesion and proliferation

versus treatment with lean uterine levels of AGEs. Obese uterine AGE levels impacted primary human endometrial stromal cell decidualization and activated endoplasmic reticulum stress within these cells. Obese uterine levels of AGEs also inhibited trophoblastic spheroid adhesion to hormonally primed endometrial epithelial cells and trophoblast cell line HTR8/SV-neo invasion.

Conclusion: These data corroborate clinical data suggesting the presence of an altered uterine environment in obese women and demonstrate that elevated uterine levels of AGEs within these women may detrimentally impact endometrial function, embryo implantation and placental development.

Keywords: Obesity, Infertility, Endometrial Receptivity, Advanced Glycation End Products, Inflammation

I-31: Fertility Preservation in Pre-Pubertal Girls

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During the last decade, there has been a considerable increase in the survival of childhood cancer as a result of improved anti-cancer Treatments Multi agent chemotherapy increased 5-year survival rate for childhood .One of the major concerns in girls suffer from childhood cancer is the adverse effect of cancer therapy on their fertility .Chemotherapy and radiation therapy, are established risk factors result in acute ovarian failure. The effect of cancer therapy on gonadal function varies, ranging from no effect to total loss of ovarian function. These effects related to several factors, including the type and dose of chemotherapy, radiation, type of surgical intervention, and the field of radiotherapy. Due to the potentially detrimental effects of chemotherapy and radiotherapy on future fertility, international societies, recommend discussion on the risks of cancer therapy on fertility and options for preservation with all patients before the initiation of any therapy. Ethical issues in this population of cancer patients should be considered .because parents make decision for their children; therefore, adult factors maybe effect on the decision. Child hood fertility preservation methods are experimental procedures and have uncertain outcomes. For this reason, children should be involved in the discussion. Methods to Preserve the Reproductive Potential in female children Undergoing Gonadotoxic Cancer therapy are cryopreservation of ovarian tissue, oophoropexy, gonadal shielding. For cryopreservation of ovarian tissue in prepubertal girls, extraction of less than half an ovary (20-30%) may be enough. The risk of contamination of ovarian tissue with malignant cell fallowed by reintroducing malignant cells in blood borne cancer is high. Promising result has been reported with an artificial ovary in animal models. Gonadal shielding consists of physical protection of gonadal tissue with suitable shields during radiation. Oophoropexy is used when pelvic or abdominal irradiation is scheduled: Ovary is transposed via laparoscopy or mini-laparotomy toward the pelvic walls laterally or behind the uterus medially. It is also important to emphasize that the existence of uniform guidelines is essential for proper clinical practice and will make it possible to use a common and shared approach to this intricate subject.

I-32: Adenomyosis and Endometriosis: The Association and Impact on Fertility

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I-33: From Triggering to iTriggering

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Trigger is an essential step in OS for IVF to mimic LH surge; probably the most important injection. The objective of iTriggering is maintain efficacy (securing the optimal number of > 75% mature oocytes, optimal LH activity surge and a subsequent P rise and without compromising fertilization, embryo development and euploidy rates) as well as reduce the risk of OHSS (mortality rate 0.18%- 1).

Harvesting oocytes at wrong stage/time impair quality and embryo developmental potential (ideally 16-18 mm; $\geq 36h$). rhCG and uhCG similar pharmacokinetics but different pharmacologic and clinical profile. r-hCG 250mcg better tolerated, higher P and hCG levels as well as better clinical outcomes.

I-34: Freezing Your Lab

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The aim of assisted reproductive treatments is live birth of a healthy baby as fast as possible in the most effective and efficient way. Freeze all strategy has been proposed as an option not only for preventing OHSS but also increasing live birth rates.

There are no clinical data supporting the widespread use of the freeze-all strategy for all IVF patients at the moment. Based on available RCTs, it seems reasonable to implement this strategy in patients at risk of OHSS and when performing PGT-A at the blastocyst stage. All other potential indications, (repeated implantation failure, high progesterone levels on the trigger day, advanced maternal age and endometriosis) still need to be explored in well performed large RCTs.

I-35: Future of Reproductive System and Ethical Consideration

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I-36: Intracytoplasmic Sperm Injection Versus Conventional IVF for Treatment of Non-Male Factor Infertility.

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Intracytoplasmic sperm injection (ICSI) was first introduced by Palermo in 1992.

At that time, the indication of this method was to help men with severe semen parameters abnormality, including oligoasthenospermia.

In the severe sperm abnormalities, ICSI was able to achieve fertilization rates equal to those with normal semen parameters. During the last two decade ICSI has been gradually expanded to treat moderate semen abnormality or even couples with non-male infertility.

The strong evidences from USA national data showed that in non-male factor infertility treatment, ICSI use was not associated with higher rate of implantation rate compared to conventional IVF (23% Vs. 25.5% respectively) and live birth rate(36.5% versus 39.2%),respectively.

ICSI procedure is and invasive method. This procedure prevents the natural selection barrier of oocyte. Bypassing the selection barrier of oocytes may result in many genetic abnormality of the resulting embryo.

In this lecture we will try to compare the effectiveness of ICSI and IVF in patients with non-male factor infertility.

I-37: Preimplantation Genetic Diagnosis for β -Thalassemia Combined with HLA Matching

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Introduction: Beta-thalassemia is an inherited anemia with fatal consequences that can be treated by hematopoietic stem cell transplantation (HSCT) and prevented by preimplantation genetic diagnosis (PGD) of embryos of carrier parents. Searching for appropriate donors with HLA typing acceptable for transplantation is not always fruitful. The success rate of the procedure is higher if compatible HLA could be found among siblings. Combined PGD-HLA typing has been introduced and carried out throughout the world in recent years for reproducing offspring both healthy and suitable for transplantation.

Materials and Methods: In our center a female blood transfusion dependent beta-thalassemia patient with parents eager for another child was selected for HSCT from the product of in vitro fertilization after PGD-HLA typing. Six to eight cells from trophoctoderm of 12 blastocyst of one IVF cycle were obtained without damaging the inner cell mass. Primarily genome DNAs underwent whole genome amplification while embryos were frozen till the genetic tests results were available. All samples were tested for beta-thalassemia mutations using primers specifically designed for this purpose. one healthy, 3 minor thalassemia, and 8 major thalassemia was the outcome. In order to avoid possibility of allele drop-out disturbing the assessment of mutation for thalassemia 10 predesigned loci of heterogene short tandem repeats for parents and embryos were examined. Common chromosome anomalies for chromosomes 13, 15, 16, 18, 21, and 22 were checked as well as sex chromosomes. Among 4 embryos that did not have major thalassemia 2 were boys. Twenty HLA loci were assessed and 8 were applied for HLA typing with the affected child of the family. Matched HLA were detected in 1 healthy boy and 1 minor thalassemia girl.

Result: The pregnancy is ongoing after the healthy male em-

bryo was implanted and in the 7th week pregnancy was confirmed. Amniocentesis result was healthy embryo with HLA matched for HSCT.

Conclusion: HLA typing in adjunct to genetic testing for patients in need of HSCT by PGD is an appropriate novel approach for reproducing healthy child with matched HLA for the siblings in need of HSCT.

I-38: How to Manage Thin Endometrium?

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Implantation and pregnancy are influenced by two main factors, embryo and endometrium. A healthy embryo and receptive endometrium are necessary for implantation.

Endometrial pattern and thickness are evaluated by ultrasound examination. There is no agreement about the most recipient endometrium, however, most clinicians prefer endometrial thickness more than 7 mm for embryo transfer.

In frozen embryo transfer cycles, patients should be counselled that endometrial thickness <7 mm may have a negative impact on pregnancy and live birth rates. Some *in vitro* fertilization (IVF) cycles are cancelled due to inadequate endometrial growth despite several treatments. For patients with a history of thin endometrium in assisted reproductive technology (ART), there is insufficient evidence that any specific protocol (natural cycle or hormone replacement) for endometrial preparation provides better pregnancy outcomes.

Different protocols such as extended estrogen treatment, Pentoxifyline, low-dose Aspirin, Tamoxifen, vaginal Sildenafil, intrauterine perfusion with granulocyte-colony stimulating factor (GCSF), and platelet-rich plasma (PRP) have been performed for the management of thin endometrium, but there is little consensus on the most effective one.

I-39: State of The ART Management of Monochorionic-Diamniotic Twins and Fetal Surgery

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Twin-to-twin transfusion syndrome (TTTS) increases perinatal morbidity and mortality for 10 to 15% of monochorionic (MC) gestations. The purpose of this abstract is to provide an update on the evolving treatments in TTTS as it pertains primarily to laser photocoagulation, as well as to provide recently published information on outcomes.

MC gestations are at risk due to the angioarchitecture of the shared placenta, with anastomoses of varying type, size, and quantity. The chronic unbalanced distribution of blood volume across placental anastomoses between the donor and recipient fetuses leads to multisystem organ impairment including maladaptive changes in both fetuses.

Fetoscopic laser photocoagulation of placental anastomoses is an effective treatment addressing the underlying pathophysiology. Further research is needed to improve survival rates, re-

duce risks of fetoscopy, and gain understanding of the prediction, assessment, and optimization of long-term outcomes for TTTS survivors.

I-40: The Epidemiology of Perinatal/Neonatal Outcomes among Twins Stratified by Mode of Conception and the Effect of Advanced Maternal Age in The United States

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Background: To compare neonatal outcomes among twin pregnancies conceived as a result of fertility treatments, including Assisted Reproductive Technology (ART), ovulation induction (OI), intrauterine insemination (IUI) and twin pregnancies conceived spontaneously A retrospective population-based study

Materials and Methods: This is a population-based analysis of neonatal outcomes in twin pregnancies in the United States based on their mode of conception: 1) Fertility enhancing drugs for OI/IUI, 2) ART (including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), and 3) spontaneous conception (SC). We used "Nativity" files from the National Center for Health Statistics from 2014-2016. All twin gestations with reported variable for mode of conception were included. The primary outcome was the rate of composite neonatal morbidity (CNM) and was defined by presence of at least one of the followings: gestational age (GA) at delivery < 28 weeks, 5 min Apgar score < 7, assisted ventilation > 6 hours, neonatal intensive care unit (NICU) admission, antibiotics administration or seizure. Statistical analysis was performed using R-3.4.1.

Results: Included in this study were 388,045 twin deliveries of which 334,677 (86.2%) resulted from SC, 36,883 (9.5%) from ART and 16,485 (4.2%) were conceived by OI/IUI. CNM was positive in 134108/334677 (40.1%; 95%CI 39.9-40.2) of SCs, 16283/36883 (44.1%; 95%CI 43.6-44.7) of ART and 7108/16485 (43.1%; 95%CI 42.4-43.9) of OI/IUI fertility treatments. The differences between these 3 groups were statistically significant before and after adjustment for potential covariates.

Conclusions: In this retrospective study, we observed that the rate of neonatal morbidity was slightly higher in twins conceived by IVF/ICSI and OI/IUI compared to SC. This slightly increased rate however, is not clinically significant and overall reassurance can therefore be provided regarding neonatal outcomes to the patients undergoing fertility treatments.

P value is calculated based on Multinomial Logistic Regression with Mode of Conception as dependent variable and the following covariates: maternal age, body mass index (BMI), race/ethnicity, marital status, gestational hypertension and diabetes.

Part B- Title: Perinatal Mortality among Twins Stratified By Mode Of Conception In The United States (2014-2015)

Objective: To perform an epidemiological study of perinatal mortality rate among twins based on their mode of conception: Assisted Reproductive Technology (ART), less invasive fertility treatments including ovulation induction (OI) and intrauterine insemination (IUI), and spontaneous conception (SC).

Design: A retrospective population-based study

Materials and Methods: We performed a population-based retrospective analysis of twin perinatal mortality in the United States (2014-2015) among newborn twins without congenital malformations and/or chromosomal abnormalities. We utilized 'Period Linked Birth – Infant death' and 'Fetal Death' data files

from the National Center for Health Statistics for years 2014 – 2015. Perinatal mortality was defined as stillbirth at ≥ 22 weeks gestation or neonatal death up to 28 days after birth. Perinatal mortality was calculated for different categories of mode of conception: 1) ART (including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), 2) fertility enhancing drugs for OI/IUI, and 3) SC. Statistical analysis was performed using R-3.4.1.

Results: Included in this study were 388,045 twin deliveries of which 334,677 (86.2%) were from SC, and 36,883 (9.5%) and 16,485 (4.2%) were results of ART and OI/IUI treatments, respectively. Rate for perinatal mortality was 3792/334677 (11 per 1000; 95%CI 10-12) in SCs, 464/36883 (13 per 1000; 95%CI 12-14) in ART fertility treatments and 215/16485 (13 per 1000; 95%CI 11-15) for twin deliveries conceived from OI/IUI. (Chi² = 8.05, P=0.01).

Conclusions: We observed that perinatal mortality rate is higher among twins conceived following fertility treatments, including ART and OI/IUI, compared to twins conceived without these treatments. Although this slightly increased risk is clinically insignificant, counselling patients before undergoing any form of fertility treatment should include discussion of the risks of perinatal death.

I-41: Intraovarian Injection of Autologous Platelet-Derived Growth Factors

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I-42: Approach to Increase the Chance of Euploidy

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I-43: The Matter of Oocyte Number: More Is Beter

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Genetics

I-44: Weighted Gene Coexpression Analysis Reveals New System Level Properties of Endometriosis

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In endometriosis (ES), an endometrial like tissue grows outside the uterus. The disease is not well-known and there is a crucial need to understand the underlying molecular mechanism of the disease in order to find treatment methodology or develop diagnostic tests. This study compares gene expression patterns between the normal endometrium (NEM) and ES at minimal/mild (MMES) and mild/severe (MSES) stages. We used microarray datasets to analyse the preservation of gene expression patterns during disease development at these stages using the signed weighted gene co-expression network analysis (WGCNA). We identified sixteen co-expression modules. Nine modules were non-preserved in both MMES and MSES. Five modules were preserved in NEM, MMES and MSES. Importantly, two non-preserved modules were found in either MMES or MSES, highlighting differences between ES stages. Some modules uncover novel co-expressed gene clusters that have not been discovered before. This study will contribute to better understanding of the ES gene regulatory mechanism and suggest a number of important target genes with future clinical implications.

I-45: Application of Genomic Studies in Uncovering Sperm Defects Mechanisms

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Background: It is estimated that about 40 % of male infertility cases are idiopathic. While asthenozoospermia is the most frequent condition observed in infertile male, very few genes in relation to this phenotype have been introduced. The main reason for this in many cases is the large number of potential candidate genes. It is assumed that more than two thousand genes are involved in spermatogenesis. Therefore, lots of potential candidate genes may exist in each case making it very difficult to identify the pathogenic variation behind the condition. However, novel gene and variation identification by using newly available technologies is a beneficial approach to uncover the asthenozoospermia pathophysiology potentially leading to new treatments. Accordingly, in the last decade the advent of Next Generation Sequencing (NGS) technology and particularly Whole Exome Sequencing (WES) has revolutionized our knowledge of reproductive genetics. This unprecedented success is due to the nature of this method in which the exons of known human genes are sequenced with adequate coverage making it possible to study various candidate genes simultaneously. In recent years, we have actively recruited consanguineous families with multiple infertile male patients. Routine tests have failed to establish the cause of infertility in all of these patients while generations of consanguineous marriages indicated it to be of genetic nature. Therefore, we applied WES to study these families which led to the identification of novel and rare pathogenic variations in genes involved in natural sperm functions.

Materials and Methods: To establish the idiopathy of infertility, all patients underwent routine diagnostic procedure includ-

ing semen analysis, hormonal assays and karyotyping. Consequently, high quality DNA was procured from patients and close family members for WES. Rigorous bioinformatics analysis on WES results led to a shortlist of candidate variants that were verified by conventional PCR and Sanger Sequencing. To confirm the pathogenicity of the identified variants, dynamic protein modeling and appropriate experiments were performed in accordance with the nature of each variation. In one case the sperm sample of patients was treated with the analogue of the predicted defective enzyme and in another, ROS evaluation test was performed on sperm samples.

Results: Using WES, we managed to identify a pathogenic frameshift variant in ADCY10 which codes for the soluble adenylate cyclase (sAC), the main source of cAMP in sperm tail and an essential enzyme for the regulation of sperm motility. Functional analysis experiment using treatment of patients' sperm samples with a cAMP analogue significantly raised the percentage of progressively motile spermatozoa and confirmed the pathogenicity of the variation. Moreover, a rare missense variant in GFPT2 was discovered in another studied family and dynamic protein modeling identified this variation to inflict negative effects upon the resulting protein. This gene is involved in the antioxidant defense system and its inactivity can lead to high levels of Reactive Oxygen Species in sperm with adverse effects on sperm motility.

Conclusion: On the whole, using WES we managed to provide the first evidence of ADCY10 and GFPT2 involvement in human asthenozoospermia. The frameshift variant in ADCY10 was particularly considerable as it had already been shown by a mouse model study that this gene was implicated in asthenozoospermia.

Keywords: Male Infertility, Genomics, WES, Familial, Asthenozoospermia

Health and Ethics

I-46: Ethics and Indications of Embryo Donation in Iran

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I-47: Civil Liability of Fertility Clinics in Embryo Donation Errors

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Despite years of practicing all methods of ART, Iranian legislator just passed a short vague act called "Act of embryo donation to infertile couples" in 2005. Unfortunately, this rule is quiet about legal effects of errors in the process of embryo donation which could result in an unwanted outcome. The vast range of errors may include race, similarities, health and certain qualifications of the embryo. Considering Iranian law that prohibited applicant parents from acquiring any information of donor's identity, problem of identity errors has no grounds unless in an

IVF process without intervention of a third party. However, any mistake in a simple IVF regarding identity of the embryo could be accounted as a tort as well as all above mentioned situations. As this study suggests, the legal approach through these variety of errors ought to be different based on significance of error, its' effects on the pre-planned life of the couple and health of the child. Moreover, measuring all other variables like limits of medical knowledge and random nature of some outcomes should be considered as well. For instance, an error in sex selection of the embryo in a case where the applicants insist on having a girl baby because of their records of Haemophilia, may affect health of the resulted boy baby seriously. Here, sex selection plays a vital role and inevitably, the wrongdoer must recover for the damages. Meanwhile, sometimes occurred error leads to a minor change in child qualifications which does not justify any legal action against the fertility clinic. Say an applicant couple who decide to have a tall baby. Does it seem rational to remedy an error which leads to a shorter child?

Such challenges inspired the author to define borders of civil liability in cases of embryo donation errors.

Obviously, comparative studies especially by a look to United States' jurisprudence help to gain a more comprehensive conclusion.

Keywords: Embryo Donation, Donation Error, Civil Liability, ART

I-48: Seminal Plasma, Immune System and Fertility

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One of the most important male factors for a successful pregnancy is seminal plasma (SP). SP provides a rich environment for a successful pregnancy and contains several different proteins originating from the testis, epididymis and male accessory glands. In efforts to ensure safe passage, multiple gene expression pathways are initiated in female epithelial cells upon contact with seminal fluid. More than 700 genes found to be differentially expressed in ectocervical tissue after intercourse, most are involved in the presentation of pro-inflammatory antigens and the expression of cytokines and chemokines. The soluble immune components of seminal fluid modulate the maternal response in such a way that pregnancy can progress. SP also promotes recruitment and activation of the immune cells, including macrophages, dendritic cells, and T cells. Moreover, SP plays a pivotal role in the induction of immune tolerance to paternal alloantigen through expanding the pool of inducible regulatory T (Treg) cells. These semen-induced changes in gene expression and resultant cellular response are known as the leukocyte reaction. Interestingly, SP also affects the sperm quality and functions before insemination. It means that SP can both stimulate and inhibit the capacity of the spermatozoa for fertilization. During the transit of the spermatozoa from the epididymis, sperm gain its motility and fertilizing capacities under the influence of proteins from the epididymal fluid, which can thereafter be observed in the SP and bind to the sperm surface. Understanding the role of SP in a normal pregnancy may provide new options for diagnosis and treatment of infertility.

I-49: Sexual Health Service Delivery in ART Settings

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I-50: Status of Ethics Committee in Biomedical Technologies in Iran And France

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The Bioethics Committee is an interdisciplinary body composed of several experts from different disciplines (mainly medicine, genetics, law, psychiatry, theology, ethics and philosophy) that follows progress in the life sciences and its applications in order to ensure respect for human dignity and human rights. The organizational structure, the duties and functions of the Ethics Committees would vary from one country to another. This paper studies the Biomedicine Ethics Committee, in particular in the area of artificial reproduction technologies, in Iran and France. We first discuss the status of ethical concerns in biomedicine legislation in Iran and France, as well as the major differences between Islamic ethics and Western secular ethics. Subsequently, specific laws and regulations regarding the organizational structure, function and role of the ethics committee in biomedical technologies in Iranian and French law are discussed.

France was the first country to create a National Consultative Ethics Committee. The National Consultative Ethics Committee for health and life sciences (CCNE) was established in France on February 23, 1983, and played an important and effective role from the beginning. The mission of CCNE is to provide advisory opinions on the ethical and social issues raised in the field of biology, medicine and health and to hold public debates within the framework of the legislation review in this area.

In Iran, the National Ethics Committee for Biomedical Research, affiliated to the Ministry of Health and Medical Education, started its activities in 1998. This committee is responsible for preparing and issuing guidelines and codes of practice concerning biomedical research activities, monitoring their implementation, handling complaints raised in this area, and issuing licenses of regional ethics committees (Article 3, regulation 2013). Academic and organizational ethics committees are formed under the supervision of the National Ethics Committee. However, non-research activities (as therapeutic activities) are beyond the scope of regulatory provisions on ethics committee in the country.

This comparative study shows the importance of ethics committees in two legal systems. Despite their differences, French law provides more comprehensive provisions regarding biomedicine ethics committee especially in term of scope of functioning. However, Iranian Ethics committee for Biomedical Research operates on a more binding basis than the French CCNE. The legislative gap or deficiencies in most areas of biomedical activities including infertility treatment in Iranian law point out the crucial need for establishment of national ethics committee for non-research activities also.

Keywords: Biomedicine, Comparative Law, Ethics Committee,

Islamic Ethics, Western Ethics.

I-51: The Association Between Infertility and Sexual Disorders from The Perspective of Mental Health

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I-52: Anonymity and Donation Advantages & Disadvantages

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Donation programs including sperm, egg and embryo donation has been practiced as infertility treatment throughout the world. Iran is the only Islamic country in which donation programs are practiced. Embryo donation has law but egg and sperm donations are accepted by decree of clergy scholars. For embryo donation a practice guideline is released by ministry of health attached to the law in which anonymity is mandatory and the documents of this treatment is highly classified. But for egg and sperm donation there is no national guideline and every infertility center has its own protocol. Anonymity has some advantages and disadvantages: the most important advantage is to prevent future lawsuits claiming for the child. There are cases of claiming for return of embryo donation resulting child and it seems to be more in the future. When the donor and recipient do not know each other, there cannot be such a claim. Also some lawsuits about inheritance, guardianship, expenditure and custody can also be prevented and most probably can bring peaceful life or the child. On the other hand anonymity has some disadvantages: the most important disadvantage is ignoring the right of knowing genetic lineage for the child. The child can be deprived from bone marrow or can be marriage between genetically brothers and sisters. All the advantages and disadvantages can be changed by updating the law and solving the lineage problem for donation resulting children by accepting of legal parents which is not currently accepted by clergy scholars and law.

Keywords: Embryo Donation, Law, Anonymity, Sperm Donation, Egg Donation

I-53: Ethical and Legal Issues in Pre- Pubertal Cancer Patients

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Recently oncology is well developed bringing hope for cancer patients to live longer and have a family life and possibly chil-

dren. Unfortunately cancer treatments like chemotherapy and radiotherapy destroy gametes and may cause secondary infertility. Fertility preservation (FP) is introduced to prevent this complication. There are different FP procedures and also different types and stages of cancer making the decision to choose the best protocol. Ethical issues about FP mostly are focused around choosing the method and patient. The prognosis of the cancer and survival rate so life expectancy is very important. It doesn't seem to be ethical to put a patient with little life expectancy in the FP procedure. The situation of the patients and their wish is also important, if they are married, if they have children and if they wish to have children in the future considering their possible short life or not. The possibility of transmission of the cancer to the children is always raised. There are two ways of transmission of cancer one is from cancer cells among the gametes and inside gonads and the second is to transmit genetically. The patient must know the transmission possibility to make his/her own decision. For immature patients there are no FP procedure other than cryopreservation of their gonadal tissue. This procedure is still in research phase and there are not high possibility to be successful in the future conception. For married people embryo freezing is the best and most promising way. But some prefer to freeze their oocytes for covering the possibility of future divorce. Both ways need controlled ovarian stimulation (COS) using hormonal drugs which cannot be used in hormone sensitive cancers. Also, COS takes time and maybe more than one cycle is needed so, invasive cancer patients cannot wait and postpone their chemotherapy to finish the oocyte retrieval. Sperm freezing is more practical and has been practiced for ages. So, it seems that the decision for FP and the type of FP procedure should be made in a triangle consist of oncologist, gynecologist and cryobiologist to make the best decision. Informing the patient about FP is the responsibility of oncologist otherwise there is no way for the patient to know about FP. Informed consent with full information covering any possibility and complication must be taken from the patient for prevention any misguide. Posthumous reproduction can be raised by these procedures because there are frozen gametes and the patient is at risk of death. There is a need for a law and guidelines about FP in Iran.

Keywords: Fertility Preservation, Oncology, Cryopreservation, Gametes, Cancer

I-54: Sexual Dysfunctions and Infertility

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Sexual dysfunction and infertility sexual dysfunction is more common in infertile couple. Infertility treatment cause sex less spontaneous and less enjoyable .couples stop sexual pleasure and lovemaking and focus on baby making. They may have problem in sexual desire arousal or orgasm it means sexual dysfunction physically, for many couples, sexual performance is enjoyable so they may have sexual dysfunction emotionally. Studies report the prevalence of SD during ART to be between 11 and 87% in women and 7 and 75% in men, culture, religion and social state effect on it.

Infertility and its treatment could change sexual self-esteem, sexual relationship and sexual function.

Healthy sexuality is central to psychological well-being and quality of life WHO state.

The sexuality is an integral part of being human and concerned with biological ,psychological ,spiritual and cultural aspect of life and it has three major dimensions ;sexual self-concept ,sexual relationship and sexual function.

How is sexual self-concept impacted in infertile couples?

Does infertility have a negative impact on the sexual relationship?

Is sexual function affected by infertility? We will discuss.

I-55: Lineage Related Ethical and Legal Issues of Donation Programs

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There is an Act about Embryo Donation 2003 in Iran law. Although there is nothing in Acts about gamete donation, some of infertility clinics practically do that; especially Egg donation. In such a position, arise many ethical and legal problems because: at first, Iranian Embryo Donation Act (EDA) is very incomplete, summary (only in 5 articles), inconclusive and vague. For example, the Act is silent about lineage (filiation) between future child and his/her genetic or social parents. Likewise about inheritance, marriage banning, family name, custody, and other child-parents rights. So there are many questions about future child's fate and his/her socio-economic and even human rights. Secondly, based on Iranian Constitution, when Acts are vague or incomplete or silent, courts judges should refer to "Authentic Sources of Islamic Jurisprudence" and "Valid Judicial Decrees" (Reliable Fatwa). The problem is that there are disagreement in jurisprudence and decrees. In addition, majority of Islamic jurists believes that there is complete lineage between future child (embryo or gamete) and his/her genetic parent (donors). So generally all of lineage legal effects should enforce to them, not to any other persons, including social parents (donee). These situations actually will breaches purpose and basis of donations. Thirdly, upon Embryo Donation Act's bylaw, all of donation related data including donor name and address and identity and even donation process, are confidential as legally secret information. So we will involve in a paradoxical situation: in one hand, EDA is vague and Constitution finally leads to majority Islamic jurist's decree that indicated complete lineage between donor(s) and child. In other hand, upon Act's bylaw, all of data about donation is confidential. So there is no access to donors (alleged parents) because of confidentiality, and there is no effect in donee access because he/she is irrelevant. In conclusion, it seems that Iranian legal system must pass from this paradox. This problem has only two solutions: 1. Setting aside donation and abrogate Embryo Donation Act until decrees adjustment and changing position. 2. Amend the Act and to recognize lineage for social parents (donee). Until now, legislature refused accepting any of these two solutions.

Keywords: Embryo Donation Act (EDA), Donation, Donor, Donee, Lineage, Gamete, Confidentiality.

I-56: Immune Modulation Treatment in RIF and Recurrent Abortion

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I-57: The Shadows of Infertility Over Sexuality.

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Studies have shown that infertility is a stressor to effect on quality of life in marital state. In fertility, marital satisfaction and sexual satisfaction form a triangle which involves sexual and family health professions. It has been defined that sexuality has three major dimensions including sexual self-concept, sexual relationships and sexual function.

Sexual self-concept refers to the image one has of oneself as a man or a woman and the evaluation of one's adequacy in masculine or feminine roles, including body image, sexual self-schema and sexual self-esteem. Sexual self-esteem decreases in infertile men and women and it associate to infertility treatment result. Thus sexual self-concept is affected.

Sexual relationships is defined as the interpersonal relationships in which one's sexuality is shared with another. Infertility have a negative impact on sexual relationships in couples. Sexual dissatisfaction is obviously in male with infertility problems. It was hypothesized that this was due to the psychological pressure associated with efforts to conceive, or to reduction of power according to evolutionary theory. In female with infertility, the most profound change of sexual satisfaction depends on the treatment stage. Infertility and its treatment have anegative influence on sexual relationship and satisfaction because the shared stress of infertility can involve both couples with the same problem.

The third part of sexuality is sexual function. It is about the ability of an individual to give and receive sexual pleasure, including various physical and psychological progresses in the sexual response cycle. Totally the findings from the different studies showed that infertility could influence sexual activity in infertile couples, and that fertility problem stress tended to decrease frequency of intercourse. Premature ejaculation and erectile dysfunction in men and sexual interest and arousal disorder in women are prevalent in infertile couples.

According to these issues, relation between infertility and sexuality needs further investigation and clinically, couples with infertility needs to evaluate and treat regarding their sexual problems.

Keywords: Infertility, Sexuality, Sexual Problems.

I-58: Environmental Contaminants and Infertility: Hypothesis or Cause for Concern?

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Background: Infertility is a unique feature in epidemiology; as it involves a couple (instead of just one person), it may re-

main asymptomatic if the couple do not seek for children, it involves a spectrum (in terms of etiology and severity), and it has a wide variety of definitions (clinical, epidemiological and demographic). Both genetic and environmental/life style factors are suspected to have a role in producing this disorder. Environmental factors are victimized to cause many communicable and non-communicable disorders. It is estimated that one forth to one third of the global burden of diseases are attributed to environmental factors. We searched medical literature in order to know whether there is any conclusive relation between environmental factors and infertility.

Materials and Methods: Keyword related to fertility or infertility; and environment or pollution were looked in Pubmed and Scopus. No language limitation were imposed. Then the results were limited to systematic reviews and the papers that has been published recently (last 4 years). All titles have been reviewed to find the relevant clinical/epidemiological studies. Finally all abstracts –and if needed full text papers- were reviewed completely. The factors were classified based on their similarity in terms of chemical property and the results of similar studies were compared.

Results: We found 699 (scopus) and 185 (Pubmed) papers in the first step. All pubmed papers were included in scopus, so we continued on Scopus papers. After excluding non-relevant studies, 24 papers were included in final step. The environmental factors were classified as:

- Endocrine disrupting chemicals (EDCs)
- Air pollutants
- Pesticides and Herbicides
- Heavy metals
- Some scattered studies (for example on Nanoparticles or Melamin)

Although some reviews reported a strong contribution for some environmental factors (e.g. some air pollutants, heavy metals or EDCs), there were not any consistency between the results of different studies in the same category.

Conclusion: More studies addressing both environmental and genetic factors and a personalized approach are needed to give a better answer to this question.

Reproductive Imaging

I-59: How Diagnostic Imaging Methods Can Reduce Unnecessary Surgeries Before ART?

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Several diagnostic methods have been recommended for investigation of uterine abnormalities for women with infertility. Many investigators prefer to assess uterine using laparoscopic and hysteroscopic procedures to achieve diagnosis and treatment means simultaneously, but these are not offered by national guidelines as initial evaluation methods.

Multiple imaging modalities are being used to assess the uterine including conventional transvaginal ultrasound, three-dimensional ultrasound, saline infusion sonography (hysterosonography), hysterosalpingography, magnetic resonance imaging, and computed tomography. These imaging methods provided well

tolerate, cost-effective and less invasive ways with high diagnostic value and accuracy. Ultrasonography (conventional and transvaginal) as a highly developed technology is an accurate and simple screening method for possible ovarian, endometrial or uterine cavity abnormalities with more advantages than other imaging modalities in the work-up of infertile women with and without symptoms of pelvic pathology. Ultrasound is able to measure structures within millimeter accuracy and high resolution. This method also can help to differentiate acute and chronic abnormalities in the fallopian tubes. This is of high importance in the assessment of the infertile couple.

Three-dimensional ultrasound (3D USG) is a valuable imaging modality with high sensitivity and specificity for diagnosing congenital uterine anomalies. This method provides high-resolution images in three different planes and helps to defining and mapping uterine lesions.

Hysterosalpingography is an accurate test for diagnosing of tubal disease in the absence of tubal patency. This method also helps gynecologists and infertility specialists to study the congenital and acquired uterine abnormalities.

Hystero-contrast sonography (HyCoSy) is an ultrasonic procedure that allows to assess the uterine cavity and fallopian tube patency by infusing saline as contrast medium through the cervical canal.

MRI (also known as NMR) is cross-sectional imaging modality with high accuracy to evaluate both the uterus and ovaries.

We aimed to state the diagnostic values of imaging modalities in infertility work up versus to surgery procedures.

I-60: Application of Color Doppler Imaging in Optimizing ART Success

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Transvaginal ultrasonography plays an essential role in modern fertility management and color Doppler ultrasound has made a significant improvement in the assessment of infertility.

Measurement of uterine perfusion by color Doppler, have a predictive value regarding fertility in patients undergoing different methods of medically assisted reproduction. Color Doppler permits visualization of small intraovarian and endometrial vessels, allowing depiction of normal and pathological changes in reproductive organs.

During the normal menstrual cycle, there is a sharp increase in end-diastolic velocities of the uterine arteries between the proliferative and secretory phases. Interestingly, the lowest blood flow impedance occurs during the time of peak luteal function, during which implantation is most likely to occur. In unovulatory cycles, these changes are not present, and a continuous increase in the RI is seen.

Measurement of ovarian stromal blood flow in the early follicular phase is related to subsequent ovarian responsiveness in IVF treatment. Poor responder seems to have low ovarian stromal blood flow PSV.

Follicular vascularity may represent a parameter in the assessment of folliculogenesis and there is a possible link between follicular vascularity and implantation potential.

A good blood supply towards the endometrium is usually considered as an essential requirement for normal implantation. Absent subendometrial and intraendometrial vascularization on

the day of hCG administration appears to be a useful predictor of failure of implantation in IVF cycles, irrespective of the morphological appearance of the endometrium.

Andrology

O-1: Comparison of Zeta Potential and MACS Sperm Selection Novel Methods for ICSI.

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Background: Sperm preparation procedure is one of the most important steps before insemination during intra cytoplasmic sperm injection (ICSI) which is mainly performed through density gradient centrifugation (DGC) technique and swim up method. These methods select sperms based on viability and normal morphology but they cannot separate healthy sperms from DNA damaged spermatozoa. Zeta potential and magnetic activated cell sorter (MACS) are two novel sperm selection procedures which have recently used for ICSI and act on the basis of membrane surface charge and surface apoptotic marker in infertile men respectively. In this study we aimed to compare sperm functional tests between two novel sperm selection procedures.

Materials and Methods: Semen samples were collected from 20 infertile men and each sample was divided into three sections. First section was washed with Ham's F10+10 % albumin (as unprocessed sample), second section was used for the MACS-DGC procedure and the third section was subjected to DGC- Zeta procedure. Percentage of sperm with abnormal morphology (papanicolaou staining), protamine deficiency (Chromomycin A3 staining) and DNA fragmentation (TUNEL staining) were evaluated on each section and the final results were compared between sections.

Results: The sperms isolated by MACS and Zeta procedures had significantly lower percentage of sperm with abnormal morphology, DNA fragmentation and protamine deficiency compared to unprocessed section ($P < 0.05$), but they did not have significant differences with each other.

Conclusion: The result of this study recommended that using these novel sperm selection procedures along with DGC could be beneficial for treatment of infertile men with high percentage of protamine deficiency and DNA fragmentation.

Keywords: Zeta Method, MACS Method, Protamine Deficiency, DNA Fragmentation, Morphology

O-2: Association between Oligospermia and Testicular Cancer: Using Bioinformatics Analysis

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Background: The incidence of testicular cancer has been increased all over the world during the last 4 to 5 decades, and in industrialized countries, testicular tumors are the most common malignant disease among men aged 20 to 34 years. A number

of reports have suggested a direct correlation between testicular cancer and infertility among oligospermia or azoospermia. The aim of this study was to determine the common genes in oligospermia and testicular cancer as a therapeutic target for testicular cancer prevention in people with oligospermia.

Materials and Methods: Genes involving in oligospermia and testis cancer obtained by Genecard database. Common genes were identified of these two diseases by the venn diagram. Original nodes were determined by string web server and cytoscape software. Finally, KEGG database was used to determine original nodes pathways involved in two disease.

Results: The results of Genecards have shown 469 and 14102 genes are involved in oligospermia and testicular cancer respectively. Among them, 422 genes are common between oligospermia and testicular cancer. TP53, INS, ALB, IL6 and EGFR has genes with highest degree. TP53 is the important gene in apoptosis, INS gene plays a role in Cell adhesion and Mismatch repair, ALB gene involved in metabolites metabolism and exogenous drugs, IL6 gene involved in block of differentiation and, EGFR gene mediated at cell proliferation.

Conclusion: Pathways involved by genes with highest degree have an important role in progression of cancer. Therefore, determining the key targets can be very effective in preventing testicular cancer among people with oligospermia.

Keywords: Testicular Cancer, Oligospermia, Infertility, Genecards

O-3: The Effect of Sperm DNA Fragmentation on Embryo Aneuploidy in ICSI-CGH Array Cycles

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Background: There is clinical evidence to show that sperm DNA damage could be a marker of sperm quality and extensive data exist on the relationship between DNA damage and male fertility status. Sperm DNA fragmentation can be the most common reason for the transmission of anomalies of the father's DNA to a child that seen in a high percentage of sperms in infertile men.

Materials and Methods: In this study, 40 patients with recurrent implantation failure (RIF) were selected; they had at least 3 times of good quality embryo transfer with failure in the implantation. both women and men appeared to have no problem. All women were between 25-35 and stimulated with GnRH agonist and oocyte isolation was performed after follicles were picked up. Semen samples were also analyzed and DFI measured using TUNEL by flow cytometry. According to their TUNEL results, 2 groups were defined, 1: DFI > 20 % and 2: DFI < 20%. Intracytoplasmic sperm injection was also performed and then the day 3 embryos were subjected to blastomere biopsy and evalu-

ated by CGH array (Genomic Comparative Hybridization). Semen parameters, TAC, ROS and malondialdehyde (MDA) formation were analyzed between both groups also the correlation between embryo aneuploidy and semen parameters were evaluated.

Results: The results of this study showed that sperm with high DFI (DFI > 20) significantly increased the number of aneuploidy embryos than sperm with low DFI ($P < 0.001$). Also, by increasing the DFI, the level of MDA significantly increased ($P < 0.001$). The level of ROS and TAC were increased and decreased respectively but they are not significant.

Conclusion: These data indicate that sperm DNA damage has a significant effect on embryo chromosome aneuploidy.

Keywords: Aneuploidy, DNA Fragmentation, Malondialdehyde, TUNEL

O-4: Short Period of Abstinence Reduces Sperm Deoxyribonucleic Acid (DNA) Fragmentation in Ejaculate of Varicocele Patients

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Background: Varicocele is the leading cause of male infertility throughout the world. Various mechanisms have been proposed to explain the testicular dysfunction in varicoceles, including Increase testicular temperature, hypoxia and increase level of reactive oxygen species (ROS). There are controversy over the effectiveness of surgical treatments, patient selection, and when to administer treatment, particularly in the era of assisted reproductive technology. Therefore finding a strategy to improve sperm parameter of varicocele patients may improve assisted reproductive technology outcomes. The aim of the present study was to assess the effect of ejaculatory abstinence (EA) periods on routine and advanced sperm tests among varicocele patients.

Materials and Methods: This study included 76 ejaculates from 38 men with grade I or II varicocele who provided ejaculates after observing 3 days and 2 hours of abstinence. A standard semen analysis was performed on all samples according to World Health Organization (WHO) protocols. Each specimen was assessed for levels of malondialdehyde (MDA) as an index of ROS, total antioxidant capacity (TAC) and sperm DNA fragmentation (SDF). Statistical analysis was done using SPSS version 23.

Results: The shorter period of EA (2 hours) resulted in statistically significant decreases in semen volume, sperm count and sperm motility in comparison with 3 days of abstinence ($P < 0.05$). Results also showed no differences in TAC level between groups. Lower levels of SDF and MDA were observed after 2 hours periods of abstinence in compared with 3 days EA ($P < 0.05$).

Conclusion: Lower seminal ROS obtained after a shorter period of EA could diminish oxidative stress induced SDF in varicocele patients. Despite the significantly lower semen volume, sperm concentration and lower motility, the significant-

ly higher percentage of spermatozoa with lower SDF in the short period ejaculate, may provide more effective selection of higher quality spermatozoa. Therefore, use of short period of EA for therapeutic fertilization would be a clinically valuable strategy to improve the DNA quality and subsequently ART outcome.

Keywords: Varicocele, Abstinence, Male Infertility, Sperm DNA Fragmentation

O-5: Enrichment of Human Spermatogonial Cells by Culture of Testicular Cell Suspension in Obstructive Azoospermic Patients

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Background: 50 million of men are infertile, and notably, azoospermia comprises 25% of male infertility cases. Therefore, it is of great interest to generate functional gametes for patients with male infertility especially for azoospermia. Spermatogenesis is a complex process regulated by multiple interactions between developing germ cells and the surrounding microenvironment. Production of sperm *in vitro* would not only provide male gametes for azoospermic patients but also offers various methods for the *in vitro* derivation of male germ cells have been developed. So, in the present study, we investigated different methods to proliferation of SSCs.

Materials and Methods: Human testicular samples were obtained from men with obstructive azoospermia. after enzymatic digestion process, cells assigned to following groups: culture of SSCs in the dish without cover (control group), co-culture of SSCs with infertile sertoli cells (I), co-culture of SSCs with fertile sertoli cells (II), culture of SSCs on nanofiber (covered with laminin) (III), culture of testicular cell suspension (IV). Then cells were cultured for two weeks in 34-StemPro medium and evaluated colony formation of human spermatogonial cells and gene specific methylation and quantitative genes expression of pluripotency (Nanog, C-Myc, Oct-4) and specific germ cell (Integrin $\alpha 6$, Integrin $\beta 1$, PLZF) genes in five different culture systems.

Results: We found that the highest number and diameter of colonies and cellular proliferation associated with IV group which were significantly different with control group and other groups while it was fewest in control group, III, II, I groups respectively. Expression of germ specific genes in IV group were significantly increased ($P \leq 0.05$) and levels of expression of pluripotency genes were significantly decreased in this group ($P \leq 0.05$) compared with other groups. Gene specific methylation pattern of examined genes did not show any changes during culture period in culture systems.

Conclusion: Our findings indicate that testicular cell suspension can reconstruct a microenvironment capable of regulating proliferation of cell colonies.

Keywords: Spermatogonial Stem Cells (SSCs), Colonization, Obstructive Azoospermia, Culture Systems

Animal Biotechnology

O-6: Can Hyaluronidase PH-20 in Embryo Culture Medium Improve Developmental Competence of Ovine Oocytes?

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Background: Hyaluronic acid (HA) plays an important role in regulating various reproductive events. There are different results from supplementation of embryo *in vitro* culture (IVC) medium with HA. Instead of using HA in IVC, supplementation of IVC with HA system components, including hyaluronan synthases (HAS), HA-degrading enzymes (hyaluronidases; HYALs) and HA receptors (HAS) is of great importance and should be investigated. PH-20, is a hyaluronidase, present in spermatozoa and degrade HA into pieces of tetra-saccharide that may play an important role in cell proliferation and mitosis. Therefore, in this study we evaluated the effect of supplementation of IVC medium with two type of HYALs: human recombinant PH-20 produced at Royan institute (rhPH-20) and PH-20 sigma (sPH-20) on early embryonic development in terms of blastocyst rate.

Materials and Methods: Cumulus-oocyte complexes (COCs) were retrieved from sheep slaughtered ovaries. Selected COCs were cultured in maturation medium. Briefly, after 22–24 hours of maturation, oocytes were fertilized using fresh semen and incubated for 18 to 21 hours. Then, denuded presumptive zygotes were divided into three groups: two groups with 300 unit/ml of rhPH-20 and purified HYALs from testis from sigma company (sPH-20) and their control counterpart.

Results: Our results demonstrated a significant decrease ($P < 0.05$) in blastocyst rate in both treatment groups (rhPH-20 and sPH-20) (16.38 ± 3.06 and 16.11 ± 3.94 , respectively) in compare to control group at and (27.53 ± 3.56).

Conclusion: Our data revealed that supplementation of IVC medium with either rhPH-20 or sPH20 couldn't improve blastocyst rate in contrast to similar studies. Previous studies used hyaluronidase 2 and found significant improvement in production of blastocyst in cattle. This difference may be raised from different type of HYALs which we used in this study in compare to previous studies. Previous studies used hyaluronidase 2 and may be its product (20 kDa HA) has an efficient impact on embryonic development, which probably connects the product to the CD44 receptor, which can effectively guide the signaling pathways, while in this study we used PH-20 which is similar to hyaluronidase 1 and its product is approximately 0.8 kDa which may have lower interaction with CD44 receptor. In addition previous studies also showed that hyaluronidase 1 has lower expression in compare to hyaluronidase 2 at morula to

blastocyst stage. Therefore, using hyaluronidase 2 instead of hyaluronidase 1 in IVC medium may promote the developmental competence of ovine oocytes which needs more investigations to be elucidated.

Keywords: Hyaluronic Acid System, Hyaluronidase PH-20, Ovine, Embryonic Development

O-7: Vitrification of *In Vitro* Derived Ovine Blastocysts by High Osmolality Solution

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Background: At present, optimal cryopreservation protocol for livestock species is use of high osmolality vitrification solution following high cooling and warming rates. This technique supports the emergence of a glassy state and inhibits ice crystals growth. Recently, Ficoll PM70 as a non-penetrating cryoprotectant (CP), a high molecular weight sucrose-polymer, has improved blastocyst cryopreservation outcomes. In this study, we aimed to optimize vitrification protocol with Ficoll for ovine blastocysts in terms of re-expansion rate.

Materials and Methods: Expanded blastocysts derived from *in vitro* fertilization in ovine species were used for assessing the efficiency of vitrification with Ficoll™. Briefly, blastocysts were put in equilibrium solution (ES) (8% EG + 8% DMSO or 20% EG + 20% DMSO) for 6 minutes and then embryos were held in vitrification solution (VS) (40%EG+20% DMSO+1%Ficoll or 16%EG+16% DMSO+10%Ficoll or 40%EG+20% DMSO+10%Ficoll or 16%EG+16% DMSO+1%Ficoll) for 35 seconds, and subsequently were immersed in liquid nitrogen. During warming procedure, embryos were incubated in 1 and 0.5 mol/l sucrose solution for 1 and 3 minutes, respectively. Finally, embryos were washed in PBS- containing 20% FBS, and cultured for 48 hours in synthetic oviductal fluid (SOF) medium under mineral oil at 38.5°C, 5% CO₂, 5% O₂ and maximum humidified air. The percentage of re-expansion rate in various treatment groups was assessed and analyzed statistically with one-way ANOVA test.

Results: Re-expansion rate was significantly ($P < 0.05$) higher in ES: 8% EG+ 8% DMSO, VS: 16%EG+16% DMSO+1%Ficoll group ($91.12 \pm 2.41\%$) in compare to other groups ($19.45 \pm 3.09\%$, $55 \pm 3.33\%$ and $29.17 \pm 3.53\%$, respectively).

Conclusion: It seems that high percentage of Ficoll, EG and DMSO (10, 40, 20, respectively) were not an acceptable combination for the vitrification solution, while their low percentage (1, 16, 16, respectively) were the best. Using high osmolality vitrification solution for cryopreservation of embryos in animal facilities and biomedical laboratories should be optimized for other stages of embryos or in other species.

Keywords: Blastocyst, Vitrification, Ficoll, Ovine, Re-Expansion Rate

O-8: Aggregation of Somatic Nuclear Transfer Embryos with *In Vitro* Fertilized Embryos at Two-cell Stage Increases the Efficiency of Blastocyst Rate in Goat

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Background: Efficiency of somatic cell nuclear transfer (SCNT) has remained low due to a strong resistance of somatic donor cells to epigenetic reprogramming. Many approaches have been developed for improving *in vitro* and *in vivo* development of SCNT embryos. One of the promising methods for increasing SCNT efficiency is embryo aggregation. In the mouse, aggregation of two or three SCNT embryos leads to increased cell numbers, normalized Pou5f1 gene expression, and higher developmental rate. In bovine, the aggregation of parthenogenetic blastomeres with *in vitro* fertilization (IVF)-derived blastomeres increased survival rate after embryo transfer. In this study, we aimed at deciphering whether aggregation of SCNT derived goat embryos at 2-cell stage with 2-cell stage IVF embryos improves the efficiency of SCNT in terms of blastocyst rate.

Materials and Methods: After producing SCNT embryos with handmade cloning (HMC) method and also IVF embryos with fertilization technique, IVF and SCNT 2-cell stage goat embryos were aggregated by co-culturing them in aggregation plates. Six days after aggregation, the blastocyst rate were determined in IVF-SCNT aggregated and single SCNT embryos.

Results: The percentage of blastocysts was higher ($P < 0.05$) in IVF-SCNT aggregated embryos ($27.72 \pm 3.45\%$) compared to that of single SCNT embryos ($16.65 \pm 4.58\%$).

Conclusion: Our results showed that aggregation of goat SCNT embryos at 2-cell stage with IVF embryos promotes the *in vitro* development in terms of blastocyst rate. However, more investigations need to be performed in order to assess the expression of important developmentally genes in aggregated IVF-SCNT embryos in compare to single SCNT embryos.

Keywords: SCNT, IVF, HMC, Blastocyst

Embryology

O-9: Effect of Pure Follicular Fluid from Normal and PCOS Women on Human Sperm Kinematics and Viability

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Background: Follicular fluid (FF) is transferred into ovarian tubes after ovulation, and in physiological condition, spermatozoa is exposed to this fluid during fertilization. Chemical composition of FF changes with some diseases such as polycystic ovary syndrome (PCOS). The rate of successful fertilization in PCOS patients is lower than healthy women. The purpose of this study was to evaluate sperm velocity, motility and survival rate after incubation in follicular fluid from normal and PCOS women.

Materials and Methods: The FF and semen samples were obtained from 20 healthy and PCOS volunteers (BMI < 30 kg/m², 18-36 years old) and 20 fertile men (20-40 years old) who were referred to Shiraz Infertility Center. The FF from 10 healthy women and 10 patients were separately filtered using 0.45 and

0.22 μ m filters, pooled and stored at -20°C until usage. Semen samples were washed with Ham's F10 and after swimming up were diluted to 10×10^6 sperm/mL. Sperm were incubated in Ham's F10, as control group, and pooled FF, which obtained from normal and PCOS patients for two hours. The sperm kinematics and survival -rate were evaluated by sperm analyzer, (VT-Sperm 3.1) and eosin staining. Results were expressed as mean \pm SEM using SPSS version 24 software. $P < 0.05$ was considered as significant.

Results: The percentage of live sperm (73.31 ± 2.90 vs. 63.81 ± 2.93) and sperm with progressive (67.94 ± 1.40 vs. 63.44 ± 3.21) and non-progressive (8.83 ± 1.40 vs. 11.00 ± 1.33) motility incubated in FF of normal and PCOS follicular fluid did not show any significant differences compared to each other and relative to control group. Furthermore, the straight-line velocity (μ m/s) (VSL) (100.43 ± 6.76 vs. 86.06 ± 5.66) and curvilinear velocity (μ m/s) (VCL) (143.10 ± 8.92 vs. 127.07 ± 6.75) showed no significant differences.

Conclusion: To our best of knowledge, this study is the first one that compare the normal and PCOS FF as sperm incubation medium. Pure filtered human FF from normal or cystic follicles could be considered as suitable incubation medium for sperm. The changes in chemical components of FF from PCOS patients do not damage to survival or motility of sperm.

Keywords: Human Sperm, Motility, Viability, Follicular Fluid, PCOS

O-10: Evaluation of Correlations between Routine Semen Analysis, Lipid Peroxidation, Chromatin Maturation and DNA Integrity in Infertile Man

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Background: Male infertility is the sole or contributing factor in 50% of infertile couple. The first conventional assessment for evaluation of male infertility is semen analysis (SA) which cannot provide complete information about the fertility potential. Many reasons are described as male infertility factors, one of the major causes is DNA damage. DNA damage has been shown to be associated with several conditions such as failed fertilization, disruption of preimplantation development, increase of miscarriage, reduction of live birth rate and etc. The main causes of DNA damage are presence of sperm chromatin deficiency, excessive oxidative stress and apoptosis. Excessive production of free radicals has been linked with disturbance of sperm functions like loss of motility, low fertility potential, which is due to induction of lipid peroxidation and at the end sperm DNA damage. The aim of this study was to investigate the relationship between sperm parameters, lipid peroxidation as ROS, DNA integrity and chromatin packaging in infertile man.

Materials and Methods: 30 infertile male referred to the Isfahan Fertility and Infertility Center, who had the inclusion crite-

ria to enter the study and taking the consent form. In the current study. Semen samples was assessed for sperm parameters by CASA software. DNA fragmentation was assessed by TUNEL and SCSA used for Chromatin condensation. Maturation was assessed by CMA3 and aniline blue, respectively and Lipid peroxidation was assessed using BODYPI.

Results: During this study, we observed significant correlations between percentage of immature sperm with sperm concentration and percentage of sperm with abnormal morphology. Significant correlations were also observed between percentages of abnormal morphology with Intensity of sperm LO. In addition significant positive correlations was observed between percentages of SCSA with percentage of TUNEL positive sperm, percentage of sperm with LO and Intensity of sperm LO respectively. We found significant correlations between percentage of TUNEL positive sperm and percentage of sperm LO. Correlation between CMA3-positive spermatozoa and intensity of lipid peroxidation were also significant. In addition, there was a close to significant negative correlations between sperm motility with percentage of sperm with DFI and intensity of sperm LO.

Conclusion: Significant correlations between sperm functional tests in infertile men indicated antioxidant supplementation or other confirmed ways for reduction of oxidative stress can improve chance of pregnancy success rates.

Keywords: Sperm Parameters, DNA Damage, Protamine Deficiency, Lipid Peroxidation

O-11: Effects of Dietary Lipoic Acid Supplementation on Seminal Parameters and Fertility Potential in Aging Broiler Breeder Roosters

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Background: High levels of polyunsaturated fatty acids in avian sperm cause more susceptibility to lipid peroxidation. The antioxidant capacity of sperm decreases with age-increasing and thus the fertility is reduced in aging roosters. The purpose of this study was to investigate the effects of different levels of lipoic acid (LA) as a feed supplement to improve the semen quality and fertility parameters of aged broiler breeder roosters.

Materials and Methods: A total of forty-two roosters in 56 weeks were randomly assigned into seven treatments (0, 25, 50, 75, 100, 125 and 150 mg LA/bird per day) for eight weeks. Semen parameters, malondialdehyde (MDA) in seminal plasma and body weight were assessed weekly and artificial insemination was performed at the end of experiment to evaluate the fertility potential.

Results: The dietary administered of LA had no significant effects on body weight, semen volume, and sperm concentration between groups ($P>0.05$), but LA levels were linearly associated with a decreased seminal concentration of MDA ($P<0.05$). The total motility, progressive motility, viability, membrane integrity and morphology of sperms had an incremental trend until the level of 100 mg LA ($P<0.05$). The same trend was observed in the fertility rate and the level of 100 mg LA showed the best performance after artificial insemination ($P<0.05$).

Conclusion: These results suggest that 100 mg dietary lipoic acid as an antioxidant supplement can improve semen quality and the fertility rate of aging breeder roosters.

Keywords: Antioxidant, Lipid Peroxidation, Malondialdehyde, Progressive Motility

O-12: Adipose Mesenchymal Stem Cells Condition Medium on Azoospermia

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Background: Busulfan is a chemotherapeutic drug which can induce subfertility and even sterility and azoospermia in the males. Conditioned media (CM) which is a good source of growth factors, cytokines, various enzymes, and other soluble factors secreted by adipose tissue-derived mesenchymal stem cells (AT-MSCs) may be effective in testes exposed to busulfan. So we aimed to evaluate the effects of AT-MSCs-CM on histopathologic changes of mouse testes exposed to busulfan according to stereological study.

Materials and Methods: In this study, we utilized 40 adult male NMRI mice (28–30 g). All the animals were divided into 5 experimental groups randomly: (1) control, (2) DMEM (injected by DMEM into testes), (3) DMSO (injected by DMSO intraperitoneally), (4) busulfan (BSU) (single intraperitoneal dose of 40 mg/kg busulfan), and (5) conditioned media (CM) [(single intraperitoneal dose of 40 mg/kg busulfan) + AT-MSCs-CM]. All mice were kept for 35 days following injection. Finally, sperm samples were collected from the tail of the epididymis, the animals were euthanized and testis samples were taken for histological and stereological studies.

Results: BSU group showed a significant decrease in volumes of the testis, interstitium, and tubules compared to the control, DMEM, and DMSO groups ($P<0.05$), although there was no significant difference between BSU and CM groups ($P>0.05$). In addition, the number of spermatogenic, and leydig cells decreased in BSU group compared to the control, DMEM, and DMSO groups, while CM could significantly improve that parameters in the CM group. Sperm count and motility, and also the total length of seminiferous tubules increased in CM group compared to the BSU group.

Conclusion: AT-MSCs-CM showed regenerative characteristics on histopathologic changes of mouse testes exposed to busulfan. Considering less concern about CM application compared to the stem cells, it can be an alternative choice for therapeutically and research purposes in the disturbed testes.

Keywords: Busulfan, Adipose Tissue (AT), Conditioned Medium (CM), Mesenchymal Stem Cells (MSC), Spermatogenesis

O-13: Cyclopiazonic Acid Impaired Male Reproductive Structure and Altered Hormonal Statuses

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Background: Cyclopiazonic acid as a mycotoxin is produced

by various fungi including *Aspergilla* and *penicillium* Spp. Consumption of the CPA-contaminated food and feedstuffs products raised some health concerns. In the current study we aimed to investigate the various dose level of CPA on the structure and function of testis.

Materials and Methods: Forty adult male mice were randomly aliquoted into control and test groups (n=8). In control group animals did not receive any treatment. In the control-sham, animals received daily 0.05% DMSO (as the CPA solvent) intraperitoneally (i.p.). In the third, fourth and fifth groups, animals received 0.3, 0.6 and 0.12 mg/kg, BW of CPA (i.p.), respectively for 28 days. Twenty-four hours after the last treatment, all the animals were weighed. After taking the blood samples, the mice were euthanized and autopsied. Morphometric and morphological changes in the testicular tissue, testis spermatogenesis indices and testosterone level were investigated.

Results: Reduction in absolute weight of the body and testicles was observed in the CPA-received groups. Serum testosterone levels showed a significant decrease ($P < 0.05$) in the animals that received high and medium doses of CPA. Histomorphometric analyzes showed reduction in the number of Leydig and sertoli cells, diameters of seminiferous tubules, thickness of epithelium of tubules and thickness of the testis capsule that was significant ($P < 0.05$) in the group that received the highest dose of CPA. Tubular Differentiation Index (TDI%), Spermiogenesis Index (SI%), and Repopulation Index (RI%) showed a decrease in the CPA-received groups, which was only statistically significant ($P < 0.05$) in the group received the highest dose of CPA. Changes in the pattern of spermatogenesis series cells, dissolution of seminiferous tubules, reduction of epididymal sperm, apoptosis in spermatogenesis series cells, changes in interstitial tissue were observed in experimental groups.

Conclusion: Our data indicate that CPA affected negatively the structure and function of testicular tissue, suggesting its detrimental effects on male reproductive system.

Keywords: Cyclopiazonic Acid, Histomorphology and Histomorphometry, Mice, Mycotoxin, Testosterone

O-14: Improvement of *In Vitro* Oocyte Maturation from Mice with Polycystic Ovary Syndrome by Co-Culture with Normal Granulosa Cell-Conditioned Media

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Background: In the PCOS ovaries, growth of early follicles is mostly arrested. On the other, the outcome of *in vitro* maturation (IVM) of PCOS oocytes is poor. Because the oocytes are usually matured *in vitro* without cumulus cells. In this ways, the studies are focusing to optimize the culture maturation media, and aid in the final stages of oocyte maturation *in vitro*. Therefore, the purpose of this study is to answer the following questions: Will there be a connection between normal cumulus-granulosa cells and germinal vesicle oocytes from mice with polycystic ovarian syndrome (PCOS) in co-culture medium? How much will the connection between immature oocytes and cumulus-granulosa cells affect maturation rate of oocytes?

Materials and Methods: In this study, the cumulus and granulosa cells were harvested from ovaries of normal NMRI mouse, and cultured for 24 hours in an IVM medium (minimum essential medium: α -MEM) supplemented with 10% bovine serum albumin. Then, oocytes at germinal vesicle stages were harvest-

ed from PCOS mice (induced to estradiol valerate for 60 days), and incubated with cultured cumulus-granulosa cells for extra 24 hours. The maturation rate of the PCOS germinal vesicles was evaluated. The presence of connection between cultured intact cumulus-granulosa cells and PCOS oocytes studied using the scanning electron microscope (SEM) images. The viability of cumulus-granulosa cells was also assayed using trypan blue staining.

Results: In the end of the 60-day treatment period, the failure of estrous cycle, the presence of multiple cystic follicles, atretic follicles, degenerated granulosa cells and oocytes, and increased testosterone, progesterone and estradiol levels confirm PCOS model. Data show that MII oocytes significantly ($p < 0.05$) increased in the presence of normal cumulus-granulosa cells when compared with the control group (cultured PCOS oocytes). In addition, the viability of cumulus-granulosa cells preserves during culture period. The SEM images shows the connection between normal cells and PCOS oocytes during culture periods.

Conclusion: The results of our study highlight the importance of maturation media formulations and culture protocols via co-culture of PCOS immature oocytes with intact cumulus-granulosa cells to promote culture microenvironment for meiotic resumption.

Keywords: PCOS, Co-Culture, Cumulus-Granulosa Cells, Germinal Vesicle, IVM

O-15: Agar; A Suitable Scaffold for Culturing of Human Ovarian Tissue

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Background: Nowadays ovarian tissue banks have been set up in many countries to improve chances of child bearing for cancer patients. As transplantation of cryopreserved ovary intensifies the possibility of malignant cells reintroduction, researchers are focusing more on ovarian tissue *in-vitro* culture methods. By tissue engineering and biomaterial improvement, embryologists attempt to use various scaffolds to mimic the ovarian extracellular matrix and preserve the tissue structure in the culture period. Our aim was to achieve an optimum culture system for human ovarian tissue to activate higher numbers of primordial follicles.

Materials and Methods: Donated ovarian tissues from 6 transsexual women were dissected, cryopreserved by slow-freezing method and cultured on either matrigel coated insert or agar-soaked scaffold for 7 days. The numbers of morphologically healthy and degenerated follicles, their developmental stages, the hormonal levels of the spent medium and also the relative expression of the apoptotic pathway and proliferation genes were evaluated and compared.

Results: Significant decrease in the number of primordial follicles was observed in both cultured groups in comparison to non-cultured control group. Besides, the number of degenerated follicles was higher in both cultured groups than the non-

culture control group, but it was only significant for primary follicles in insert cultured group. The number of primary and secondary healthy follicles was significantly higher in the agar cultured ovarian strips compared to the non-cultured control group. A significant increase was seen in estrogen level in the agar cultured group at day 7 when compared to the second day of culture. The Q-PCR analysis showed a down regulation of apoptotic genes and an increase in Ki67 gene during culture in both groups compared to the non-cultured control group.

Conclusion: Since there was no significant difference between these two scaffolds in evaluated parameters and on the other hand there was a significant follicular growth in agar cultured group in comparison to non-cultured control; agar scaffold can be recommended as a suitable scaffold for human ovarian tissue culture. Meanwhile it must be mentioned that agar is less expensive and more accessible compared to other scaffolds used in this field.

Keywords: *In Vitro* Culture, Cryopreservation, Human Ovarian Tissue, Agar, Scaffold

O-16: Using of Decellularized Human Amniotic Membrane Hydrogel as A Natural 3D Bioscaffold to Improve Ovarian Follicle Culture

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Background: 3D culture of ovarian follicles using natural hydrogel-based scaffolds to attain fertilizable oocytes is considered as a promising strategy in women suffering from infertility. Human amniotic membrane due to its unique biological composition and regenerative properties, has been used extensively in tissue engineering. In this study, mouse secondary follicles were cultured using decellularized human amniotic membrane (DHAM) hydrogel in order to improve ovarian follicle culture.

Materials and Methods: Ovarian follicles (120-140µm) were cultured in three different doses of DHAM hydrogel (15, 30 and 45 mg/ml) in combination with alginate (1.5%) (1:1) and compared to control group (Alginate 0.75%). At the last days of the culture period (14 days), follicular morphology was evaluated.

Results: The results have been showed that antral/total rate of follicles in 45 mg/ml of DHAM hydrogel (42.77%) were significantly ($P<0.05$) higher than the other doses but there wasn't any significant difference between 45mg/ml and control one (46.08%). Also, the rate of degenerated follicles in 45mg/ml (51.85%) reached to the control group (42.62%) and was the lowest rate as compared to the other hydrogel-alginate combinations in which was comparable against 30 mg/ml group (68.69%).

Conclusion: DHAM-based hydrogel as a bioscaffold may apply instead of alginate which can improve the rate of antral follicles and decrease the rate of degenerated follicles in 3D culture system.

Keywords: Secondary Follicles, 3D Culture, Human Amniotic Membrane, Mouse

O-17: Evaluating the Potential of Dj-1 and Sap as Two Biomarkers for Human Sperm Selection

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Background: The selection of sperm with good genomic integrity and surface antigens is a critical factor in the success of assisted reproductive technology (ART) especially in male factor infertility. This study assessed the potential DJ-1 and serum amyloid P compound (SAP) two sperm surface proteins as bio markers for sperm selection.

Materials and Methods: Sperm were obtained from 114 men (72 asthenozoospermic (AT) and 42 normospermic(N) who presented at Avicenna Fertility Clinic for their treatment. The semen characteristic, DNA fragmentation (DFI), chromatin maturation index (CMI), bio marker levels on sperm, and their correlation with embryo quality were assessed in both groups. The paired samples t-test and independent-samples t-test were used for analyzing the data and P values<0.05 were considered significant.

Results: Outcomes exhibited the major reduction in DJ-1 and SAP following reduction in sperm quality and DNA integrity ($P<0.001$) with cut-off value of 12% (DJ-1) and 10% (SAP). The specificity of these three biomarkers was 73.8 and 88.1%, respectively. Also, DFI ($P<0.001$), CMI ($P<0.05$), cleavage ($P<0.05$), and embryos quality ($P<0.001$) decreased significantly in AT group in compared with NS group. It was shown that DFI was 76.5% in DJ-1 and 94.1% in SAP, and CMI was 75.50% and 87.5%, respectively. The significant correlation was found between of the two bio markers and CMI ($P<0.001$), DFI ($P<0.001$) and embryos quality ($P<0.001$).

Conclusion: By comparing the efficiency of these two bio markers for selecting sperm with the lowest level of chromatin damages, it seems that selection based on SAP has significance over others.

Keywords: Serum Amyloid P Component, DJ-1, Chromatin Maturation, Sperm DNA

O-18: Supplementation of Maturation Medium with CoQ10 Enhances Developmental Competence of Ovine Oocytes Through Improvement of Mitochondrial Function

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Background: *In vitro* maturation (IVM) can impair the balance between anti-oxidant capacity and oxidative stress, which may jeopardize embryo development by increasing oxidative stress, reducing energy metabolism and causing improper meiotic segregation. Balance in energy production and reduction of oxidative stress can be achieved by supplementation of Coenzyme Q10 (CoQ10), an electron transporter in the mitochondrial inner membrane. With the aim to improve the *in vitro* production of ovine embryos, we studied the effect of CoQ10 supplementation during the maturation sheep oocytes.

Materials and Methods: A minimum of 100 cumulus oocyte complexes (COCs) were matured in the presence of 15, 30 or 50 μ M CoQ10 in three to five replicates, followed by *in vitro* fertilization and culture in a subset of oocytes.

Results: Our data revealed that when compared to control oocytes or other concentrations of CoQ10, supplementation with 30 μ M CoQ10 resulted in a significant increase in blastocyst and hatching rates, improved the distribution, relative mass and potential membrane of mitochondria, decreased levels of reactive oxygen species (ROS) and glutathione (GSH) and also the percentage of oocytes with misaligned chromosomes after spindle assembly. The relative expression levels of apoptosis markers CASPASE3 and BAX were significantly reduced in treated oocytes and cumulus cells while the relative expression level of GDF9, an oocyte specific growth factor, significantly increased in treated cumulus cells and oocytes.

Conclusion: In conclusion, supplementation with CoQ10 improves the quality of COCs and the subsequent developmental competence of the embryo.

Keywords: Coenzyme Q10, *In Vitro* Maturation, Mitochondrial Status, Oocytes, Oxidative Stress

O-19: The Effects of Coenzyme Q10 on Mouse Embryos Following Vitrification

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Background: Cryopreservation has some adverse effects on embryo including reduction of cells metabolism, damage to mitochondria and plasma membrane, formation of reactive oxygen species, damage to the structure of proteins, lipids and DNA, and eventually, necrosis and apoptosis. In the present study, therefore, the effect of coenzyme Q10 as an antioxidant was investigated on mouse embryos following vitrification.

Materials and Methods: After superovulation, mice mating and vaginal plaque examination, morulae were collected from uterine horns on the third day of gestation. The morulae were classified into 9 groups and then vitrified. The culture and vitrification media of the experimental groups consisted of two different doses (10 and 30 μ M) of coenzyme Q10. After one week, the embryos were warmed and then cultured. After 48 hours of culture, the blastocyst rate, total cell number (TCN), viability, and DNA fragmentation index (DFI), and after 72 hours of culture, the hatching rate were evaluated.

Results: Blastocyst rate was significantly reduced in the groups containing 30 μ M coenzyme Q10 in their culture media compared to other groups ($P < 0.005$). The hatching rate in the groups containing 10 μ M coenzyme Q10 in their culture and vitrification media was significantly higher than other groups, and in the groups containing 30 μ M coenzyme Q10 in their culture

media was significantly lower than other groups ($P < 0.005$). In groups containing 10 μ M Coenzyme Q10 in their culture media, the viability and DFI were higher and lower than other groups, respectively ($P < 0.005$).

Conclusion: It seems that coenzyme Q10 is able to improve embryo quality and some aspects of mouse embryo development following vitrification through its antioxidant and anti-apoptotic properties, and through the production of ATP and ionic balance which this function of coenzyme Q10 is dose-dependent.

Keywords: Coenzyme Q10, Cryopreservation, Vitrification, Blastocyst, DNA Fragmentation

O-20: Co-Culturing with Ovarian Cells Improves *In Vitro* Growth and Development of Mouse Preantral Follicles

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Background: Isolation and *in vitro* culture of ovarian follicles is one of the potential alternatives for fertility preservation in patients with types of cancer that can metastasize to ovaries; hence, many studies have been conducted to optimize follicle culture conditions. The present study aimed to investigate the effects of ovarian cells (OCs) on *in vitro* growth and development of mouse preantral follicles.

Materials and Methods: A total number of 193 preantral follicles (100-130 μ m in diameter) were mechanically isolated from the ovaries of 2-week-old NMRI mice, individually encapsulated in 5 μ l droplets of freshly prepared alginate hydrogel (0.5% w/v) in the absence or presence of OCs (-OCs and +OCs, respectively), and cultured for 14 days. The morphology, diameter, survival and antrum formation rates and hormonal secretion of follicles, and also the maturation and abnormality rates of oocytes in terms of cortical granules distribution, spindle formation, and chromosomal alignment were assessed during culture. All statistical analyses were conducted in SAS version 9.4. Differences were considered significant at $P < 0.05$.

Results: Data indicated that in the presence of OCs follicles had a more spherical shape and significantly larger diameter on day 13 of culture (327.59 ± 8.74 vs. 402.73 ± 22.63 μ m for -OCs and +OCs; $P < 0.05$); however, co-culturing with OCs did not affect the follicles survival and antrum formation rates. On the other hand, co-culturing with OCs increased estradiol (10.08 ± 0.79 vs. 31.74 ± 0.30 ng/ml for -OCs and +OCs; $P < 0.05$) and progesterone (1.59 ± 0.03 vs. 2.86 ± 0.35 ng/ml for -OCs and +OCs; $P < 0.05$) secretions, but it did not significantly alter androstenedione secretion on day 13 of culture. Surprisingly, in the presence of OCs, follicles were more likely to break down their GVs and reached GVBD/MII stages (%GV oocytes: 29.62

vs. 10.14%; %GVBD/MII oocytes: 55.55 vs. 72.46% for -OCs and +OCs; $P < 0.05$). Co-culturing with OCs also decreased cortical granules abnormality rate (66.66 vs. 40% for -OCs and +OCs; $P < 0.05$), although it did not have any significant impact on spindle formation and chromosomal alignment.

Conclusion: OCs improve *in vitro* growth and development of follicles probably via secreting special growth factors and cytokines that activate signaling pathways involved in follicles growth and development. So, these cells could be used properly in the follicle culture systems.

Keywords: Follicle, Alginate, Ovarian Cells

O-21: Bone Marrow Mesenchymal Stem Cells and L-Carnitine Co-Treatment Suppress Oxidative Stress and Inflammation in Mice with Induced Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common cause of infertility due to anovulation. Bone marrow mesenchymal stem cells (BM-MSCs) have anti-inflammatory and anti-oxidant properties which may inhibit oxidative stress and inflammation and L-Carnitine can improve the survival rate of transplanted MSCs. Since inflammation and oxidative stress are involved in the pathogenesis of PCOS, we decided to investigate the effect of BM-MSCs and L-Carnitine co-treatment on oxidative Stress and inflammation in mice with induced PCOS.

Materials and Methods: PCOS was induced through daily injections of testosterone enanthate (1 mg/100g S.C. for 5 weeks). NMRI mice (3 weeks old) were divided into 5 groups (n=6): Control, PCOS, PCOS + L-Carnitine, PCOS + BM-MSCs and PCOS + L-carnitine + BM-MSCs. Treatment of L-Carnitine was carried out with the dose of 500 mg/kg by i.p injections every other day for 4 weeks. BMMSCs were injected through the tail vein (10⁶MSCs/30g body weight) at 1 and 14 days after induction of PCOS. At the end of treatment, serum levels of IL-6 and TNF- α , Malondialdehyde (MDA) and the antioxidant capacity were measured relatively using the ELISA kit, thio-barbituric acid (TBA) and Ferric reducing antioxidant power (FRAP) assay. Data were analyzed using one way ANOVA and Tuckey's test and the means were considered significantly different at $P < 0.05$.

Results: The Serum levels of MDA, IL-6 and TNF- α significantly increased in the PCOS groups compared to the control group. These parameters significantly reduced in treated PCOS groups. IL-6 and TNF- α only in the PCOS + L-carnitine + BM-MSCs group were compensated to control level. Antioxidant capacity also reduced significantly in the PCOS group when compared to the control while it significantly increased in the treated PCOS groups to the control level.

Conclusion: We conclude that BM-MSCs and L-carnitine have protective effects against inflammation and oxidative stress in mice with induced PCOS.

Keywords: Polycystic Ovary Syndrome, BM-Mscs, Stress Oxidative, Inflammation, Mouse

O-22: Sperm Motility Improvement on Nano-Diamond Coated Glass Substrates

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Background: The employment of Nanotechnology as a valuable tool could be beneficial to patients and also offer new alternatives for assisted reproductive technology (ART). Focusing on the male infertility, particularly on sperm, Nanoparticles are mostly used for sperm purification, labeling, sorting, and imaging. However, toxicity of these particles are questionable along with their insignificant improvement of ART outcomes. This in turn sets aside their unique properties offered at Nanoscale and underestimates their potentials. In this essence, using nanoparticles as surface modifiers or coatings seems promising and less addressed. Surface modification by nanoparticles leads to the alteration of surface characteristics (e.g. roughness, elasticity, and surface charge). These alterations affect sperm behavior especially its motility since sperms exhibit wall following behavior and surface accumulation. Moreover, surface modification is an attempt towards mimicking the complex *in vivo* environment of the female tract (highly folded and ciliated) with continually changing surface topology. Here, we modified microscopic glass substrates with Nano-Diamond (ND) particles and investigated the effect of surface modification on sperm motility.

Materials and Methods: ND particles with average size of 28.5 nm were coated non-homogenously on microscopic glass substrates through a heat-and-quench process. The procedure was started by preparation of Nanofluid containing ND particles, followed by heating the glass substrate and finally quenching the substrate in the Nanofluid. The washed and dried glass substrates were incubated for 10 minutes. Afterwards 3 μ l of the processed human sperm sample was applied on the substrates (witness and modified). The imaging was done by a microscope digital camera and ImageJ software was used for image processing.

Results: The results show that the sperm motion is significantly improved on modified substrates compared to that of the witness mainly due to the lowering of the surface roughness.

Conclusion: The results suggest that the usage of these improved surfaces can be good means for diagnosis of deficiencies and can further be used for sperm selection in microfluidic devices. The selection in this manner would occur due to the distinction between motility of the sperms i.e. helping the better ones to reach the collection chamber in lesser time.

Keywords: Nanotechnology, Assisted Reproductive Techniques, Nano-Diamond, Sperm Selection, Microfluidics

O-23: Supplementation of Maturation Medium with Alpha Lipic Acid Increases The Developmental Competence of

Ovine Oocytes

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Background: Alpha-Lipoic acid (α -LA), a naturally occurring disulfide molecule, is a powerful antioxidant for clinical therapy of some metabolic diseases and advanced cancers by reducing the level of reactive oxygen species (ROS) and increasing glutathione peroxidase activity (GSH). α -LA acts as an important coenzyme for enzymes inside the mitochondria, such as pyruvate dehydrogenase and α -ketoglutarate dehydrogenase; therefore, it has been used as a vitamin-like substance but there are few reports about the effect of α -LA on *in vitro* maturation (IVM) in ruminants.

Materials and Methods: In this experimental study cumulus-oocyte complexes (COCs) were matured in control and α -LA (25 μ M) groups. After oocyte maturation we assessed cumulus expansion index (CEI), ROS and GSH level. After *in vitro* fertilization (IVF), non-treated treated matured oocytes were assessed for developmental rate, hatching rate and DNA fragmentation in derived blastocysts.

Results: The results revealed that 25 μ M α -LA during IVM have increased CEI, decreased ROS level and elevated GSH content in matured oocytes. Furthermore, supplementation of maturation medium with 25 μ M α -LA increased the blastocyst formation and hatching rates in compared to control. In addition DNA fragmentation have decreased in blastocysts derived from α -LA treated group in compared to control group.

Conclusion: Oocyte IVM is a complex physiological process in which oocyte undergoes nuclear maturation and cytoplasmic maturation. During oocyte IVM, oocyte is much more susceptible to oxidative stress, which disrupts oocyte and subsequent embryo development. In this study we have showed that α -LA could efficiently promote oocyte IVM and improve oocyte quality in terms of blastocyst and hatching rate and decrease DNA fragmentation in resultant embryos.

Keywords: α -LA, CEI, ROS, GSH, DNA Fragmentation

O-24: The Effect of Preincubation Time on Oocyte Quality and Fertilization Potential of Mouse MII Oocytes in The Simple and Myo-Inositol Supplemented Media

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Background: It is demonstrated that non-optimal preincubation time in *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles can lead to reduction in the oocyte quality, regarding to oxidative stress condition and mitochondrial alteration, and consequently can decrease the oocyte fertilization potential. Nevertheless, there is not any explanation of standard preincubation time in ART guidelines. Myo-inositol, as an antioxidant, exists naturally in the follicular fluid and is a marker of good quality in the oocytes. This study evaluated the oxidative stress condition, mitochondrial alterations and fertilization potential in mouse MII oocytes following 0, 4 and 8 hours preincubation time in the simple and myo-inositol supplemented media.

Materials and Methods: Cumulus Oocyte Complexes (COCs) which were retrieved from 6-8 weeks-old superovulated female NMRI mice were pooled and divided randomly in five experimental groups: (1) control, (2) 4 hours preincubation in simple medium, (3) 4 hours preincubation in 20 mmol/l of myo-inositol supplemented medium, (4) 8 hours preincubation in simple medium, (5) 8 hours preincubation in 20 mmol/l of myo-inositol supplemented medium. COCs in each group were denuded and intracellular reactive oxygen species (ROS), glutathione (GSH), mitochondrial membrane potential (MMP) and mitochondrial distribution were measured by a fluorometric assay. ATP content of oocytes also was measured using the ELISA method. 2PN rate was calculated to assess oocytes fertilization potential.

Results: Results showed that intracellular H₂O₂ and glutathione levels, mitochondrial distribution, mitochondrial membrane potential, ATP content, as well as fertilization rate were different between groups. Nonetheless, myo-inositol supplementation could improve levels of H₂O₂, glutathione, mitochondrial distribution, ATP content and fertilization rate. Unlike other variables, mitochondrial membrane potential of oocytes was not reduced after 4 hours of preincubation in either simple or supplemented medium, but 8 hours of preincubation time could decrease it significantly. Addition of myo-inositol to the medium could not ameliorate mitochondrial membrane potential in oocytes preincubated for 4 and 8 hours. While, ATP content did not decline in oocytes preincubated for 4 and 8 hours, supplementation of myo-inositol in medium could increase it in both groups.

Conclusion: Finally, the analysis addressed that 4 hours or more preincubation time can influence the oocyte quality related to alternation in H₂O₂, glutathione, mitochondrial integrity and mitochondrial membrane potential which ultimately leads to reduced oocyte fertilization potential. Supplementation of myo-inositol in medium improves the oocyte quality in comparison to the simple medium and saves 4 hours for preincubated oocytes.

Keywords: Oocyte Preincubation Time, Myo-Inositol Supplementation, Oocyte Quality, Fertilization Potential

O-25: Upregulation of Two Oxidative Phosphorylation Genes, NCF2 and TXNIP in Cumulus Cells of The Patients with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is associated with oxidative stress. Cumulus cells (CCs) are biologically differentiated from other follicular cells and have specialized roles in oocyte maturation during folliculogenesis. Reactive oxygen species (ROS) can greatly damage the ovarian follicles and change the expression of oxidative phosphorylation (OXPHOS) genes. A recent study of granulosa cells by microarray and subsequent qPCR validations revealed overexpression of two OXPHOS genes, neutrophil cytosolic factor 2 (NCF2) and thioredoxin interacting protein (TXNIP) in PCOS with insulin resistance (IR) and/or non-IR. NCF2 is a rate-limiting cofactor of NADPH oxidase, and increased expression of NCF2 potentiates NADPH oxidase activity, leading to oxidative stress. TXNIP encodes a cytoplasmic protein that inhibits the activity of thioredoxin disulfide reductase and thereby increases oxidative stress. Due to lack of data on CCs and their importance in folliculogenesis, we studied expression of NCF2, TXNIP and succinate dehydrogenase B (SDHB), a high abundant OXPHOS gene as internal control, in CCs of IR and non-IR PCOS.

Materials and Methods: Eight women with PCOS-IR, 8 women with PCOS non-IR based on Rotterdam criteria as case groups and 8 women without PCOS with male factor infertility as control group, all underwent GnRH antagonist protocol treatment at Royan Institute were included in this case-control study. Gene expression of CCs was studied by real time PCR. Normalization was performed considering Hypoxanthine phosphoribosyltransferase 1 (HPRT1) expression as a tissue specific reference gene based on previous evidences. Kruskal-Wallis was used to compare between groups. All statistical tests were two-sided and $P < 0.05$ was considered statistically significant.

Results: Expression of NCF2 in IR was higher than non-IR and control groups ($P=0.005$, $P=0.002$, respectively) with a fold change over 40. TXNIP was overexpressed in IR compared to controls ($P=0.012$) with a fold change around 15 while no significant difference was observed in SDHB expression, as expected.

Conclusion: Overexpression of NCF2 and TXNIP genes in CCs of PCOS was in accordance with previous findings in granulosa cells of PCOS. Fold changes of upregulation in CCs were much more than those of previously reported in granulosa cells (2.13-3.32). It seems in CCs of PCOS, response to oxidative stress conditions is very stronger than those granulosa cells.

Keywords: Polycystic Ovary Syndrome, Cumulus Cells, NCF2, TXNIP, SDHB

O-26: Evaluation Three Methods of Ovarian Decellularization by Triton, Ammonium Hydroxide and SDS

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Background: Decellularization is a novel technique in regenerative medicine. Recently decellularized ovary introduced as a scaffold in the field of human fertility preservation. So the aim of the present study was producing a decellularized ovarian scaffold for fertility preservation approaches.

Materials and Methods: In the present study 2 mm pieces of bovine and human ovarian cortex were prepared. In the SDS group, ovarian cortex was decellularized with 0.1% SDS for 24 h, in the SDS-Triton-ammonium group these pieces were decellularized with 0.5% SDS for 2 hours, 1% Triton and 0.1% Ammonium hydroxide for 22 hours and in SDS-Triton group pieces were decellularized with 0.5% SDS for 3 h and 1% Triton for 9 hours. Human ovarian cortex was decellularized in three steps. First, it was treated with 0.1% SDS for 24 hours then according to freeze and thaw, it was treated with 0.2% SDS for 10 hours. After decellularization all of the scaffolds were washed with deionized water for 24 hours. The intact ovarian cortex was used as a control group. H&E and DAPI staining were applied to prove decellularization. Elastin and Masson's trichrome staining was carried out to evaluate the presence of elastin and collagen respectively in decellularized tissue. Furthermore, MTT test was done to assess the *in vitro* scaffold's cytocompatibility.

Results: According to the H&E staining results, the bovine ovarian cortex was decellularized in all groups. No residual nuclei were observed by DAPI staining. Preservation of the ECM content was evaluated by Masson Trichrome and Gomori's aldehyde-fuchsin staining. Elastic and collagen fibers were kept after the decellularization process in all groups. OD values of eluted formazan of MTT test showed that fibroblasts on the SDS-Triton-Ammonium decellularized scaffolds were more viable than other groups. Human ovarian cortex was decellularized completely with mentioned protocol. Furthermore elastic and collagen fibers were kept in decellularized human ovarian cortex too. There was no significant difference in the Proliferation rate of the fibroblast cells in the human decellularized scaffold and two-dimensional conventional culture system.

Conclusion: In conclusion, human ovarian cortex was decellularized with the combination of different SDS solution. Structure of ECM was not damaged in any groups. However, MTT test results of SDS-Triton-Ammonium was better than other groups.

Keywords: Decellularization, Ovary, Scaffold

O-27: The Effect of L-Arginine on The Ovarian Histology in Mouse Model of the Endometriosis

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Background: One of the most common diseases among women is endometriosis, which affects women at the age of fertility. The cause of infertility in women with endometriosis may be due to anatomical problems that lead to adhesions and fibrosis, hormonal or immune system problems. Endometriosis also causes complications such as ovarian disorders. Based on the antioxidant and anti-inflammatory effects of L-arginine, it can be a solution to reduce the malignant effects of endometriosis on the ovaries. The purpose of this study is to investigate the effect of L-arginine on the ovarian histology in mouse model of endometriosis.

Materials and Methods: At the first step, induction of endometriosis in mice was done by injection of suspension of endometrium separated from the uterus of donor mice. In the second step, the control group was divided into four treatments: control without any treatment, control receiving L-arginine intraperitoneally (250 mg/kg, every day), control receiving danazol intraperitoneally (25 mg/kg, every other day) and control receiving L-arginine together with danazol. The endometriosis group was divided into four treatments: endometriosis without any treatment, endometriosis receiving L-arginine intraperitoneally (250mg/kg, every day), endometriosis receiving danazol intraperitoneally (25mg/kg, every other day), and endometriosis receiving L-arginine together with danazol. At the end of the treatment period, the mice were dissected then ovaries were separated and histologically examined.

Results: Microscopic studies showed that the endometriosis symptoms was reduced in the endometriosis group which receiving L-arginine and simultaneous L-arginine and danazol by increasing ovulation, increasing corpus luteum formation, decreasing the number of ovarian cysts and improvement in the status of the cortex and stroma.

Conclusion: According to our findings, danazol and L-arginine can increase the quality of oocytes and induces ovulation. In addition, because of its antioxidant properties, L-arginine can reduce the damages caused by oxidative stress in this disease and thus help to compensate tissue changes induced by endometriosis. L-arginine also causes follicular growth and ovulation, leads to increasing fertility rates.

Keywords: Ovary, Endometriosis, L-Arginine, Mouse

O-28: Effect of Selenium on Sperm Parameters and DNA Fragmentation in Infertile Men

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Background: Infertility is an important medical and social problem that has an impact on well-being. A significant development in the last 10 years in the study of human infertility has been the discovery that oxidative sperm DNA damage has a critical role in the etiology of poor semen quality and male infertility. It is now documented that selenium as a potent antioxidant protect the organism against oxidative stress via the inhibition of propagation of ROS reactions. We explored the efficacy of selenium for improving sperm parameters and DNA fragmentation in infertile men.

Materials and Methods: The study included 353 infertile men with idiopathic oligoasthenoteratospermia who received sup-

plemental daily selenium (400 µg) for at least 100 days. The mean age of cases was 28.5 years (range 20–45), and the median age was 30 years. These cases had presented with male factor infertility (primary or secondary) for at least 1 year. The longest and shortest duration of infertility was 10 years and 1 year, respectively. The median time of diagnosis of infertility was 1 year with a mean of 2.5 years.

Results: We observed 258 cases total improvement in sperm motility, morphology, count and DNA fragmentation in comparison with no treatment. No response to treatment occurred in 95 cases after 14 weeks of antioxidant therapy. On the basis of paired t-test results, antioxidant therapy with oral selenium was effective for treatment of oligoasthenoteratospermia.

Conclusion: Supplemental selenium may improve semen quality and have beneficial and protective effects, especially on sperm motility. We advocate their use for the treatment of idiopathic male infertility diagnosed with oligoasthenoteratospermia or asthenospermia in semen analysis.

Keywords: Sperm, Infertility, Selenium, Teratospermia, Asthenospermia

O-29: High-Throughput Rheotaxis-Based Sperm Separation Using A Simple Microfluidic Device

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Background: Human infertility affects almost 200 million people worldwide, and nearly one-third of these population are male related. Several technologies offer to assist the reproduction cycle, and in all of them, the most crucial part which controls the success rate is sperm sorting and selection process. Current clinical solutions for sperm sorting and selection have faced few problems through the years; especially they fall short when sorting for sperm free of reactive oxygen species, DNA damage, and epigenetic aberrations. Hence, recently microfluidic systems have attracted researcher's attention due to their numerous capabilities, for sperm selection purposes. So far, several microfluidic platforms have succeeded in the selection process, but often they are not straightforward to use or fabricate which prevents them from clinical adoption. Inspired by the natural physics of female reproductive tract and sperm's rheotaxis behavior, a novel microfluidic system was designed to fulfill the needs for sperm sorting and separation. This device takes advantages of oval-shaped microgrooves to optimize the shear stress acting on sperms and to create rheotaxis zones for the separation of highly motile sperms

Materials and Methods: It has been confirmed that in the absence of the flow or when the shear rate is below a critical value, sperms swim in all directions but if the shear rate exceeds the critical value, they were seen to reorient and swim against the flow. Based on these observations and researches and by the use of a finite-element method simulation, we investigated how flow can provide a rheotaxis zone in a microchannel and how to use that as a lever to separate sperms with different motilities. By imitating the microgrooves existed on the uterine wall, a microfluidic system featuring oval-shaped microgrooves that efficiently and noninvasively isolates highly motile and morphologically normal sperm, with lower epigenetic global methylation, from raw semen, is presented.

Results: The primary tests show that comparing to convention-

al methods the device is simple to use, requires only a small amount of semen, has a high throughput and the processing time of 15 minutes. Also, since the whole process is passive, in further experiments, we expect to see great improvements in DNA integrity and morphology of the sample.

Conclusion: The proposed system shows to be a promising substitute for conventional techniques.

Keywords: Sperm-Separation, DNA-Fragmentation, Rheotaxis, Microfluidics

O-30: Methanolic Extract of *Stachys Schtschegleevii* Improves Sperm Parameters in Type 2 Diabetic Rats by Reduction of Oxidative Stress in Epididymis

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Background: Approximately 70% of diabetic men experience some form of sexual dysfunction and infertility at some point in time. These patients tend to avoid diagnosis due to social stigma and use traditional medicine to improve their complications. Our previous studies showed that *Stachys schtschegleevii* (SS) has anti-diabetic, anti-inflammatory and antioxidant effects. This study was conducted to investigate the effect of SS on sperm parameter and oxidative stress status in epididymis of diabetic rats.

Materials and Methods: Thirty adult male rats were divided into 6 groups including: control, diabetic, diabetic treated by glibenclamide and diabetic treated by 100, 200 and 300 mg/kg methanolic extract of SS for 40 consecutive days by oral gavage. Type II diabetes was induced by high fat diet and low dose of streptozotocin (35 mg/kg). At the end of experiment, animals were euthanized and their left epididymis was removed and dissected in Ham's F10 and incubated at 37°C. Total count, motility, viability and morphology of sperm as well as trazoospermia (TZI) and sperm DNA fragmentation index (SDFI) were assessed according to the WHO standard methods. Right epididymis were rapidly homogenized and stored at -80 °C to measure glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA) and total antioxidant capacity (TAC). Data were statistically analyzed by SPSS using one-way ANOVA test and Tukey's post-hoc.

Results: Results showed that high dose of SS could significantly increase total sperm count compare to diabetic group but all dose of SS could significantly improve sperm motility, viability and morphology and reduce TZI and SDFI in a dose dependent manner. Analysis of oxidative stress indices indicated that SS also reduced MDA level and increased GPx, SOD and TAC level of epididymis in a dose dependent manner but this changes was significantly only in mid and high dose of SS compared to diabetic group. Our results revealed that all effects of mid and high dose of SS were better than glibenclamide treated groups.

Conclusion: Based on our results it can be concluded that administration of SS can be considered as a suitable protective strategy for improvement of infertility or subfertility complication in diabetic males.

Keywords: *Stachys Schtschegleevii*, Diabetes, Male Infertility, Sperm Analysis, Oxidative Stress

O-31: The Effect of Methanolic Extract of Poulak on Mean Volume and Total Number of Leydig Cells and Plasma Lev-

el of Testosterone in Type 2 Diabetic Rats

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Background: Diabetes mellitus is a common metabolic disorder of endocrine system which increasing so dramatically all over the world and can gradually cause damage to male reproductive system. *Stachys schtschegleevii* which commonly known as poulak has been used in Iranian traditional medicine due to its analgesic, antibacterial, anti-inflammatory, antioxidant and hypoglycemic properties. The aim of this study was to evaluate the effect of methanolic extract of poulak (MEP) on the number, morphology and function of Leydig cells in diabetic male rats.

Materials and Methods: Thirty adult male rats were divided into 6 groups including: control, diabetic, diabetic treated by glibenclamide and diabetic treated by 100, 200 and 300 mg/kg MEP for 40 consecutive days by oral gavage. Type II diabetes was induced by high fat diet and low dose of streptozotocin (STZ). After 2 weeks of dietary manipulation, and an overnight fasting, the rats were injected intraperitoneally with 35 mg/kg STZ. At the end of experiment, animals were anaesthetized and plasma samples were taken from caudal vena cava for measurement of testosterone and then testes were removed and fixed in 10% neutral buffered formalin. Tissue were processed by standard paraffin embedding and serially sectioned at 20 µm thickness. Selected sections by systematic random sampling were stained with H&E and mean volume and total numbers of Leydig cells were estimated by optical dissector and stereoinvestigator software. Finally, one-way ANOVA and Tukey's post-hoc were performed for data analysis.

Results: Results showed that diabetes reduced mean volume and total number of Leydig cells as well as plasma level of testosterone significantly compared to control groups. It was also indicated that administration of MEP could improve and increase size and number of Leydig cells and testosterone level of diabetic animals in a dose dependent manner. Our analysis revealed that all doses of poulak have better effect than glibenclamide treated groups.

Conclusion: Based on our results it can be concluded that administration of poulak due to improvement of structure and function of Leydig cells and enhancement of spermatogenesis process can be considered as a suitable protective strategy for increasing of fertility in diabetic males.

Keywords: Poulak, *Stachys Schtschegleevii*, Diabetes, Leydig Cells, Testosterone

O-32: The Composition of Human Uterine Fluid Compared to Clinically Used Preimplantation Embryo Culture Media

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Background: Embryo culture media used in IVF not only affect IVF efficacy but also have a direct effect on child outcomes. Composition analysis of routinely used embryo culture media showed that no two media had the same composition, making clear that the optimal composition has not been determined yet. Considering that the *in vivo* conditions could provide valuable information for the improvement of currently used embryo culture media, we analyzed the composition of human uterine fluid.

Materials and Methods: We determined the composition of human uterine fluid of 22 non stimulated women on the third day of the luteal phase of the menstrual cycle when the endometrium is considered to become receptive for embryo implantation. Fertile women of reproductive age with normal uterine anatomy were included. We compared our findings to the composition of 15 human preimplantation embryo culture media that are commonly used in IVF. In total, 37 components including ions, metabolites, immunoglobulins, proteins and amino acids were measured. The effects of potential confounders, such as female age and BMI, were evaluated using linear mixed models and the Mann-Whitney U test was used for comparing the concentrations of different components in uterine fluid with concentrations in culture media

Results: Compared to the embryo culture media, calcium, phosphate, lactate, pyruvate, albumin and 21 amino acids were present at significantly different concentrations in human uterine fluid. Immunoglobulins, citrulline, ornithine and uric acid were absent from all analyzed culture media, while being present in uterine fluid. The mean concentration and variation of all 37 components in uterine fluid seemed not to be affected by age or BMI

Conclusion: The concentration of 32 analysed components differed between human uterine fluid and human embryo culture media. These differences suggest that current *in vitro* culture conditions might be suboptimal to provide support for the developing embryo *in vitro*. Our findings provide valuable information for the improvement of embryo culture media

Keywords: Human Uterine Fluid Composition, Human Preimplantation Embryology, Embryo Culture Media, IVF

Female Infertility

O-33: Estimating of Total Trans Fatty Acid in Subcutaneous Adipose Tissue of Pregnant Women with PCOS and Non-PCOS Pregnant Women

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Background: Polycystic ovary syndrome (PCOS) is a complex and heterogeneous endocrine disorder with well-established metabolic abnormalities. The increasing epidemic of PCOS has led to a great deal of interest in the function of adipose tissue (AT) and quality of AT seems involved in the pathogenesis of PCOS. Fatty acid composition of adipose tissue triacylglycerol's (AT-TAG) partly reflect dietary fatty acid intake of about 2 years, especially for those which cannot synthesize endogenously like trans fatty acids. The distribution of tFA produced via partial hydrogenation of vegetable oils (mainly elaidic acid; t9) differs compared to ruminant derived tFA (mainly vaccenic acid; t11). Our study not only measured the trans FA percentage in Iranian mother abdominal AT but also we analyzed for the first time individual isomers of trans C18:1 in PCOS and non-PCOS women.

Materials and Methods: Abdominal subcutaneous AT samples from 13 PCOS were compared with 32 non-PCOS women who underwent cesarean section. The FA composition of each biopsy was measured using gas chromatography. Data with normal and abnormal distribution were analyzed using t test and Mann-Whitney U test, respectively.

Results: No significant differences were found with respect to age and body mass index before pregnancy and at delivery day among non-PCOS and PCOS. Total trans FA, C18:1 t9 and C18:2t were lower in PCOS than non-PCOS whereas C18:1 t11 and C18:1 t6 were unaltered ($p < 0.05$).

Conclusion: While the most report of trans fatty acid of adipose tissue belong to Iranian men and women in 2008, our result in compare to other researchs show not increasing rate. Our data confirms lower industrial trans isomer in AT of PCOS mother which may regarding more attention on nutrition in this group.

Keywords: PCOS, Adipose Tissue, Trans Fatty Acid

O-34: The Predictive Values of the Serum Levels of Estradiol and Progesterone during The Modified Natural Cycles for Clinical Pregnancy after Vitrified-Warmed Embryo Transfer

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Background: The study was conducted to examine the predictive value of serum estradiol and progesterone on the day of human chorionic gonadotropin (hCG) administration and embryo transfer for clinical pregnancy rate in modified natural-cycle frozen embryo transfer (NC-FET). **Material and Methods:** In a longitudinal prospective study, all eligible women who underwent

Materials and Methods: In a longitudinal prospective study, all eligible women who underwent NC-FET cycles with hCG triggering in Royan Institute, Tehran, Iran, from June 1, 2015, to December 31, 2016, were evaluated. Serum estradiol and progesterone levels were measured at menstrual cycle initia-

tion, on day of trigger with hCG, on day of embryo transfer, and in pregnant women every 7 days until the observation of a gestational sac with embryonic heartbeat.

Results: In total, 101 modified natural FET cycles were assessed, and the clinical pregnancy and live birth rates achieved were 34 (33.6%) and 32 (31.6%), respectively. The demographic and laboratory characteristics of the pregnant and non-pregnant women following NC-FET were compared together. The mean estradiol level ($P=0.041$) and size of the dominant follicle ($P=0.034$) on the day of hCG administration in pregnant women were significantly greater than those of non-pregnant women. In addition, there was a difference between the groups in terms of the mean level of basal serum AMH, but this did not reach the significance level ($P=0.075$). No significant difference was seen between groups regarding other variables. The changes in estradiol level during early pregnancy showed an increase by an average of 200 pg/mL per week. Multivariable logistic regression analysis showed that only the estradiol level on the hCG day was a significant predictive variable for clinical pregnancy following NC-FET ($P=0.04$).

Conclusion: Serum estradiol level on the day of hCG administration was a significant predictor of clinical pregnancy in modified NC-FET. The study findings demonstrated that estradiol level on hCG administration day was associated with the developmental competence of the corpus luteum, and therefore an optimal luteal structure-function during the modified natural cycle and the initial phase of pregnancy after FET.

Keywords: Vitri-fied-Warmed Embryo Transfer, Modified Natural Cycle, Serum Estradiol, Serum Progesterone, Live Birth

O-35: Alterations of Toll-Like Receptor 3 Related Genes in Endometriosis Patients

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Background: Toll-like receptor 3 (TLR3), comprises a family of receptors through directly recognizing exogenous and endogenous ligands, plays the key role in realization of immune responses, and also involves in the processes of cell proliferation, survival, apoptosis and tissue remodeling. Aberrant expression of TLR3 and some its downstream genes involved in the pathogenesis of immunological diseases. Endometriosis, a common gynecologic disorder that is characterized by the ectopic growth of endometrial tissue, has described as a genetic, hormonal and also immune disease. This study aimed at evaluation of TLR3 and some signaling pathway genes in eutopic endometrium (EU) and ectopic (EC) as well as serum parameters in women with endometriosis.

Material and Methods: Twenty endometriosis lesions from EC and EU biopsies were obtained from endometriosis women,

as well as endometrium from healthy women ($n=16$, as control group, CE) in the proliferative phase of menstrual cycle (days 5 to 14). Q-PCR was performed for determination of mRNA expression level of TLR3 signaling pathway genes (TLR3, TICAM, NF- κ B1A, CXCL10, IRF3, IFN- β , IL-6 and IL-8 genes) in tissue samples. Also, serum protein levels of TLR3, IFN- β , IL-6 and IL-8 were determined by ELISA Assay.

Results: mRNA gene expression of TLR3, NF- κ B1A, IFN- β , IRF3, TICAM1, IL-6 and IL-8 were significantly higher in EU compared to ectopic ones and also compared to CE ($P<0.05$ or $P<0.01$). There was no significant difference in CXCL10 expression between EU vs. CE whereas its mRNA expression was significantly higher in EU than ectopic ones. Serum protein levels of TLR3, IFN- β , IL-6 and IL-8 were significantly increased in patients with endometriosis in comparison to the control ($P<0.05$).

Conclusion: In this study, moreover to elevation of IL-6 and IL-8 cytokines, significant and clear changes was observed in the mRNA expression of other genes in TLR3 cascade in diseased eutopic endometrium, demonstrating that EU similarly to EC was in an intensive inflammatory state. Interestingly, the results showed that expression of aforementioned genes have significant difference between ectopic and eutopic endometrium of endometriosis patients, suggesting that fundamental alterations in the concept of immune response in eutopic endometrium may lead to its activation and escapes from apoptosis. These changes maybe have potential contribution to misplaced implantation of endometrium, and then ectopic tissues become stable to a certain degree from the immunological point of view.

Keywords: Endometriosis, TLR3, Toll-Like Receptor 3, Signaling Pathway, Expression

Genetics

O-36: Mitotic Arrest Deficient 2 Like 1 (MAD2L1) Gene Variations in Products of Conception with Aneuploidy

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Background: Genomic abnormalities certainly aneuploidy are one of the most common cause of spontaneous abortion. Although aneuploidy is associated with advanced maternal age it is frequent in young women. Spindle assembly checkpoint (SAC) complex has a critical role in fidelity of chromosome segregation. Several genes are involved in SAC, such as MAD1L1, MAD2L1 and BUB1. Since evidences of a decrease in the level of BUB1 and MAD2 proteins in clinical samples of spontaneous miscarriages and the probable role of MAD2L1 last exons deletions in fibroblasts of the patients with a history of trisomic abortion, we investigated MAD2L1 gene variations

in products of conception (POCs) with aneuploidy.

Materials and Methods: To detect aneuploidy, POC of mothers younger than 36 years were analyzed using quantitative fluorescence polymerase chain reaction (QF-PCR) and/or array comparative genomic hybridization (aCGH). Those with aneuploidy were enrolled in genotyping. MAD2L1 exons were genotyped using PCR and Sanger sequencing. Frequency of the observed single nucleotide variations (SNVs) were compared with the highest population minor allele frequency (MAF) using Chi Square.

Results: According to QF-PCR and aCGH results, 38 aneuploid samples were enrolled in genotyping. We observed rs903147, rs2908989, rs758373815, rs752146697, rs78047690 and rs2908990 SNVs with following allele frequencies and P values of comparison with the highest population MAF: C:0.64/A:0.36 (P=0.0184), A:0.46/G:0.54 (P=0.0265), T:0.99/G:0.01 (P=0.7613), G:0.96/A:0.04 (P=0.0083), G:0.97/A:0.03 (P=0.1410), A:0.54/G:0.46 (P=0.4913), respectively.

Conclusion: Frequencies of rs903147, rs2908989 and rs752146697 SNVs were significantly higher than the highest population MAF. These SNVs seem to be associated with aneuploidy in POC.

Keywords: Abortion, Aneuploidy, MAD2L1, Single Nucleotide Variation

O-37: Cross-Link between Heat Shock Protein Hsp70 and Epigenetic Alterations in Testicular Tissue; Evidence for A Diabetic Condition

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Background: Diabetes has been known to adversely affect germ cells and sperm DNA integrity via inducing oxidative stress. The Heat shock (HSP) family proteins are known as stress responders, which are highly expressed in oxidative stress condition. Aside from oxidative stress-induced DNA fragmentation, it has been shown that histamine-protamine exchanged failure (epigenetic pathways) is able to negatively affect DNA integrity. Thus, here in the current study, the cross-link between DNA methylation, histone acetylation/deacetylation, and the Hsp70 content of testicles was investigated.

Materials and Methods: For this purpose, 12 mature male Wistar rats were divided into control and diabetes-induced groups (No:6 rats in each group). The experimental diabetes was induced by streptozotocin. The germ cells global DNA methylation was assessed by immunohistochemical staining. The mRNA levels of DNA methyltransferase-1 (DNMT1), histone acetyltransferase (HAT) and Histone de-acetyltransferase (HDAC) were evaluated by qRT-PCR. The Hsp70 expression was evaluated both at mRNA and protein levels. Finally, the germ cells DNA integrity was analyzed by TUNEL staining.

Results: Observations showed a significant (P<0.05) reduction in global DNA methylation in the diabetes-induced group. The animals in the diabetes-induced group represented a remarkable (P<0.05) reduction in mRNA level of HAT, DNMT1, and HDAC versus the control animals. Moreover, the diabetes-induced animals exhibited decreased expression of Hsp70 (P<0.05). Finally, the diabetes-induced group showed a severe DNA fragmentation at the germ cell level.

Conclusion: Our data showed that simultaneous with decreased Hsp70 level, the global DNA methylation and mRNA levels of HAT, HDAC and DNMT1 were decreased in diabetic condition. In addition, simultaneous with DNMT1 reduction, the diabetes-induced animals exhibited a remarkable reduction in global DNA methylation and a significant enhancement in DNA fragmentation. All these findings represent a direct correlation between Hsp70 expression, epigenetic changes, DNA methylation and DNA fragmentation in the diabetic testicle.

Keywords: Experimental Diabetes, Epigenetic, DNA Fragmentation, Hsp70, Rat

O-38: Enhancing Expression of NRF2 Antioxidant Gene by NAC Supplementation in Infertile Men

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Background: Reactive oxygen species (ROS) play an important role in male infertility. Nuclear factor erythroid 2-related factor 2 (NRF2) can up-regulate the expression of antioxidant genes and protect cells from oxidative damage. The current study suggests that effect of N-acetyl-cysteine on sperm quality in asthenoteratozoospermia men is associated closely with NRF2 gene expression.

Materials and Methods: The study was carried out in the unit of ACECR Infertility Research Center, Qom, Iran. The patients consisted of 50 infertile men with asthenoteratozoospermia, who received NAC (600 mg/d) orally for 3 months, after which they were compared with pre-treatment status. Semen was analyzed according to WHO (2010) for DNA integrity [terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)]. Quantitative real-time reverse transcriptase polymerase chain reaction was used for detecting the level of NRF2 mRNA expression in ejaculated spermatozoa. Seminal plasma levels of antioxidant enzymes were determined by ELISA kit.

Results: After NAC treatment, patients' sperm concentration and motility increased significantly whereas abnormal morphology and DNA fragmentation showed significant decreases compared to pre-treatment levels (P<0.05). NAC treatment significantly increased seminal plasma of antioxidant enzyme levels compared with baseline values (P<0.05). Furthermore, NAC treatment significantly unregulated NRF2 levels in sperm in comparison to untreated NAC (P<0.05). Significant correlations were observed between the level of NRF2 mRNA expression, specific sperm parameters and levels of antioxidant enzymes (P<0.05).

Conclusion: The results demonstrate that NAC oral supplementation protects against oxidative stress by activating the Nrf2 antioxidant defense system and improving sperm parameters in infertile men.

Keywords: Nuclear Related Factor 2, N-Acetyl-Cysteine, Oxidative Stress, Asthenoteratozoospermia

O-39: Application of Fluorescence in Situ Hybridization Technique for The Screening of Prenatal Diagnosis

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Background: In order to obstetric care of pregnancy in women with high-risk are suggested prenatal diagnosis through amniotic fluid sampling for cytogenetic analysis of fetal cells. The purpose of this study was to assessing effectiveness of the rapid fluorescence in situ hybridization (FISH) technique for detecting Chromosomal numerical abnormalities (13, 18, 21, X and Y) in high-risk pregnancies.

Materials and Methods: In this study a total of 136 samples were received for a FISH and/or a full karyotype for the screening of prenatal diagnosis from high-risk pregnancies problem who were introduced to the Medical Genetic Laboratory of Dr. Keshavarz in Rasht between May, 2017 and May, 2019. In 97 samples both conventional culture techniques for getting karyotype through G-banding techniques were applied in conjunction to FISH test, to compare the both the techniques carried out.

Results: Among 97 patients, 81 normal for the five major chromosome abnormality and 12 patients were found to be abnormal (6 trisomy 21, 1 trisomy 13, 1 trisomy 18 and 4 monosomy X) and all the FISH results correlated with conventional cytogenetics. A total of 136 patients for the chromosomal abnormalities analyzed by both/or cytogenetics and FISH there were 114 (83.8%) normal, 12 (8.8%) cases were abnormal and another 6 (4.4%) cases were suspicious mosaic and 4 (3%) cases of culture failure. The detection rate of prenatal diagnosis with FISH in 97 patients was 95.8%. Based on the results of this study, there were no false-positive and false-negative autosomal or sex chromosomal results, within our established criteria for reporting FISH signals.

Conclusion: Therefore FISH as a reliable and rapid method in prenatal screening method for detecting numerical chromosomal abnormalities and has now been performed as a routine diagnostic procedure for detection of fetal aneuploidy.

Keywords: Screening, Prenatal Diagnosis, Fluorescence in Situ Hybridization

O-40: Various Inflammatory Response of Fallopian Tube Epithelial Cells to Sperms with DNA Fragmentation

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Background: The fallopian tubes have important role to prepare a safe environment for fertilization. In addition, they have different functions such as sperm saving, capacitation and so on. The interaction between the male and female gametes and embryos in the female reproductive system plays an important role in fertility, embryonic development, and implantation. The immune system in reproductive tract has different challenges such as overcoming infections and chronic inflammatory conditions as well as being able to tolerate the allogeneic spermatozoa. Cytokines are one of the most important variables of immune cells that provide effective host protection. During fertilization sperms carry proteins which are allogenic to the female immune system. Therefore, cytokines play an important role in the fallopian tube specially in the presence of sperm. In current study we investigated the expression of anti-inflammatory cytokines by fallopian tube under the effect of sperms with and without DNA fragmentation.

Materials and Methods: Fallopian tube epithelial cells were cultured into the tissue culture flasks containing DMEM/F12 with 10% FBS medium. During cell preparation, sperm samples from 20 donors which were normal according to WHO criteria were collected. The extent of sperm DNA fragmentation was measured by the TUNEL assay. Afterward, samples divided to two groups of normal and abnormal DFI, and the cell lines without sperm classified as control group. Different sperms were co-incubated with fallopian tube cells for 24h. RNA extraction from cells was done followed by cDNA synthesized. Finally, PCR array was performed to evaluate cytokine expression profiling. In addition, this data was validated by q-PCR.

Results: The data analysis indicated that the expression of anti-inflammatory cytokines in the presence of sperm significantly changes. Sperm DFI is also effective in expressing of some cytokines and the results showed various expression of them in different groups. Anti-inflammatory cytokines including TGFB2, IL11 and IL13 had lower expression in sperm group than the control. However, other cytokines like IL2, IL12A, IL22 and IL24 were upregulated in response to sperms with abnormal DFI and they were down-regulated in response to normal sperms.

Conclusion: This study point that abnormal DFI can change the expression of cytokines which have essential roles in sperm preservation and fertilization. Therefore, the abnormal sperm seems to be suppressed by the immune system of fallopian tube.

Keywords: Inflammatory Response, DNA Fragmentation, Fallopian Tube, Sperm

O-41: Modulation of The Fallopian Tube Micro-Environment in Response to DNA-Fragmented Sperms by Chemokines

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Background: The human fallopian tube is a part of female reproductive system in which fertilization occurs. The cross-talk between sperms and fallopian tube epithelial cells is very valuable because it has different roles in reproductive process such as; fertilization and early embryo development. Therefore, the existence of a sperm recognition system that apprise the female reproductive tracts of the sperm entry looks essential. This system should prepare the safe environment in fallopian tubes in which sperms and oocytes transport and eventually fertilization occur. It has been suggested, some kind of chemokines can regulate the ability of female reproductive tract to tolerate the allogenic spermatozoa. Therefore, in this study we evaluate influence of the DNA-fragmented sperms on fallopian tube epithelial cells by assessing chemokines expression with PCR-array and also we investigate association between sperm DNA Fragmentation Index (DFI) and pregnancy rate outcome.

Materials and Methods: In this investigation, sperm samples from 20 donors with normal features according to WHO standards were collected. Based on DNA fragmentation we evaluated normal and abnormal sperm with tunnel assay method. To assess the effect of spermatozoa on human fallopian tube epithelial cells, sperms were co-cultured with fallopian tube cell line (OE-E6/E7). After 24 hours' cells were washed and RNA extraction was performed. Finally, cDNA was synthesized and PCR array was performed. In the present study we performed statistical analysis of CCL21, CCL22, CXCL2 and CXCL5.

Results: The outcome of data analysis shows that DNA fragmentation of sperms can change the expression of chemokines relatively. The highest expression of CCL21 and CCL22 were seen in the presence of normal sperms. However, there was no significant difference between abnormal DFI and the control groups. On the other hand, the expression of CXCL2 and CXCL5 were the lowest under the effect of sperms with DNA fragmentation.

Conclusion: The results show that fallopian tube response to the presence of sperms with different mechanism including chemokine alteration which might lead to modulate the fallopian tubes environment for fertilization. Different expression of chemokines in normal and abnormal DFI groups also shows that the necessity of DFI screening for ART procedure.

Keywords: Chemokines, Sperm, Fallopian Tube, DNA Fragmentation, PCR Array

O-42: The Best Transcript Between Pluripotency Related Genes for Prediction of Developmental Competency of Bovine Nuclear Transfer Derived Embryos

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Background: Developmental competency of reconstructed embryos with different types of donor nucleus is often lower than *in vivo* and *in vitro* counterparts. Presence of a reliable in-

dicator in level of transcription of donor cell or preimplantation embryo can help to prediction the postimplantation developmental fate of cloned embryo.

Materials and Methods: In this study, the developmental ability and the pattern of some pluripotency related genes (Oct4, Sox2, Nanog) in bovine cloned embryos reconstructed mesenchymal stem cells (MSCs), and adult fibroblasts (AF) were compared with *in vitro* fertilized (IVF) counterparts. For these purposes, *in vitro* matured abattoir-derived oocytes were considered as recipients and hand-made cloning technique was used for oocyte enucleation and cell attachment in NT procedure.

Results: Despite the lower developmental rate in NT derived embryos (12.05 and 19.23% For AF- and MSCs-NT derived blastocysts, respectively) than IVF counterparts (26.7% Blastocyst rate) (P<0.05), but the use of stem cells improved developmental competency of reconstructed embryos, significantly (P<0.05). As well as, the relative abundance (RA) of the Oct4 transcripts at the blastocyst stage exhibited higher level of expression in *in vitro* produced embryos. Though IVF embryos did differ significantly higher than AF embryos (P<0.05), whereas no difference was observed between IVF and MSC-NT embryos.

Conclusion: By evaluation of the relationship between the expression of genes involved in pluripotency and developmental competency in produced blastocysts, it can be concluded that Oct4 can be regarded as an indicator of the embryos produced quality.

Keywords: Nuclear Transfer, Oct4, Developmental Competency, Bovine, Embryo

O-43: Successful Application of whole Exome Sequencing in Identification of the Underlying Genetic Cause in A Family with Infertility in Both Sexes

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Background: The most severe phenotypes of infertility in women are premature ovarian insufficiency (POI) and non-obstructive azoospermia (NOA) in men. POI is characterized by premature amenorrhea reflecting an early depletion of the follicular reserve in the ovaries of women under the age of 40. Non obstructive azoospermia is defined as lack of spermatogenesis or mature spermatozoa in the testes, low testicular volume and high follicle stimulating hormone values. Due to the absence of sperm in testis and follicles in the ovaries, these two disorders are considered similar in terms of the phenotype. POI and NOA are heterogeneous diseases caused by a variety of mechanisms however, there is a strong genetic component for both as families with multiple affected children exist. Despite many studies into the genetics of POI and NOA few genes have been identified that can explain a substantial proportion of cases leaving many cases idiopathic. Here, our objective is to identify the genetic cause of NOA and POI in six affected members of a consanguineous Iranian family.

Materials and Methods: Whole exome sequencing (WES)

was performed the affected and their parents. In silico analysis was also used to predict the effect and pathogenicity of the discovered variants followed by Polymerase Chain Reaction (PCR) and Sanger sequencing to validate the segregation of the variant with the disease in the pedigree.

Results: Given the consanguinity of the pedigree, high priority was given to homozygous variants observed in the affected of both sexes. A final list of pathogenic variants was prepared and one specific variant in a highly conserved domain of a gonad specific protein was confirmed to be mutual among the affected.

Conclusion: With the application of WES we identified a pathogenic variant in Iranian family with multiple instances of POI and NOA. Our finding reinforces the clinical role of WES in the molecular diagnosis of highly heterogeneous genetic diseases for which conventional genetic approaches have previously failed to provide a molecular diagnosis.

Keywords: Premature Ovarian Failure, Non-Obstructive Azospermia, Whole Exome sequencing, Next Generation Sequencing, Familial Studies

O-44: Different Gene Expression of Lipopolysaccharide Induce Tumor Necrosis Factor-Alpha Factor (LITAF) in Endometrial Tissues of Women with and without Endometriosis

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Background: Endometriosis is an estrogen-dependent and chronic inflammatory disease of women in reproductive age. Endometriosis is defined as the presence of endometrial glands and stroma outside the uterine cavity. Immune system alterations in peritoneal cavity is one of the main due of growth and proliferation of endometrial lesions in women with endometriosis. It is shown that macrophages number and activation are increased in peritoneal fluid of women with endometriosis. Tumor necrosis factor- α (TNF- α) is one of the main inflammatory cytokines secreted by macrophages. It has been reported that the concentration of TNF- α is increased in peritoneal fluid of women with endometriosis. Our study showed that gene expression of TNF- α is increased in ectopic lesions of women with endometriosis in compare to control group. Lipopolysaccharide induce tumor necrosis factor-alpha factor (LITAF) is a transcription factor that regulates the expression of TNF- α gene. The objective of present study was gene expression of LITAF in eutopic and ectopic endometrial tissues of women with endometriosis in compare to women without endometriosis.

Materials and Methods: In this study, ten women with endo-

metriosis (endometriosis group) and 10 women without endometriosis (control group) were enrolled after signing consent form. Both groups underwent laparoscopy surgery at Royan Institute. Ectopic endometrial samples were collected during laparoscopy. Eutopic and control endometrial samples were collected by pipelle. Then, RNA extraction and cDNA synthesis were done for all the tissues. Gene expression of LITAF was measured by real time-PCR. P value less than 0.05 was considered statistically significant.

Results: Ectopic endometrial tissues showed highest LITAF gene expression level in compare to eutopic and control samples. LITAF gene expression was significantly higher in ectopic tissues in compare to control samples. In addition, the level of LITAF gene expression was higher in eutopic endometrial tissues of women with endometriosis than control group but this difference was not significant.

Conclusion: The results of this study suggest that over expression of LITAF in endometrial tissues of women with endometriosis could involve in inflammatory pathways of endometriosis.

Keywords: Endometriosis, LITAF, TNF- α , Ectopic, Eutopic

O-45: Five Years of Experience in Preimplantation Genetic Diagnosis for Single Gene Disorders: Royan Report

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Background: Preimplantation genetic diagnosis (PGD) has been practiced in those couples who are affected/carrier to the single gene disorders (SGD) wishing to have a normal baby since 1987. It is the golden standard method in those with fertility problems or affected to SGDs in which the legal permission for induced abortion is not allowed. In Iran, due to popularity of consanguine marriages, carrier status to the SGDs is frequent especially for thalassemia which has a high incidence in some provinces. Therefore, PGD will be highly applicable in mentioned couples who are usually reluctant to induced abortion following prenatal diagnosis. Here, we report our experience in carrying out PGD for single gene disorders for couples referred to Royan infertility center from June, 2014 till March 2019.

Materials and Methods: Couples underwent assisted reproductive treatment following standard protocols. Single blastomere biopsies were done on third day in embryology laboratory and embryo transfers achieved on 5th day. 125 indications from various SGDs were assessed for informative short tandem repeats and mutation confirmation in a preliminary step for each family to be able to determine the pattern of segregation of linkage markers. On PGD day, the genotype of each embryo was detected based on direct mutation analysis (if applicable for the particular SGD) and confirmed by analysis of the pattern of inherited linkage markers.

Results: In total 302 cycles of PGD including 156 for beta thalassemia were done in which 1918 embryos were assessed for SGD status including 992 for beta thalassemia. Among them, 751 embryos were diagnosed as unaffected and selected for transfers including 437 for beta thalassemia, of them, 347

embryos were transferred including 204 for beta thalassemia disease. Pregnancy (chemical/clinical) was achieved in 80 couples (28.2% per ET) including 40 (26.4% per ET) in beta thalassemic couples. Positive outcome (take home baby) was reported in 46 (16.2% per ET) and 28 (17.9% per ET) for beta thalassemic couples. We also detected the rates of allele drop out, contamination and no amplification as 4.7%, 1.4% and 9.8% in respect.

Conclusion: PGD for single gene disorders is an attractive option for carrier/affected couples and may be the only one in those with infertility problems.

Keywords: Single Gene Disorder, Preimplantation Genetic Diagnosis, Infertility

Andrology

P-1: Identification of Key Genes and Construction of Potential miRNA–mRNA Regulatory Network in Teratozoospermia

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Background: Teratozoospermia is characterized by the presence of spermatozoa with abnormal morphology over 85 % in sperm. mRNAs that aid in the detection of sperm abnormalities are potential biomarkers to evaluate the quality of sperm in the of male infertility. The current study was aimed at identifying key genes and molecular mechanism in teratozoospermia.

Materials and Methods: We downloaded two gene expression profiles (GSE6872 and GSE6967) from the Gene Expression Omnibus (GEO). We conducted differential screens of the expression of genes (DEGs) between two groups using the online tool GEO2R based on the R software limma package. Target miRNAs of DEGs were predicted using miRNet. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed for the DEGs to investigate the biological processes involved in the development of teratozoospermia, subsequently, pathways interrelation analysis of hub genes was carried out using plug-in ClueGO v2.3.3. Finally, miRNA–mRNA regulatory networks were visualized using Cytoscape.

Results: With the criteria of adjusted P value (adj.p) <0.01 and absolute log value of fold-change (log|FC|) >2, 970 and 4799 DEGs from GSE6872 and GSE6967 were obtained respectively. A total of 509 intersection DEGs were shared by two datasets. 2256 target miRNAs were predicted for DEGs. The mRNA–miRNA networks were composed of 2256 miRNA nodes and 454 mRNA nodes. The DEGs were mainly enriched in the cytosol and cytoplasm and were involved in the regulation of translational initiation, related to protein binding, and participated in the regulation of the Ribosome pathway.

Conclusion: The current study revealed the potential key genes, pathways, and miRNA–mRNA regulatory networks in teratozoospermia, which may contribute to a more comprehensive understanding and treatment of male infertility

Keywords: Teratozoospermia, Bioinformatics Analysis, Disease Makers, miRNA–mRNA pairs, Functional Enrichment Analysis

P-2: Association between ICSI Clinical Outcomes in Total Globozoospermia Due to DPY19L2 Deletion and Phospholipase C Zeta Sperm Marker involved in Oocyte Activation

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Background: Globozoospermia is one of the main reported causes of fertilization failure after intracytoplasmic sperm injection (ICSI) which is characterized by the presence of 100% acrosomeless round headed spermatozoa in an ejaculate and can be overcome by artificial oocyte activation (AOA). Low expression level of Phospholipase C zeta (PLC ζ), which is one of the main sperm factors involved in oocyte activation, is mostly account for fertilization failure. Deletion of the DPY19L2 gene is reported as a causative genetic factor in over 70% of infertile men with globozoospermia. This study aimed to evaluating the expression of sperm PLC ζ at RNA and protein levels in 32 DPY19L2 deletion-mediated globozoospermic men and reporting the corresponding clinical outcomes following ICSI with AOA.

Materials and Methods: Semen samples were obtained from 32 men with total globozoospermia (presenting with 100% round-headed spermatozoa) and 32 fertile men and the expression of PLC ζ at RNA (Real-time PCR) and protein levels (western blot) was compared between them.

Results: PLC ζ expression (at RNA and protein levels) in globozoospermic men was significantly lower compared to fertile men (P<0.05). Fertilization rate in globozoospermic couples following ICSI–AOA was significantly lower compared to fertile men (P<0.05). However, implantation (26.2%) and pregnancy (53.8%) rates were not endangered by DPY19L2 deletion in these couples.

Conclusion: ICSI–AOA Application in globozoospermic couples improves the post-ICSI fertilization rate and results in satisfactory live birth frequency.

Keywords: ICSI–AOA, Globozoospermia, Fertilization, PLC ζ

P-3: Effects of Long-term Administration of Aspartame on Sperm Parameters, Sex Hormones and Testis Histomorphological Indices in Mice Model

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Background: Aspartame is a non-nutritive sweetener particularly used in ‘diet’ and ‘lowcalorie’ products and also in a variety of foods, drugs and hygiene products. The aim of the present study was to assess potential adverse effects of aspartame on sperm parameters, sex hormones and histomorphometrical indices of testis tissue in mice model.

Materials and Methods: Thirty-six adult male NMRI mice were randomly divided into 4 groups of 9 animals each, including control group (received 0.3 ml normal saline) and aspartame groups (received 40, 80 and 160 mg/kg b.w, respectively). All animals were daily treated by gavage. After 90 days, animals were killed by cutting neck vessels under light anesthesia, serum samples were collected for hormonal evaluations. Thereafter, testicles and caudal epididymis were removed for histomorphometrical and sperm analysis.

Results: The results showed that there was a significant (P<0.05) reduction in sperm count at all administrated doses

(40, 80 and 160 mg/kg) compared with the control group. Moreover, aspartame significantly ($P < 0.05$) decreased sperm motility, viability and maturation while significantly ($P < 0.05$) increased sperm abnormality DNA damage at 80 and 160 mg/kg in comparison with control group. Also, long-term administration of aspartame significantly ($P < 0.05$) lowered serum concentrations of FSH and LH at doses of 80 and 160 mg/kg, and testosterone at dose of 160 mg/kg. In other hand, histomorphological indices including TDI (Tubular differentiation index), SPI (spermiogenesis index) and RI (Repopulation index) were significantly reduced at doses of 80 and 160 mg/kg.

Conclusion: Our findings revealed that long-term administration of aspartame at high doses adversely affects sperm parameters, sex hormones and testis histomorphometrical indexes in male mice model.

Keywords: Aspartame, Sperm Parameters, Mice

P-4: Induction of Oxidative Stress following Long-term Administration of Aspartame at High Doses in Male Reproductive System of Mice

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Background: Aspartame is a non-nutritive sweetener which widely used in a variety of foods, beverages, and medicine. In this study, effects of long-term administration of aspartame on oxidative stress biomarkers in testis tissue and sperm samples of male mice were evaluated.

Materials and Methods: Thirty-six mature male mice were assigned to four groups, 9 mice in each. Group I was the control and received 0.3 ml of distilled water. Groups II, III and IV received aspartame at doses 40, 80 and 160 mg/kg b.w, respectively. All groups received treatment via oral gavage. Animals were euthanized following 90 days. Total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities and nitric oxide (NO) and malondialdehyde (MDA) levels were measured in testis tissue and sperm samples of animals.

Results: The results showed that aspartame at doses of 80 and 160 doses significantly decreased testicular and seminal TAC and antioxidant enzymes and also increased NO and MDA.

Conclusions: Based on the results, long-term administration of aspartame at high doses could induce oxidative stress in reproductive system of male mice.

Keywords: Aspartame, Oxidative stress, Mice

P-5: Reproductive Outcome in Infertile Men with Acephalic Spermatozoa Syndrome Referring to Royan Institute

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Background: Male infertility problems may be contributory to 30- 40% of infertile couples. Teratozoospermia is one of the main causes of male infertility. Acephalic spermatozoa syndrome is an apparently rare and severe type of teratozoospermia, and it is characterized by a predominance of headless spermatozoa. ART seems to be the main treatment for these patients to dominate infertility. The purpose of current study was evaluating the ART outcome in infertile men with acephalic spermatozoa syndrome referring to Royan institute.

Materials and Methods: To this end, we undertook a complete review of the available data on the ART outcomes of 18 infertile couples. We retrospectively evaluated the ART outcomes of patients with 25-90% acephalic spermatozoa. Semen analysis done based on WHO guidelines.

Results: From a total number of 18 couples, 9 couples underwent ART (2 IVF and 18 ICSI cycles). 135 oocytes, 91 injected oocytes and 24 embryo transfers (ETs) were included in the analysis representing. From these 20 cycles, in 5 cycles, oocytes were frozen, in one case, no embryo was formed. Totally, one pregnancy achieved (from a case with 36 % acephalic sperm) resulted to one healthy boy.

Conclusion: In conclusion, with the emergence of ICSI, births were possible, although results are still not completely satisfactory. Pregnancy rate with ICSI was low but when pregnancy occurred, embryonic development happened properly and led to the birth of a healthy baby. This study can be a hopeful plan in reproductive medicine to treatment patients with acephalic spermatozoa syndrome.

Keywords: Acephalic Spermatozoa Ayndrome, ART, Male Infertility

P-6: Humanin Levels Is a Suitable Indicator to Semen Quality via Participate in Regulating Oxidant/Antioxidant Balance

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Background: Humanin, a mitochondrial derived 24-amino acid peptide, prevents cell damage from stress/injury in different tissue/cells, including male germ cells. The current study was designed to investigate the correlation between Humanin and the qualitative and quantitative properties of seminal fluid, in parallel with the oxidant and antioxidant balance in the semen of patients with asthenozoospermia.

Materials and Methods: The current study, which was conducted for a year, involved 60 fertile and 60 asthenozoospermic subfertile men belonging to Hilla City, Iraq. Seminal fluid was obtained from the participants before and after treatment with zinc supplements. The Humanin, total antioxidant, total reactive oxygen species, and peroxiredoxin levels were assessed using the spermatozoa and seminal plasma samples.

Results: Humanin levels, total antioxidant status and peroxiredoxin levels were significantly lower while the concentration of reactive oxygen species were significantly higher in subfertile men than in healthy men.

Conclusion: Humanin levels is correlates with oxidant/antioxidant balance and linked to semen quality via.

Keywords: Humanin, Asthenozoospermia, Total Antioxidant Status, Peroxiredoxin, Reactive Oxygen Species

P-7: The Key Role of Vitamin E in Enhancement of Semen

Quality via Elevation Seminal Zinc Binding Proteins Levels in Iraqi Infertile Men

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Background: The current research was designed to investigate the effect of vitamin E supplementation on the quantitative and qualitative characteristics of semen along with Zinc Binding Protein levels in the seminal plasma in asthenozoospermic patients. Zinc in human seminal plasma is divided into three types of ligands which are low (LMW), intermediate (IMW), and high molecular weight ligands (HMW).

Materials and Methods: Semen samples were obtained from 60 fertile and 60 asthenozoospermic infertile men with matched age. The subfertile group was treated with vitamin E. Semen samples were collected (before and after vitamin E supplementation). After liquefaction seminal fluid at room temperature, routine semen analyses were performed. Sephadex G-75 and Sephadex G-25 was performed for determination of the amount of zinc binding proteins, the gel filtration of seminal plasma on. All the fractions were investigated for protein and for zinc concentration by atomic absorption spectrophotometry. Evaluation of chromatograms was made directly from the zinc concentration in each fraction.

Results: The mean value of semen volume, progressive sperm motility percentage and total normal sperm count were increased after vitamin E sulfate supplementation. A significant high molecular weight zinc binding ligands percentage (HMW-Zn %) was observed in seminal plasma of fertile males compared with subfertile males. However, seminal low molecular weight ligands (LMW-Zn) have opposite behavior.

Conclusion: Vitamin E supplementation elevates HMW-Zn% & LMW-Zn% in seminal plasma of asthenozoospermic subjects.

Keywords: Vitamin E, Asthenozoospermia, Zinc Binding Protein, Sephadex G-75, Sephadex G-25

P-8: The Effect of Misoprostol on Serum Level of Testosterone and Spermatogenesis Indices in Adult Male Mice

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Background: There is good evidence that men with a low prostaglandin E (PGE) concentration in their semen tend to be infertile and any drug which causes an increase in seminal PGE concentration might therefore be of use in the treatment of male infertility. Misoprostol is a synthetic PGE1 methyl analogue indicated for the prevention of gastric ulcers induced by non-steroidal anti-inflammatory drugs. This study was conducted to investigate the effect of misoprostol on serum level of testosterone and spermatogenesis indices in testes tissue of adult male mice.

Materials and Methods: In this study, 20 adult male mice were divided into 4 groups including: control and three treat-

ment groups which received 1, 10 and 100 µg/Kg/day for 40 consecutive days by oral gavage. At the end of experimental period, animals were anaesthetized and blood samples were directly taken from heart for measurement of testosterone and then testes were removed and fixed in 10% neutral buffered formalin. Tissues were processed by standard paraffin embedding and tissue sections were stained with Weigert's iron hematoxylin and then tubular differentiation index (TDI), spermiogenesis index (SPI) and repopulation index (RI) as spermatogenesis indices were analyzed by light microscopy. Finally, one way ANOVA and Tukey's post hoc were performed by SPSS software and differences were considered to be statistically significant when P<0.05.

Results: Results showed that low and mid doses of misoprostol could not change serum level of testosterone, TDI, SPI and RI significantly compare to control group. It was also indicated that however high dose of misoprostol could not significantly change the serum level of testosterone but it increased TDI, SPI and RI significantly compare to control group (P<0.01).

Conclusion: Based on our results it can be concluded that administration of misoprostol in high dose can improve male fertility by increasing spermatogenesis indices in germinal epithelium of seminiferous tubules.

Keywords: Misoprostol, Prostaglandin E, Testosterone, Spermatogenesis Indices, Testis

P-9: The Evaluation of Diazinon Effects on Sperm Maturation and Sperm DNA Denaturation in Infertile Men

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Background: Exposure to pesticide is one of the important reason for infertility. The aim of this study investigation of diazinon effects on the sperm maturation and sperm DNA denaturation in infertile men in Babol city

Materials and Methods: 90 adult males (18-52 years old) were divided in to three groups (n=30). Group 1 (infertile and Diazinon-exposed men), Group 2 (infertile and Diazinon-unexposed men) and Group 3 (fertile and Diazinon-unexposed men) as a control. Sperm maturation and sperm DNA denaturation were evaluated by aniline blue staining and acridin orange test respectively. Collection Data were analyzed by spss software (One way ANOVA test). Signification different at P<0.05.

Results: The percentage of immature sperms increased significantly in diazinon-exposed infertile group and the diazinon-unexposed infertility group compared to control group (p <0.000). Also, percentage of this parameter was higher in diazinon-exposed infertile group than diazinon-unexposed infertility group, but was not significant. The percentage of sperm DNA denaturation increased significantly in diazinon-exposed infertile group (P <0.000) and diazinon-unexposed infertile group (P <0.002) compared to control group, and the percentage of mentioned parameter was higher in the diazinon-exposed infer-

tility group than diazinon-unexposed infertility group, but was not significant.

Conclusion: The results of this study show that Diazinon significant effect on the sperm maturation, sperm DNA denaturation and fertility in men. Consequently it is necessary to restrict the use of Diazinon in a systematic program.

Keywords: Diazinon, Sperm Maturation, Sperm DNA Denaturation

P-10: Cross-Link between Epigenetic Modifications and Hsp70 Expression in Varicocele Condition after Testosterone Therapy

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Background: Epigenetic, with a specific and extensive role in genomic signaling and modifications, has gained interests in many diseases, especially varicocele (VCL). VCL is a well-known male fertility disorder, in which the oxidative stress and failed Hsp70 expression, in the sole and simultaneously, are known to result in germ and sperm cells DNA fragmentation that finally is able to end with temporal and/or complete infertility. Here in the present study, we tried to reveal the cross-link between nuclear DNA-related epigenetic changes and Hsp70-2 expression in varicocele-induced testes, after testosterone therapy.

Materials and Methods: For this purpose, 18 mature male Wistar rats were divided into three groups (n=6 for each group), including control (Con), experimental VCL-induced, and testosterone-treated VCL-induced (VCL+T). The Real-time PCR was used to analyze the expressions of DNMT1, HAT, and HDAC, as epigenetic markers, and Hsp70, as stress responder, in all groups. The germ and sperm cells DNA integrity was analyzed by DNA ladder test. Finally, the correlation between epigenetic markers and Hsp70-2 were analyzed.

Results: Our observations showed a significant (P<0.05) reduction in DNMT, HAT, HDAC, and Hsp70 in VCL group versus the control animals. However, the HAT, HDAC, DNMT1 and Hsp70 expression were remarkably increased in VCL+T group versus VCL animals. Moreover, the animals in VCL+T group represented ameliorated DNA fragmentation (at both germ and sperm cells) versus VCL-sole animals.

Conclusion: Observations revealed that testosterone, by boosting the Hsp70 expression, ameliorates the VCL-induced disintegrity. On the other hand, promoted Hsp70 expression, in turn, amplifies the epigenetic markers. Thus, the testosterone could stabilize and/or protect the DNA content by retaining the Hsp70 and epigenetic markers.

Keywords: Varicocele, HAT HDAC, DNMT1, Hsp70, Testosterone

P-11: Assessment of Oxidative Stress and Chromatin Status in Varicocele-Induced Rats

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Background: Varicocele (VCL), dilation of the veins of the pampiniform plexus within the spermatic cord, occurs more often in the left testicle. Etiologically of male infertility in these individuals is multifactorial. Several studies showed that increase in oxidative stress (OS) level could effect on quality of sperm and lead to low sperm concentration, and motility and high percentage of sperm abnormal sperm morphology and DNA damage. Therefore, the aim of this study was assessment of sperm lipid peroxidation, protamine deficiency, residual histone and DNA damage in VCL- induced rats.

Materials and Methods: 36 wistar male rats were used and rats were divided into three groups including VCL, control and Sham. Left VCL model were induced by partially ligating left kidney veins for the VCL group. After two months, rats from three groups were sacrificed and sperm functional tests including lipid peroxidation, protamine deficiency, residual histone and DNA damage evaluated by BODIPY (581/591) C11, Chromomycin A3, aniline blue and acridine orange, respectively, were assessed on epididymal sperm. All of the statistical analyses were carried out using the Statistical Program for Social Sciences (SPSS Inc., Version 25.0) and one-way ANOVA test was used for comparison of parameters between three groups. P < 0.05 was considered statistically significant.

Results: Mean percentage of sperm lipid peroxidation were significantly higher in VCL in comparison of control and sham groups (P<0.001). In addition, mean percentage of protamine deficiency (P<0.001), residual histone (P<0.01) and DNA damage (P<0.001) were significantly higher in VCL group in comparison of control and sham groups.

Conclusion: Testicular heat stress has harmful effects on testis and spermatogenesis via high production of oxidative stress.

Keywords: Varicocele, Chromatin Status, Sperm Parameters, Residual Histone, DNA Damage

P-12: Effects of Zinc Nanoparticles on The Testis of Wistar Rats

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Background: The purpose of this study was to investigate the effect of Zinc oxide nanoparticles on the reproductive system in the male Wistar's rat

Materials and Methods: 50 male Wistar rats weighing 220±20 g were divided into 5 groups of 10, including control group, sham, 25 mg/kg ZnO (IP), 50 mg/kg ZnO (IP), 100 mg/kg ZnO (IP) for twice a week. All stages of the test were performed in 4 weeks. The testes were evaluated by optical microscopy after tissue preparation steps.

Results: Histopathologic findings showed, zinc oxide nanoparticles in the testis, reduced cell population dose-dependent, respectively. In the group receiving 50 mg/kg, 100 mg/kg zinc oxide nanoparticles edema, Hyperemia and vacuolar degeneration were observed.

Conclusion: Due to these observations it could be exhibited that ZnO nanoparticles may have the potential to induce reproductive failure in Wistar rat.

Keywords: Zinc oxide, Testis, Rat, Nanoparticle

P-13: Sperm Telomere Length and Chromatin Status in Individuals with Varicocele

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Background: Varicocele is referred to as abnormal ventricular expansion of the testicles with incidence of 10-20% in male population oxidative stress are considered as an effective mediator for Varicocele associated infertility that it can effect on telomere length integrity and sperm chromatin. Considering that the level of oxidative stress is high in infertile men with Varicocele, the aim of this study was to compare the length of sperm telomere as a potential indicator for predicting genome integrity, along with assessing the status of sperm chromatin and lipid peroxidation between infertile men with varicoceles and fertile individuals.

Materials and Methods: This study was performed on 18 samples of male and female varicocele (II and III grade) and 20 fertile individuals. On a part of each semen sample, sperm motility, concentration and morphology were evaluated by using computer-aided sperm analysis (CASA), and on the remaining semen sample evaluation of protamine deficiency, DNA fragmentation, sperm lipid peroxidation, and sperm telomere length were performed by Chromomycin A3 staining, TUNEL assay, Bodipy probe and real time PCR, respectively.

Results: The results showed that the mean of sperm parameters including sperm count concentration, and sperm motility in infertile men with varicocele were significantly lower in comparison with fertile individuals. Also, the percentage of abnormal morphology was significantly higher in infertile men with Varicocele ($p < 0.05$). In addition, mean percentage of sperm lipid peroxidation, protamine deficiency and DNA fragmentation were significantly higher while mean of sperm telomere length was significantly lower in infertile men with varicocele compared to fertile men ($P < 0.05$).

Conclusion: The result of this study shows that infertile men with varicocele have telomere length less than fertile, which may be due to increased oxidative stress and its relevance to varicocele state. Therefore, by antioxidant therapy alone or in combination with varicoectomy could reduce the effects of oxidative stress on sperm telomere length and chromatin.

Keywords: Varicocele, Sperm Telomere Length, Infertility

P-14: A Histochemical Study of Testis after Experimentally Induced Varicocele in Rat

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Background: Varicocele is probably one of the most contro-

versial subjects in the area of male infertility. Varicocele is described as the dilation and tortuosity of plexus pampiniformis (especially in the left testis) and leads to some pathological problems in the testis tissue. The stasis of venous blood in the dilated pampiniform plexus impairs arterial blood flow and reduces oxygen supply to testis tissue which can lead to the testicular hypoxia and hyperthermia. Also, oxidative stress and heat stress is known as the main factors in the pathophysiology of varicocele. The adverse effect of such conditions on spermatogenesis can cause alterations in sperm concentration, motility, and morphology. Moreover, glycolysis impairment happens by limiting the glucose supply to the tissue. Therefore, the varicocele affects not only the physiology of testis but also influence the intracellular component. Hence, studies on the association of a varicocele with male infertility are still required to improve our understanding of etiology of varicocele and male infertility. In this study, we aimed to demonstrate the adverse effects of varicocele on testicular histopathology alterations.

Materials and Methods: A total of 30 Wistar rats were randomly divided into three groups: surgically induced left varicocele, sham-operated, and untreated controls. Two months after surgery, animals were euthanized with ether. Then left testes were collected, processed, and stained with Pas and Sudan Black. For the microscopic study, DinoLite digital lens and Dino Capture 2 Software were used and histological abnormalities were evaluated for each testis.

Results: The seminiferous tubules were surrounded by a thin, regular basement membrane. While the spermatogonia and spermatocytes were revealed with low cytoplasmic carbohydrate ratio and the thickened basement membrane in varicocele-induced rats. Also, our analyses have demonstrated that an increase was observed in the volume of lipid accumulation in spermatogenesis cell lineage of the varicocele-induced group in comparison with the control group.

Conclusion: Our results demonstrate that since normal spermatogenesis occurs through notable use of carbohydrates as the main source of energy, any disorder in glucose and hexose carbohydrate transport or metabolism can drive the cells to change principal energy from glucose to lipid. Accordingly, because of inadequate main energy sources, the cells with poor metabolisms undergo starvation and consequently cell death.

Keywords: Histochemistry, Varicocele, Testis, Rat, Seminefrous Tubules

P-15: Remarkable Sperm Aneuploidy in Oligoasthenozoospermic (OAT) Patients Detected by Array CGH and FISH

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Background: Embryonic aneuploidy could result in miscarriage and implantation failure, whilst contribution of sperm aneuploidy in sterility is not well defined. OAT is associated with lower pregnancy and implantation rates. High incidence of aneuploidy has been reported in OAT spermatozoa using fluorescence in situ hybridization (FISH), but limited number of analyzed chromosomes is an inherent disadvantage of FISH studies. Comprehensive aneuploidy screening has not been studied in spermatozoa of OAT patients.

Materials and Methods: A descriptive study including 5 infertile men with severe OAT according to WHO 2010 guidelines, aged 33-38 years and candidate for intracytoplasmic sperm injection were enrolled. Female partners were healthy ranged 26-35 years old. All the patients had normal blood karyotypes with 2-10.5 years of infertility and history of implantation failure. Patients with idiopathic infertility were included by exclusion of Y chromosome microdeletions, chemotherapy, exposure to toxicity, high temperature and sexual hormonal alterations. Illumina 24 sure platform array comparative genomic hybridization (aCGH) was employed for three samples of each patient, each sample containing two sperm. Depending on the aneuploidies found by aCGH, multicolor FISH was utilized to find the magnitude of the detected abnormalities in total population of spermatozoa.

Results: Aneuploidy was detected by aCGH in 66% of samples ranged 1-6 chromosomes per patient. The most frequent ones were chromosomes 8, 21(3 of 5 patients; 26.6% and 33.3% of samples, respectively). A range of 400-1286 sperm per patient were scored in FISH and 19 chromosomes were totally evaluated. The most frequent abnormalities were disomy of chromosomes 13, 20 and X/Y with mean rates of 7.3%, 4.9% and 5.2%, respectively and total aneuploidy range was 38.25-81.6%.

Conclusion: By aCGH, we observed abnormalities that hardly ever considered in sperm FISH studies, such as chromosomes 19 and 20. Considerable frequency of aneuploidies found by both aCGH and FISH techniques in OAT sperm cells, but aneuploidies of the single sperm seem to be independent of frequent aneuploidies in total sperm population. Regarding remarkable revealed aneuploidy, comprehensive preimplantation genetic screening (PGS) in OATs with idiopathic infertility specifically those with history of implantation failure is recommended.

Keywords: Sperm, Aneuploidy, Array CGH, FISH, Oligoastheno-teratozoospermia

P-16: The Effect Of Varicocelectomy on Sex Hormones Levels and Semen Parameters

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Background: Varicocelectomy is a type of surgery for varicocele treatment. This may recover fertility properties in males through improvement in semen parameters as well as sexual hormones. In this study, the role of varicocelectomy with focus on semen properties and sexual hormones have been assessed.

Materials and Methods: In this cross-sectional study, before-after data from fifteen patients with different varicocele grade, regarding to varicocelectomy have been compared.

Results: After varicocelectomy, a significant improvement in some semen parameters such as motility and concentration has been showed. However, among sexual hormones, only testos-

terone has been increased after surgical intervention (P=0.007).

Conclusion: Based on our knowledge, varicocele treatment may almost improve fertility potential in males significantly. Accordingly, it seems that varicocelectomy has positive effect on parameters such as testosterone and serum FSH levels as well as sperm count and motility. Therefore, it could be concluded that varicocelectomy is an effective approach to improve male fertility and sexual disorders.

Keywords: Varicocele, Varicocelectomy, Hormones, Infertility, Semen

Animal Biotechnology

P-17: Detrimental Effects of Oxidative Stress on The Ovine Epididymal Spermatozoa Can Be Prevented by The Extract of Iranian Wild Celery

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Background: Spermatozoa are susceptible to oxidative stress due to the high content of polyunsaturated fatty acids and the deficiency of intracellular antioxidant system. Iranian indigenous wild celery (*Kelussia odoratissima* Mozzaf) is a medicinal herb that belongs to the family of Umbelliferae and grows at high altitudes (more than 2000 m). Antioxidant potential of *Kelussia odoratissima* Mozzaf is comparable to α -tocopherol and butylated hydroxytoluene when assayed by various methods. The aim of this study was to evaluate the effects of aqueous extract of *Kelussia odoratissima* Mozzaf on ovine epididymal spermatozoa exposed to *in vitro* oxidative stress.

Materials and Methods: Testes of sexually mature rams were collected in a local abattoir and transported to the laboratory. In the laboratory, epididymal spermatozoa were recovered from the cauda epididymis and re-suspended in TCFE extender (Tris, citric acid, fructose, egg yolk). Spermatozoa at the concentration of 10×10^6 sperm/ml were incubated at 37°C in the presence of 100 μ M H₂O₂, 100 μ M H₂O₂ + 0.01% (w/v) wild celery extract or in the absence of them (control) in the TCFE extender for 30 min. Motility of fresh sperm and incubated sperms were analyzed by a computer-assisted sperm analysis system (CASA). Each treatment was replicated 5 times and the data of motility parameters were analyzed by One-way ANOVA followed by the Tukey's post-hoc test using IBM SPSS 20. Differences at the level of P<0.05 were considered significant.

Results: Incubation of sperms with H₂O₂ caused a significant decrease in the total and progressive motility (42.8 ± 1.74 and $32.6 \pm 3.16\%$ respectively) compared with the control (74 ± 4.15 and $68.4 \pm 6.60\%$) and fresh groups ($78.2 \pm 1.77\%$ and $73.7 \pm 1.78\%$). Co-incubation of spermatozoa with H₂O₂ and the extract significantly prevented this effect ($62.6 \pm 2.40\%$ and $51.5 \pm 1.98\%$).

Conclusion: This study showed that aqueous extract of *Kelussia odoratissima* Mozzaf could prevent detrimental effects of oxidative stress on the ovine epididymal spermatozoa.

Keywords: Wild celery, Sperm motility, H₂O₂, Oxidative stress, Epididymal Sperm

P-18 : Protective Effect of The Aqueous Extract of Wild Celery Against The Detrimental Effects of Incubation on The

Motility of Ovine Epididymal Spermatozoa

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Background: It has been shown that *in vitro* spermatozoa processing and incubation may lead to a progressive reduction in the motility and DNA integrity of them. A reason that has been proposed for this phenomenon is the deleterious effects of excessive ROS production during the incubation period. Spermatozoa are susceptible to the oxidative stress due to the high content of polyunsaturated fatty acids and the deficiency of intracellular antioxidant system. Iranian indigenous wild celery (*Kelussia odoratissima* Mozzaf) is a medicinal herb that belongs to the family of Umbelliferae and grows at high altitudes (more than 2000 m). Antioxidant potential of *Kelussia odoratissima* Mozzaf is comparable to α -tocopherol and butylated hydroxytoluene when assayed by various methods. The aim of this study was to evaluate the effects of aqueous extract of *Kelussia odoratissima* Mozzaf on ovine epididymal spermatozoa incubated at 37°C for 4 hours.

Materials and Methods: Testes of sexually mature rams were collected in a local abattoir and transported to the laboratory. In the laboratory, epididymal spermatozoa were recovered from the cauda epididymis and re-suspended in TCFE extender (Tris, citric acid, fructose, egg yolk). Spermatozoa at the concentration of 10×10^6 sperm/ml were incubated at 37°C in the presence or absence of 0.01% (w/v) wild celery aqueous extract. Motility of fresh and incubated spermatozoa was analyzed by a computer-assisted sperm analysis system (CASA). Each treatment was replicated 5 times and the data of motility parameters were analyzed by One-way ANOVA followed by the Tukey's post-hoc test using IBM SPSS 20. Differences at the level of $P < 0.05$ were considered significant.

Results: Incubation of spermatozoa at 37°C caused a significant decrease in the total and progressive motility (56.2 ± 3.26 and $47.4 \pm 5.6\%$ respectively) compared with extract (72.3 ± 2.94 and $67.2 \pm 3.38\%$) and fresh groups ($78.2 \pm 1.77\%$ and $73.7 \pm 1.78\%$).

Conclusion: This study showed that aqueous extract of *Kelussia odoratissima* Mozzaf could prevent detrimental effects of *in vitro* incubation of ovine epididymal spermatozoa.

Keywords: Wild Celery, Sperm Motility, *In Vitro* Aging, Oxidative Stress, Antioxidant

P-19: Positive Correlation of The Post-thaw Motility with the Motility following A Short Oxidative Challenge in Ram Epididymal Spermatozoa

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Background: The spermatozoa of different donors have different freezability. The aim of the present study was investigating the possible underlying causes of this difference. Moreover, finding a criterion that could predict the freezability of different semen samples was another aim of this study.

Materials and Methods: Testes of sexually mature rams were collected in a local abattoir and transported to the laboratory. In the laboratory, epididymal spermatozoa were recovered from

the cauda epididymis and re-suspended in TCFE extender (Tris, citric acid, fructose, egg yolk). After motility assessment using a computer-assisted sperm analysis system (CASA), a sample of recovered spermatozoa were diluted in the TCFE extender (1% egg yolk) at 3×10^7 sperm/ml and then $50 \mu\text{M H}_2\text{O}_2$ was added to the suspension. After that, the sperm suspensions were incubated at 37°C. After 30 minutes, the motility of incubated samples was analyzed. At the same time, the recovered spermatozoa were undergone freezing process. At least one week after freezing, the frozen samples were thawed and their motility analyzed. This experiment was performed in 10 replicates (10 different testes). Finally, by using IBM SPSS 20, the Pearson's correlation between the post-thaw (PT) motility parameters and post-oxidative challenge (POC) motility parameters were calculated.

Results: There was a correlation between PT total motility and POC total motility ($r=0.757$, $n=10$, $P < 0.001$). Moreover, there was a correlation between PT progressive motility and POC total motility ($r=0.775$, $n=10$, $P=0.024$).

Conclusion: The results of the present study showed that freezability of spermatozoa might be related to their sensitivity to the oxidative stress. Furthermore, the degree of sensitivity to a mild oxidative challenge might predict the freezability of, at least, ovine epididymal spermatozoa.

Keywords: Oxidative stress, Cryosurvival, Spermatozoa,

P-20: The Effects of Ethylenediaminetetraacetic Acid (EDTA) on Post-thawed Rooster Sperm Quality in Modified Beltsville Extender

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Background: Semen freezing and thawing procedures not only is associated with serious damage to sperm but also changes the membrane permeability to some ions such as calcium. Calcium plays a substantial role in sperm motility and membrane fusion process during the acrosome reaction. The aim of this study was to evaluate the effects of different concentrations of EDTA as a calcium chelator in semen extender on sperm quality factors after thawing.

Materials and Methods: Semen samples were collected from eight mature roosters (Ross 308). After initial semen assessments, samples with adequate quality were mixed together and diluted with modified Beltsville extender without EDTA (control) and supplemented with 1, 2, and 3 μM EDTA. After thawing, sperm viability and motility were measured by Eosin-Nigrosine and Computer-Aided Sperm Analysis (CASA), respectively. The data were analyzed by the GLM procedure of SAS 9.1.

Results: The results of this study showed that the level of 2 μM EDTA (53%) significantly increased sperm motility in compared with control (45.5%) and other levels of EDTA ($P < 0.05$). But the other levels of EDTA had no significant effect on sperm motility in compared with control. Using 2 μM EDTA (55.5%) significantly increased sperm viability compared to the control group (50.25%) and 3 μM EDTA (51%) ($P < 0.05$). Using EDTA at 1 μM , 2 μM , and 3 μM significantly increased sperm progressive motility (27.5%, 30.75%, and 25.5%, respectively; ($P < 0.05$)) compared to the control (20.75%).

Conclusion: Results of this study revealed that addition of 2 μM EDTA to the extender for freezing of rooster semen can im-

prove significantly the function of rooster sperm after freezing-thawing process.

Keywords: Rooster, EDTA, Sperm motility, Freezing

P-21: The Effect of Osmotic Stress on Developmental Competence of *In Vitro* Matured Bovine Oocytes

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Background: Increasing the permeability of the oocyte membrane can increase its ability to cryopreservation. Induction of sublethal controlled osmotic stress can improve the oocyte freezability through the increase the expression of genes associated with permeability of cell membranes such as aquaporins. Therefore, it was evaluated to establish whether the developmental capacity of the oocyte is affected by osmotic stress or not.

Materials and Methods: Bovine immature aspirated oocytes from abattoir-derived ovaries were initially cultured in isotonic IVM medium (bicarbonate-TCM 199 supplemented with 10% FCS and 0.1IU/ml FSH) (osmolality was 280-285mOsm) for 1 hour adaptation. After that, the oocytes of treatment group were exposed to hyperosmotic medium (500-510mOsm IVM medium contained sorbitol) for 4 hours and then transferred to IVM medium. All of the maturation procedure was carried out at 38.5°C with a 5% CO₂/air atmosphere for 22-24 hours. *In vitro* matured oocytes were exposed to motile epididymal sperm in TALP medium for 22-24 hours. After fertilization, denuded presumptive zygotes were cultured in SOFaaBSA medium in 5% CO₂, 7% O₂, and 88% N₂ for 9 days.

Results: The results showed that exposure of bovine oocytes to high osmolality had not any effect on developmental competence of osmotic stress-treated oocytes. Hyperosmotic effects resulted in 0.04±0.02 oocyte degeneration, 67.9 ± 7.36 cleavage rate, and 23.61 ± 2.79 blastocyst rate versus 0.03 ± 0.02, 72.8 ± 4.28, and 21.56 ± 3.15 for control and treatment groups, respectively) (P>0.05).

Conclusion: The results of the present study demonstrated that oocyte is resistant to controlled osmotic stress even for up to 4h in maturation procedure. Understanding this resistance will allow the development of improved assisted reproduction methods such as cryopreservation for CPA addition and removal procedures during mammalian oocyte vitrification.

Keywords: Osmotic Stress, Development, Bovine, Oocytes, Embryo

P-22: Effects of Dietary Fish Oil as Source of Omega 3 Fatty Acids on The Quality of Cryopreserved Semen in Rooster

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Background: Rooster semen is sensitive to cryopreservation which limits preservation of semen in frozen states. Polyunsaturated fatty acids (PUFAs) of the omega-3 are important for semen membrane integrity, sperm motility and viability. The purpose of this study was to evaluate the effects of dietary fish

oil on the quality indicators of rooster semen after cryopreservation.

Materials and Methods: Eighteen broiler breeder roosters were randomly assigned into three experimental treatments as following: 1) control group received a basal diet, 2) fish oil group (FO) received a basal diet supplemented with 0.5% fish oil and 3) fish oil group received a basal diet supplemented with 2% fish oil. Semen sample were collected at days of 0,15,30,45 and 60 of post feeding. The obtained semen cryopreserved according to standard protocol and thawed for evaluation. Motion characteristics, viability and morphology of sperm were measured after thawing.

Results: Dietary FO had significant positive effects on motility and viability of sperm compared to the control group (P<0.05). Adding 0.05 % FO significantly increased the percentage of total motility and viability (P<0.05) Morphology of sperm was not significantly affected by the FO (P≥0.05).

Conclusion: It can be concluded that dietary n-3 fatty acids before cryopreservation increases the cryotolerance of rooster semen which could be a suitable strategy for better preservation of semen for long time.

Keywords: Cryopreservation, Unsaturated Fatty Acid, Docosahexenoic Acid, Apoptosis

P-23: Dietary Supplementation of Fish Oil Improves The Semen Parameters in Aged Broiler Breeder Roosters

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Background: Reproductive performance is an important factor in breeder poultry production. Between different factors, Age is one of the important issue affecting fertility of breeder's roosters. While fertility performance in rooster peaks around 32 weeks of age, it significantly reduces from 45 weeks onward. Polyunsaturated fatty acids (PUFAs) play crucial roles in biological functions related to reproduction and fertility. Dietary omega-3 fatty acids (FA) have a great impact on the reproductive performance. The purpose of this study was to assess the effects of dietary fish oil (n-3) on the semen parameters and reproductive performance of aged broiler breeder roosters.

Materials and Methods: Twenty-four Ross 308 roosters from 30 to 45 weeks (eight roosters per treatment) were assigned into three treatments: 1) basal diet as control group, 2) basal diet supplemented 0.5% fish oil, and 3) basal diet supplemented with 1.5% fish oil. All the diets protected with vitamin E as an antioxidant. Roosters were fed their diets for 105 days. Semen volume, sperm concentration, total motility, progressive motility and viability were assessed biweekly.

Results: The highest percentage of total and progressive motility were obtained in group with 0.5% fish oil (P<0.05). Viability, semen volume and sperm concentration were not significantly affected by the dietary fish oil (P<0.05).

Conclusion: It can be concluded that dietary fish oil improves rooster sperm parameters and reproductive performance in aged broiler breeder roosters. This strategy could be a suitable method to preserve the fertility performance.

Keywords: Sperm, Fertility, PUFA, Omega-3 Fatty Acids

P-24: The Mitochondria Uncoupling Protein (UCP2) Is Involved in Cryopreservation of Bull Sperm Exposed to Sublethal Oxidative Stress

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Background: Cryopreservation of bull sperm associated with an increase in reactive oxygen species (ROS) which lead to induce lipid peroxidation of sperm membrane. During the freeze-thaw process, the sperm is subjected to biochemical, osmotic, physical stress in which fertility potential of thawed semen is reduced. Mitochondria is known to be an important organelle of sperm that regulates the antioxidant response during cryopreservation. The Mitochondria Uncoupling protein (UCP2) is a key protein that regulates the antioxidant activates of sperm in freeze-thaw process. The purpose of this study was to evaluate the effects of cryopreservation on the UCP2 protein of bull sperm and it's associated with antioxidant systems, motility, mitochondria activity, apoptosis status and ROS concentration. **Materials and Methods:** Bull semen was diluted in extenders containing different concentrations of Xanthine Oxidase (XO) as activator of XO and then cryopreserved. Several quality indicators of sperm were compared in cryopreserved semen in different rather than fresh semen.

Results: The results showed that UCP2, membrane potential, viability, motility and membrane integrity were negatively affected by the cryopreservation ($P < 0.05$). The reactive oxygen species (ROS) and lipid peroxidation and were significantly after cryopreservation ($P < 0.05$). Sublethal concentration of Xanthine Oxidase (XO) increased the several quality indicators of thawed semen ($P < 0.01$). Also, XO improved the membrane integrity and motility of sperm after cryopreservation ($P < 0.05$). **Conclusion:** It can be concluded that due to freezing process, UCP2 reduced post cryopreservation that may be responsible for the lower antioxidant systems and consequently lower quality of sperm post-thawing. Sublethal concentration of XO can partially restore these negative impacts.

Keywords: Apoptosis, Artificial Insemination, Semen

Embryology

P-25: Effect of Myoinositol Supplement on The Quality of Frozen- Thawed Human Sperm on Patients with Oligoasthenoteratozoospermia Syndrome

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Background: Oligoasthenoteratozoospermic syndrome is common presentation in male infertility. this syndrome occurs by decreasing motility, numbers and morphologic changes sperm. The production of oxygen species increases, in the semen ab-

normal. then it may affect motility, morphology and DNA stability of sperm. This study aimed at evaluating the effect of Myoinositol in human semen parameters and DNA fragmentation patients with syndrome after thawing procedure.

Materials and Methods: Semen samples were obtained from 40 oligoasthenoteratozoospermia patients, aged 28-45, which have been referred to IVF center of Akbar Abadi hospital. Semen samples were collected through masturbation into sterile containers, following 2-4 days sexual abstinence. All the samples after by CASA were immediately in two groups, group 1: (2 mg per ml) + freezing medium (Life Global), group 2: freezing medium. Samples were thawed. Semen parameters in two groups were analyzed by CASA for motility and morphology. Also, the level of total ROS, TAC, DNA fragmentation and MDA were evaluated by DCFHby Fluorimetry), ELISA kit, TUNEL assay by flow cytometry and ELISA respectively.

Results: Our data clearly showed that total, progressive motility were higher in the experimental group. Also, DNA fragmentation is lower in myoinositol than control (22.4% vs. 29.6%, $p > 0.01$). TAC is higher in myoinositol than control (1.12mM vs. 0.95 mM, $P > 0.05$) and MDA is significantly decreased in myoinositol than control (2.1 nmol vs. 2.3 nmol, $P < 0.05$). The level of ROS was decreased in myoinositol group but not significant (179.4mM vs. 200.8mM, $P < 0.05$).

Conclusion: These data suggest that improves sperm parameters through the mitochondrial mechanism and it can play a protective role sperm DNA and increases TAC capacity. So, This study showed that can be used as a supplement in sperm freezing process in patients with Oligoasthenoteratozoospermia syndrome.

Keywords: Myoinositol, Sperm Cryopreservation, DNA Damage, Oligoasthenoteratozoospermi Syndrome

P-26: Increasing Pregnancy Rate in Mice Using Vaginal Cytology in Different Phases of Estrus Cycle in Mice

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Background: Determining different stages of the estrus cycle is an effective method for tracking various stages of the cycle, as well as selection of mature mice that are ready for pregnancy or pseudo-pregnancy. Determining different phases of the estrus cycle is possible by examining the changes in vaginal smear epithelial cells, which is not only quick and inexpensive, but also one of the most accurate methods for identifying various stages of mouse estrus cycle. The stages of estrus period were determined according to the proportions and morphology of leukocytes and epithelial cells. In the proestrus dominance stage only nucleated cells are observed. The estrus phase consists of the Cornified cells. The next stage, Metestrus, represent the same percentage of Cornified cells, nucleated cells and leukocytes. Finally, in the diestrus stage leukocytes are dominant.

Materials and Methods: In order to prepare sterile specimens from mouse vagina, physiological sterile sodium chloride (0.9% NaCl) was used and the samples were stained using 0.1% crystal violet dye. After vaginal smear and determination of estrus cycle stages, the mice were mated individually for with vasectomized male mice overnight, and the mice vaginal plaque

was observed and recorded the following morning.

Results: 30 ten weeks old mice were selected, of which 11 mice were in the Proestrus and Estrus cycle. Of these 11 mice, 81.8% were positive for plaque and ready for mating. The rest mice were in the Metestrus and Diestrus cycle, of which only 2 (10.5%) mice showed positive plaque, i.e. ready for mating.

Conclusion: Mice that are in the Proestrus and Estrus stages are ready to mate and after mating, pregnancy or pseudopregnancy is achievable. Therefore, the proestrus and estrus cycle, can be considered as the best time for embryo transfer to recipient mice.

Keywords: Pregnancy, Mice, Vaginal Cytology, Estrus Cycle

P-27: Protective Effects of Silymarin on Spermatogenesis Parameters following Testicular Torsion-Detorsion in Mouse

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Background: Testicular torsion (TT) is an emergency and acute event that occurs most often in infants and adolescents, and if not treated promptly, it often leads to infertility. Detorsion results the blood reperfusion and ultimately more damage occurs to the testicle. Silymarin is a polyphenolic flavonoid that plays an important role in the improvement of many diseases due to its antioxidant properties. The aim of this study was to investigate the histological changes of the testis in mice that are affected by unilateral testicular Torsion-Detorsion (TD), and also to evaluate the protective effects of silymarin on the damage induced by TD.

Materials and Methods: In this study, 32 adult male NMRI mice were randomly divided into four groups. Group 1: control sham, Group 2: silymarin, in two groups without testicular torsion application, Group 3: Torsion Detorsion (TD) and Group 4: TD plus silymarin (TD+S). The testicular torsion was performed by rotating the 720 ° spermatic cord the left testis in a counterclockwise direction. After 1 hour, with a rotation opposite to the previous direction, Detorsion was done. After the detorsion, the animals received 50 mg / kg of silymarin via gavage for 35 days. At the end of experimental, all of the left testicles were removed. After fixation in 10% formal saline, paraffin sections were prepared and stained with hematoxylin-eosin. Histomorphometrical studies were performed to evaluate the spermatogenesis process. Data were analyzed by ANOVA and post hoc Tukey test (P <0.05).

Results: The mean number of spermatogonia, primary spermatocytes, rounded and elongated Spermatide in TD groups were reduced in comparison to the other groups. Silymarin in TD+S group significantly ameliorated spermatogenesis parameters compared to the TD group (P<0.05).

Conclusion: The results showed that spermatogenesis parameters improved with the administration of silymarin. This may be due to the antioxidant effects of silymarin.

Keywords: Silymarin, Spermatogenesis, Torsion, Testis

P-28: Beneficial Effect of Platelet-Rich-Plasma on Testicular Ischemia-Reperfusion Injury in Adult Mice

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Background: Testicular Torsion (TT) is a common syndrome that could lead to function loss and infertility in the testis. Ischemia due to testicular torsion and reperfusion related to the detorsion can cause different morphological changes in testicular tissue. In addition, reperfusion induces increased tissue damage after ischemia. Therefore, testicular ischemia and subsequent reperfusion (I/R) can lead to testicular tissue damage. Platelet Rich-Plasma (PRP) may has therapeutic effect due to the presence of growth factors. In this study, PRP was used to evaluate its effects.

Materials and Methods: Thirty adult male NMRI mice were randomly divided into three equal groups. The first group, is the healthy control, without testicular twisting. Group II: ischemia - reperfusion (I/R) + Pbs (phosphate-buffer-solution) group, Group III: The I/R + PRP group. In these two groups spermatic cord were twisted for one hour, and after detorsion, Pbs and PRP were injected into the rete testis, respectively. 35 days after surgery, left testis sampled for histomorphometrical examinations. After fixation in 10% formal saline, paraffin sections were prepared and stained with hematoxylin-eosin. Obtained data were statistically compared by ANOVA and post hoc-Tukey test (P <0.05).

Results: Cross-sectional area, the number of tubules and numerical density of the seminiferous tubules were significantly decreased in the I/R-Pbs group compared to the other groups (P<0.05). I/R caused a significant increase in interstitial space of testicular tissue in I/R group compared to the control and I/R+ PRP groups (P<0.05). PRP could reduce these tissue changes compared to the I/R group (P<0.05).

Conclusion: PRP is effective for the prevention of testicular torsion damage in mice testis. It seems that, PRP due to possession ample of growth factors, capable to relatively improve the undesirable effects of I/R.

Keywords: Ischemia, Reperfusion, Platelet-Rich-Plasma, Testis, Torsion

P-29: Positive Effects of Bioactive Peptides on Sperm Viability and Motility in Male Rate Treated with Cimetidine

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Background: Cimetidine is widely used for digestive tract ulcers but induces testis injury. Bioactive peptides are extracted from protein sources and a number of studies have suggested as a beneficial agent during peroxidative damage. In this study positive effects of Bioactive peptides on sperm viability and motility in male rate treated with cimetidine was investigated.

Materials and Methods: Forty-two male rats (165±20 g) were randomly divided into six groups of seven animals and were fed with treatments (Control, peptides 20 mg/kg, cimetidine 40mg/kg, cimetidine 120mg/kg, peptides+ 40 mg/kg cimetidine, peptides + 120 mg/kg cimetidine) for 6 weeks. At the end of the experiment, all the animals were anesthetized with chloroform, the vasa deferens separated. The eosin-negrosine dyeing and

Neubauer lams were used to determine sperm viability and motility respectively.

Results: The results showed that dietary treatments significantly affected on viability of sperm and the highest value was observed in the animals fed bioactive peptides treatments which had significant difference with cimetidine treated rats ($P < 0.05$). No significant difference was seen in sperm motility in animals ($P > 0.05$).

Conclusion: Based on our results, boactive peptides extracted from marine sources improve viability of sperm in cimetidine treated rats but did not affect the sperm motility. Aforementioned results can be explained according to bioactive peptides antioxidant activity which reported in several references.

Keywords: Cimetidine, Bioactive Peptides, Reproduction

P-30: Evaluation of Sperm Abnormal Morphology in Male Rats Induced by Varicocele

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Background: Varicocele refers to the abnormal inflation of spermatic veins in the scrotum. Varicocele is a common cause of infertility in men who are clinically associated with sperm abnormal morphology. So, this study aimed to evaluation sperm abnormal morphology in male rats induced with varicocele experimentally.

Materials and Methods: In this study, 30 adult male Wistar rats were divided into three groups, I: varicocele-induced II: sham and III: control. After two months of varicocele induction, rats were sacrificed and epididymides were dissected. Then, Sperm abnormal morphology assessed by WHO protocol and eosin-nigrosine test. Differences within groups were compared by one-way analyses of variance (ANOVA) using a post hoc test (Tukey). Collected data were presented as mean \pm standard error of mean (SEM) and $P < 0.05$ was considered to be significant.

Results: The result of this study showed that mean Sperm abnormal morphology were significantly higher in varicocele induction group compared to control and sham groups ($P < 0.01$). The deformity of tail and head of sperm in varicocele rats compared with sham and control rats showed a significant increase of ($P < 0.05$). However, that sperm neck deformity in varicocele groups increased but did not show significant difference.

Conclusion: In this study, by evaluating sperm parameters between groups, it was shown that the abnormal morphology of sperm in varicocele rats significantly increased, which was consistent with previous studies. Also, respectively highest sperm anomalies were the sperm tail and head, and then sperms that had been defeated from the neck was in the next rank.

Keywords: Varicocele, Sperm Parameters, Sperm Abnormal Morphology, Eosin - Necrosin Test

P-31: The Comparison of α -Tubulin Protein Expression in Normospermic and Oligoasthenoteratospermia Semen Samples

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Background: The centrosome is considered to be the microtubule organizing center and composed meshwork of proteins including α -tubulin. Sperm centrosome is located in the midpiece of the tail. It is believed that α -tubulin protein levels have been disturbed in oligoasthenoteratospermia. Hence this study was carried out to evaluate α -tubulin protein in normozoospermic and ligoasthenoteratospermic semen samples.

Materials and Methods: The semen samples were collected from 20 normozoospermic and 20 ligoasthenoteratospermic men who underwent seminal fluid evaluation at the Royan Institute. The conventional sperm parameters were assessed by computer-assisted sperm analyzer. The expression of α -tubulin protein were analyzed by Western Blotting. All values were expressed as mean \pm SD.

Results: The levels of α -tubulin are markedly lower in oligoasthenoteratospermic samples (0.59 ± 0.14) as compared to the normozoospermic samples (0.84 ± 0.32).

Conclusion: Lower α -tubulin protein expression in sperm of infertile males may be a possible cause for their reduced fertilization ability. Further studies on centrosomal protein are required to design rational approaches for the diagnosis and treatment of male infertility.

Keywords: Centrosome, α -Tubulin, Oligoasthenoteratospermia

P-32: Evaluation of Histological and Histochemical Alterations in Testis Tissue Following Long-term Oral Administration of Aspartame in Mice

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Background: Aspartame is the most famous consuming artificial sweetener that widely used in foodstuffs. Many experimental studies have reported toxicity of long-term administration of aspartame in different organ tissues, whereas little evidence is available on adverse effects of long-term consumption of aspartame on reproductive system. The present study has been conducted in order to evaluation of the effects of aspartame on histological and histochemical parameters in mice.

Materials and Methods: The adult male mice were randomly divided into four groups of nine each. In three groups, aspartame was administered orally with the doses of 40, 80 and 160 mg/kg.BW respectively for 90 days by gavage. Also a control group was considered. 24 hours after the last treatment, tissue samples were taken and used for histological and histochemical evaluations (Masson's trichrome, Alkaline phosphatase, Oil red O, Sudan black B and Periodic acid Schiff stains).

Results: Microscopic analyses revealed that long-term administration of aspartame increased thickness of tunica albuginea, edema in subcapsular and interstitial connective tissues, and

atrophied seminiferous tubules, arrested spermatogenesis, decreased Leydig cells/mm² of interstitial tissue, and causes hypertrophy and cytoplasmic granulation of Leydig cells. The carbohydrate ratio was reduced in first three layers of the germinal epithelium (GE) cytoplasm. The upper layers of the GE series were manifested with low rate of lipid accumulation in cytoplasm, while the cells which were located in first layers were revealed with higher amount of lipid foci. Also, histochemical changes were observed in testis tissue of 80 and 160 mg/kg groups.

Conclusion: The current study indicated that long-term oral administration of aspartame causes histological and histochemical adverse effects on the testicular tissue which potentially can lead to infertility.

Keywords: Aspartame, Histology, Histochemistry, Mice

P-33: PRKAR2B Expression at Different Times After HCG-Induced Ovulation in NMRI Mice

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Background: Oocyte maturation and resumption of meiotic occur by induction of hCG (Human chorionic gonadotropin) that hormone functions instead of the LH (Luteinizing Hormone). Diminishing The cAMP (Cyclic Adenosine Monophosphate) level will control the phosphorylation/dephosphorylation of cAMP-PKA effective for the activation of MPF (Maturation Promoting Factor) and the activation of MPF causes resumption of meiotic in immature oocytes. So the mechanisms cAMP-PKA regulate the meiotic status of oocytes. PRKAR2B (cAMP-dependent type 2 regulatory subunit beta) is expressed in ovarian follicles as well as other tissues such as white and brown adipose and brain. This gene produces PRKAR2B, one of the PKA members. Since hCG plays an important role in ovulation induction and according to the possible role of PRKAR2B gene in ovulation, the effect of hCG injection on the expression of PRKAR2B gene in MII oocytes was investigated. The real function of in the resumption of meiotic is unclear still.

Materials and Methods: In this study, female mice were randomly assigned to four treatment and control groups. In each group, all mice were under ovarian stimulation by 10 IU PMSG (Pregnant Mare's Serum Gonadotropin). After 48 hours, they were induced by hCG 10 IU due to ovulation. Mice were sacrificed by spinal dislocation at the hours 16, 20, 24 and 28 for the released oocytes and gene expression of PRKAR2B after ovulation induction.

Results: The lower number of MII oocytes at hour 28 in comparison with those of other hours and PRKAR2B expression in the hour 24 group was significantly higher than those of other groups were seen while the expression was significantly lowest at hour 28.

Conclusion: We could conclude this relation between PRKAR2B and the rate of cell proliferation. In respect to the results of this study seems that the lower expression of PRKAR2B will result in the less yielded MII oocytes.

Keywords: HCG, Ovulation, PRKAR2B, Mouse

P-34: The Effect of Cult-Active Medium on Fertilization and Pregnancy Rates in Couples with Azoospermia Male Factor

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Background: To evaluate efficiency of cult active medium on fertilization and cleavage rates, embryo development, and pregnancy rate after intracytoplasmic sperm injection (ICSI) in obstructive azoospermia patients.

Materials and Methods: This study was done on 157 ICSI cycles (stimulated with standard long protocol). After oocyte collection, the oocytes were randomly divided into two groups: control and artificial oocyte activation (AOA). The injected oocytes in the control group were cultured in cleave. The remaining oocytes were chemically activated by exposure to 200 µl cult-active medium for 15 minutes. Around 16 to 18 hours after ICSI, fertilization was assessed. The percentage of cleavage and embryo quality were calculated 72 hours after ICSI. Pregnancy rate was determined by Biochemical experiment.

Results: There are significant differences in the fertilization and cleavage rates after Cult-active used (P<0.05). also the pregnancy rate significantly increased (P<0.05). embryo quality no difference between ICSI AOA and control groups (P>0.05)

Conclusion: The findings showed that Cult-active treatment may fertilization and cleavage rates, which in turn, affect the implantation and pregnancy rate.

Keywords: Cult-Active, Obstructive Azoospermia, Failed Fertilization, Oocyte Activation, ICSI

P-35: Human Placenta Decellularized Matrix as A Scaffold for Use in Proliferation of Spermatogonial Stem Cells

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Background: Extracellular matrix (ECM) of tissues contains various ranges of growth factors, proteins, proteoglycans, hyaluronic acid and others. Decellularization of tissue and production of tissue engineering scaffolds from ECM components is one of the most reliable strategies in fabrication of scaffolds. Although various protocols have been developed to remove the cells from tissues with minimal degradation of ECM components, an optimal protocol is still needed to satisfy scientists. In this study, we examined various procedures for decellularization of placenta and optimized a reliable decellularization protocol.

Materials and Methods: Human placenta was obtained from mothers undergoing cesarean after obtaining informed consent. The tissues were treated with various contents of Triton X-100 and sodium dodecyl sulphate (SDS) for 15 or 30 minutes. The decellularized tissues were casted, freeze and freeze dried to fabricate a porous scaffold. The scaffolds were then cross-linked by glutaraldehyde gas. The removal of the cells from tissues was determined by H&E and 4', 6-diamidino-2-phenylin-

dole (DAPI) staining, and DNA content assay. Alcian blue, Masson's trichrome and Orcein staining was used to confirm that ECM remained intact after decellularization process. Morphology of porous scaffold was viewed under scanning electron microscopy (SEM). Cell viability and cell adhesion property of scaffold were determined by MTT and SEM.

Results: Histological analysis showed that the groups content %0.5 SDS and SDS+ Triton X-100 for 30 minutes were completely decellularized. Decellularization was further confirmed by DAPI staining and DNA content assay. Laminin, collaegen, elastin and glycosaminoglycans remained intact after decellularization process. MTT test showed cell viability changes for %0.5 SDS group. SDS+ Triton X-100 for 30 minutes have no significant difference with control group ($P>0.05$).

Conclusion: Our study proved a reliable and effective protocol for decellularization of placenta with minimal negative effects on ECM components. The decellularized placenta is suggested as a promising bio-scaffold tissue engineering applications.

Keywords: Decellularized Scaffold, Placenta, Extracellular matrix

P-36: Effects of Myo-inositol on Sperm Parameters and DNA Integrity in Asthenozospermia after Cryopreservation

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Background: Human semen cryopreservation considered as indispensable part of assisted reproductive centers, andrology laboratories and sperm bank. Despite long history of semen cryopreservation, post thawing survival rate is still limited and fails to meet the ideal expectation. Accordingly, cryoprotective media are commonly supplemented with antioxidants and it has been shown to improve cryo-survival post thawing. Therefore, the aim of this of this study was to evaluate effect of myo-inositol during human semen cryopreservation.

Materials and Methods: A total of 20 semen samples were collected from men with asthenozospermia parameters attended the Andrology Unit of the Qom Fertility and Infertility Center. Each semen sample was divided into two equal aliquots. The two identical aliquots of each semen sample were randomized into two groups (A and B). Group A was treated with cryo-protectant plus with (2 mg/mL) Myo-inositol solution, while group B was treated with cryo-protectant alone (control). The Sperm parameters after thawed analysis by WHO guidelines and DNA integrity were evaluated via acridin orange method.

Results: The results of this study showed that the sperm samples frozen with cryoprotectant (Myo-inositol) had a significantly higher proportion of sperm motility and sperm viability compared with those frozen without cryoprotectants ($P<0.01$). In addition, this study showed DNA integrity had significant difference between groups ($P<0.05$).

Conclusion: *In vitro* Myo-inositol supplementation of cryopreserved ejaculate sperm, from infertile men, resulted in a significant enhancement of post-thaw sperm quality. Such finding is interesting, and may have important implications on the future outcome of assisted reproductive techniques using cryopreserved sperm.

Keywords: Myo-Inositol, DNA Damage, Sperm Parameters, Cryopreservation

P-37: Evaluation of Dianabol Effects on Fertility Rate in Male Mice

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Background: Dianabol is an orally active, synthetic anabolic-androgenic steroid. Low doses of these drugs are used to treat diseases such as anemia, poor growth in children and to reduce the spread of HIV. The main subject of this study is determination of adverse effects of dianabol in fertility rate of adult male mice.

Materials and Methods: 18 adult male mice were divided into 3 groups of 6: Group 1: as control 0.2 ml distilled water were given daily through gavage. Group 2: received 5 mg/kg Dianabol daily by gavage. Group 3: were given 10 mg/kg of Dianabol daily by gavage. Treatment period was 6 weeks. At the end of the treatment, each mouse from each groups were coupled with three adult female mouse. Ten days after determining vaginal plaque, the animals were dissected and the number of embryos and corpora lutea were counted and fertility rate was examined.

Results: Fertility examinations revealed that 5 mg/kg BW and 10 mg/kg BW made significant decrease in fertility rate compared with control group. Also, fertility rate of group 3 was declined in comparison with group 2. Moreover, evaluation of Arrested embryos showed that Dianabol in group 2 and 3 caused significant increase the arrested embryo in comparison with control group.

Conclusion: Due to the results, it can be mentioned that dianabol considerably made dangerous effects on fertility specially increased arrested embryos. So, the athletes using dianabol must be careful in applying doses of this anabolic steroid.

Keywords: Dianabol, Fertility, Mice

P-38: Methandrostenolone High Doses Effects on Sperm Quality in Adult Mice

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Background: Methandrostenolone as Anabolic-androgenic steroid ranks among the drugs most widely abused with the goal of improving athletic ability, appearance, or muscle mass. This drug was developed as synthetic analog of testosterone and are administrated for treating refractory anemia, hereditary angioedema, breast cancer and starvation states. The doses of Methandrostenolone used by athletes is typically in excess of therapeutic doses. The aim of this study was to determine the effects of high doses of Methandrostenolone on sperm characteristics.

Materials and Methods: This study was carried on 18 adult male mouse. Animals were divided to 3 equal groups as follow: control, M10 and M20 groups that respectively received

0.2 ml distilled water, 10 mg/kgBW and 20 mg/kgBW Methandrostenolone daily through gavage for 42 days. After treatment period, animals were euthanized by cerebrosplinal dislocation and dissected. Cauda epididymis was collected and put in PBS buffer, then cut into pieces. Finally, sperm motility, vitality and count were evaluated. one drop of sperm suspension was spilled on the slide and sperm motility was evaluated with microscope equipped to hotplate and the result was expressed as percentage of motile sperms. Also, eosin-nigrosin staining was used for assessment of sperm vitality and sperm count was performed with hemocytometer.

Results: Evaluation of sperm quality in all groups indicated that M10 and M20 groups caused significant decline in sperm quality in comparison with control group. Also M20 group showed significant decrease in sperm motility, vitality and count compared with M10 group.

Conclusion: Regarding to the considerable diminution of sperm quality in animals induced with high doses of Methandrostenolone, it may affect the fecundity of the users. So, we suggest specially to the athletes to eschew for applying high doses of Anabolic Steroid Methandrostenolone.

Keywords: Methandrostenolone, Sperm Quality, Significance, Ice

P-39: Investigation of The Ovarian Histological Changes after Treatment with Ethephon in Mice

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Background: Ethephon including illegal chemicals uses in agricultural production, which is set to accelerate growth and achieve, improve the quality of the product. The most important side effects of ethephon on the body include cancer, liver disease, kidney disease, reproductive system dysfunction and infertility. The purpose of the study was to investigate the effect of ethephon on ovarian histological changes in mice.

Materials and Methods: In this study, 50 adult female mice with an average weight of 30 g were used for the experiments. Animals were divided into five groups respectively: control (without receiving any substance), sham (receiving daily serum physiology orally), ethephon 1 (receiving daily 192 mg/kg orally), ethephon 2 (receiving daily 240 mg/kg orally), ethephon 3 (receiving daily 480 mg/kg). After 21 days, ovaries were separated for histological studies. Ovaries were embedded in paraffin and cut into sections 6mm thick. The sections were stained with Hematoxylin-Eosin (HE) and observed under an optical microscope.

Results: The results indicated that in the average dose (240mg/kg) and high dose (480mg/kg), the follicular atresia increased and hyperemia in different parts of the ovary especially in medial portion of the ovary was seen. In high doses, the most atresia was seen in the secondary follicles as a cavity formation.

Conclusion: It seems that oral administration of ethephon with effect on ovaries can have a deleterious effect on fertility and embryo development in mice.

Keywords: Ovarian follicle, Atresia, Histological Changes, Ethephon, Mice

P-40: The Effect of Ethephon on Oxidative Stress Factors in Female Mice

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Background: Ethephon is a good and effective plant growth regulator that has the effects on promoting fruit ripening. The most important side effects of ethephon in the body include cancer, liver disease, kidney disease, impaired reproductive system performance, and infertility. This study was done to investigate the measurement of the oxidative stress system factors in serum samples of ethephon-receiving mice.

Materials and Methods: In this study, 50 adult female mice with an average weight of 30 g were used for the experiments. Animals were divided into five groups; control (without receiving any substance), sham (receiving daily serum physiology orally), ethephon 1 (receiving daily 192 mg/kg orally), ethephon 2 (receiving daily 240 mg/kg orally), ethephon 3 (receiving daily 480 mg/kg). Oxidative stress markers including malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured in serum samples. The data were analyzed using SPSS version 21 software and the results were expressed as mean \pm SD.

Results: The results of the experiments showed that ethephon in all three doses reduced the TAC compared to the control group ($P < 0.05$). Also the level of MDA in three doses of ethephon-treated mice increased compared to the normal control mice ($P < 0.05$).

Conclusion: It can be concluded that an oral administration of ethephon with effect on the oxidative stress system can have a deleterious effect on fertility and embryo development in mice.

Keywords: Ethephon, Oxidative stress, Fertility, Mice

P-41: Effect of Raffinose on The Cryopreserved Rooster Spermatozoa in Modified Beltsville Extender

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Background: Cryopreservation of sperm cells causes irreversible damage to the sperm. Factors such as the formation of ice crystals, the production of active oxygen species, temperature changes, lipid peroxidation, changes in membrane composition, and osmotic stress reduce the quality of sperm after thawing. Raffinose, composed of fructose, galactose, and glucose, is a sort of native oligosaccharide and is classified as a trisaccharide. Raffinose plays a cryoprotective role by interacting with membrane lipids and proteins and decreasing the risk of intracellular ice crystal formation, which causes cellular osmotic dehydration during cryopreservation. The present study was conducted to determine the effects of different levels of Raffinose on some post-thawed rooster semen quality parameters.

Materials and Methods: Semen samples were collected from eight sexually mature Ross 308 breeder roosters. After initial semen assessments, samples with adequate quality were mixed together and diluted with modified Beltsville extender without Raffinose (control) and supplemented with 50, 75, and 100 mM Raffinose. After thawing, sperm viability and motility were measured by Eosin-Nigrosine and Computer-Aided Sperm Analysis (CASA), respectively. The data were analyzed by the GLM procedure of SAS 9.1.

Results: Using Raffinose at 50 Mm, 75 mM, and 100 mM significantly increased sperm motility (60%, 50.25%, and 47.5%, respectively; $P < 0.05$), and viability (64%, 53.5%, and 51% respectively; $P < 0.05$) in compared with control. In terms of progressive motility, the extender supplemented with 50 mM and 75 mM Raffinose improved sperm progressive motility compared to the control group ($P < 0.05$). But using 100 mM Raffinose had no significant effect on sperm progressive motility.

Conclusion: The results of this study revealed that the addition of Raffinose to the diluent improves significantly the function of post-thawed rooster spermatozoa.

Keywords: Raffinose, Rooster, Semen, Cryopreservation

P-42: Histopathologic Evidence Associated with Impaired Folliculogenesis Induced by Butachlor in Rat Ovaries

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Background: Butachlor is one of the top members of the chloroacetanilide herbicide, extensively used for control of annual grasses and some broad-leaved weeds, particularly in rice crops. The studies of the possible toxic effects of butachlor have indicated that it is a suspected carcinogen and posing a potential threat to the agro-ecosystem and human health. The aim of this study was to evaluate the impact of butachlor on morphological and pathological changes of rat ovaries.

Materials and Methods: Twenty-eight female Wistar rats were housed under standard housing conditions (controlled 12:12 hour light/dark cycles and an ambient temperature of 22-24 °C). The rats were acclimatized for two weeks before the experiment. Vaginal smears were examined daily in order to select normal estrous cycles in female rats. Female rats with regular estrous cycles were randomly divided into a control group or an experimental group to receive oral butachlor 72 mg/kg /day. Seven rats from each group were sacrificed after six weeks for blood collection from the dorsal aorta and removing the ovaries to pathological examination and measurement of ovarian follicles apoptosis. The same procedures were performed in the remaining rats from each group six weeks later. Ovaries tissue sections were prepared and stained with hematoxylin-eosin for morphometry by light microscopy. Serum levels of progesterone and estradiol were measured by ELISA.

Results: Serum levels of progesterone and estradiol showed no significant difference between the experimental and the control group ($P > 0.05$). The number of primary and mature follicles statistically increased in the experimental group after 12 weeks of exposure. In addition, the primary follicles, as well as atretic follicles, had the highest mean number after 6 weeks of exposure in the experimental group compared to the control group ($P < 0.05$).

Conclusion: This study demonstrated that butachlor negatively impact ovarian folliculogenesis and maybe ovulation. Under the influence of butachlor, the appearance of the ovary has changed to the cystic ovary. Butachlor likely influences metabolic systems that can exacerbate anovulation and the PCOS phenotype.

Protective strategies and strong recommendations should be considered to decrease human exposure to this pesticide.

Keywords: Butachlor, Ovary, Rat

P-43: Cyclopiazonic Acid Lowered The Quality of Sperm Parameters and Fertility Rate in Mice

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Background: Mycotoxins are secondary active metabolites, which are produced by different species of fungi. Cyclopiasonic acid (CPA) is an indole tetrameric acid mycotoxin which is found in various nutrients (Meat, milk and eggs). This study was aimed to investigate the potential effects of CPA at different dose levels on the sperm quality parameters and fertility rate in mice.

Materials and Methods: Forty adult male mice were randomly divided into 5 groups (n=8). The control group did not receive any treatment. In the control-sham, animals received daily 0.05% DMSO (as the CPA solvent) intraperitoneally (i.p.). In the third, fourth and fifth groups, animals received 0.3, 0.6 and 0.12 mg/kg, BW of CPA (i.p.), respectively for 28 days. At the end of treatment period, sperm quality parameters, sperm damage and the fertility rate were evaluated.

Results: CPA exposure for 28 days resulted in a significant reduction in sperm count, sperm motility, sperm survival and chromatin quality. At the same time, CPA elevated the percentage of sperms with damaged DNA. Reduced fertilization rate, two-cell embryos (%), blastocysts (%) and hatching embryos (%) were recorded in the CPA-received animals compared to the control group.

Conclusion: The detrimental effects of CPA as reproductive toxicity, may attribute to the alterations in sperm parameters and *in vitro* fertility rate reduction. Therefore, the presence of CPA in foodstuffs alone and/or along with other mycotoxins could have negative effects on the male fertility factors.

Keywords: Cyclopiazonic Acid, *In Vitro* Fertilization, Mice, Mycotoxin, Sperm

P-44: Effect of Pentoxifylline on Tumor Necrosis Factor and Reactive Oxygen Species Levels in Seminal Plasma in Idiopathic Male Infertility

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Background: Pentoxifylline is a methyl xanthine derivative that influences the sperm motion characteristics. It is commonly used in treatment of male-factor infertility, including asthenozoospermia. The aim of this study was to evaluate any possible effect of Pentoxifylline on sperm characteristics, TNF (Tumor

Necrosis Factor) and ROS (Reactive Oxygen Species) Levels in Seminal Plasma in a group of patients with asthenozoospermia.

Materials and Methods: 30 infertile men with asthenozoospermia were allocated to this study. A dosage of 400 mg pentoxifylline / twice daily for duration of 3 months was administered to each patient. Two semen samples (one before and one after the pentoxifylline therapy) were evaluated under blind condition. Semen parameters ,TNF and ROS Levels(by human TNF ELISA kit and Chemiluminescence assay- Agarwal respectively) in Seminal Plasma were analyzed (pre and post intervention) for each sample. Data was analyzed statistically using one-way ANOVA and Tukey's test .

Results: Pentoxifylline increased significantly the mean sperm count and normal morphology in the men with asthenozoospermia when compared to pre-administration. pentoxifylline was significantly effective on the fast progressive motility of sperm ($P<0.01$). Based on the results, mean concentration (\pm S.D) of TNF in the seminal plasma was significantly increased in patients treated with pentoxifylline when compared to pre-administration. Moreover, ROS levels in seminal plasma of patients with asthenozoospermia decreased significantly compared to before administration of pentoxifylline.

Conclusion: Although our results demonstrate that oral therapy of pentoxifylline significantly increase the quality of sperm, especially motility from infertile men with asthenozoospermia, but more molecular studies are needed to elucidate the safety of pentoxifylline- administration.

Keywords: Pentoxifylline, Seminal Plasma, Asthenozoospermia, Infertility

P-45: Effect of Co-Administration of Pentoxifylline and Zinc on Total Antioxidant Capacity and Malondialdehyde Levels in Seminal Plasma in Idiopathic Male Infertility

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Background: Pentoxifylline is a methyl xanthine derivative that influences the sperm motion characteristics. It is commonly used in treatment of male-factor infertility, including asthenozoospermia and Zinc is known to influence several phases of sperm life, from germ cell development to spermiation. The aim of this study was to evaluate any possible effect of a combination of zinc and Pentoxifylline on sperm characteristics and Total Antioxidant Capacity and Malondialdehyde Levels in Seminal Plasma in a group of patients with asthenozoospermia.

Materials and Methods: Ninety men with asthenozoospermia in a doubleblind, randomized clinical trial were allocated for this study. They randomized to tree groups. Group I received Pentoxifylline / and zinc, group II, Pentoxifylline, group III, zinc . pentoxifylline and zinc twice daily for duration of 3 months was administered to each patient according to grouping . Finally, we compared pre and post intervention semen parameters of sperm, Levels Seminal Total Antioxidant Capacity (TAC) and Malondialdehyde levels (MDA) in all the specimen by FRAP(ferric-reducing ability of plasma assay) and TBA (Tiobarbitotic acid) methods, respectively. Data was analyzed statistically using one-way ANOVA and Tukey's test .

Results: Pentoxifylline and zinc increased significantly the

mean sperm motility, count and normal morphology in the Pentoxifylline and zinc treated groups when compared to pre-administration. The mentioned parameters increased significantly in the Pentoxifylline + Zinc group in comparison with Pentoxifylline and Zinc groups. Based on the results, mean concentration (\pm S.D) of TAC in the seminal plasma was significantly increased in the groups of pentoxifylline, zinc, and pentoxifylline + zinc compared to pre-administration. Moreover, MDA levels in seminal plasma of patients with asthenozoospermia decreased significantly compared to before administration in the groups of pentoxifylline, zinc, and pentoxifylline + zinc. also, TAC level in zinc+ pentoxifylline group was significantly higher than zinc and pentoxifylline groups. In the event that, MDA was significantly reduced in the pentoxifylline + zinc group compared with the groups of pentoxifylline and zinc.

Conclusion: It is suggested that pentoxifylline be co-administered with antioxidants such as zinc, for the treatment of male infertility.

Keywords: Pentoxifylline, Zinc, Asthenozoospermia

P-46: Sperm miR-26a Tanscript Content in Normozoospermic Fertile and Infertile Men

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Background: Infertility is a reproductive disorder with multiple genetic and environmental causes which affects 15% of couples worldwide. With male factor responsible for half of the cases, approximately 7% of men encounter with infertility and About 60-75% of male infertility cases are considered to be idiopathic. Currently, due to WHO guidelines, conventional semen parameters, including sperm concentration, motility, morphology, seminal volume and pH is being used for evaluation of male fertility potential which provide limited information and cannot distinguish fertile and infertile men on an individual basis. Consequently, clinicians are searching for more reliable seminal bio-markers to have more accurate prospective in the case of male infertility patients.

Materials and Methods: Present study was approved by the institutional review board of Biology Department, Shahid Chamran University of Ahvaz. Semen samples were collected from fertile (n=15) and infertile men (n=15) with normal sperm parameters according to the World Health Organization (WHO) guidelines. Real-time PCR was carried out to evaluate relative expression of sperm miR-26a transcript content in ejaculated sperm.

Results: Sperm concentration was significantly ($P<0.05$) lower in men with unexplained infertility compared with control group. Significant ($P<0.05$) lower content of miR-26a transcript in ejaculated sperm observed in infertile men than fertile control. Also, significant correlation ($r=0.369$, $P=0.045$) was seen between miR-26a transcript content and sperm morphology.

Conclusion: Our findings indicate that assessment of sperm expression profile reflect the quality of spermatozoa in etiology of unexplained male infertility and suggest that miR-26a sperm content transcript could be used as novel diagnostic marker for male infertility or potential treatment target.

Keywords: Unexplained Male Infertility, Normozoospermia, Sperm Transcriptome, miR-26a

P-47: Protective Effect of Heparin on Epididymal Sperm Quality in Ischaemic-Reperfusion Injury of the Rat Testicle

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Background: Testicular torsion is an urologic emergency that causes testicular damage and lead to reduced fertility or infertility. It appears that the main pathophysiology of testicular torsion is ischemia/reperfusion (I/R) injury of the testis caused by the twisted spermatic cord and its release. Surgical detorsion is currently the only treatment and allows blood reperfusion. Even with successful surgical repair, loss of spermatogenesis and a significant increase in germ cell apoptosis may happen. Heparin is a naturally occurring anticoagulant produced by basophils and mast cells. The protective effects of heparin and its derivatives in IR injury have been evaluated in different systems including hepatic, gastrointestinal, urogenital systems and in pancreas, lungs and heart but no previous study has evaluated the effect of heparin in the prevention of IR injury in rat epididymal sperm quality.

Materials and Methods: Eighteen Wistar Albino male rats weighing 250–300 g were divided into three groups: sham (group S, n = 6); torsion/detorsion (group T/DT, n = 6), and heparin pretreatment (group Hep, n = 6). The left testes were rotated 720° clockwise for 2 hours in the rats of the torsion–detorsion group (group T/DT). Rats in the treatment group underwent the same surgical procedure as the torsion–detorsion group but were also given heparin 800 IU/kg (Hep group) by an intraperitoneal route 30 min prior to detorsion. In sham group (group s), the left testes were brought out through the incision and were placed back in the scrotum. After 2 hours of reperfusion a left orchietomy was performed and the sperm was collected from the epididymis and sperm characteristics such as sperm motility, sperm vitality, sperm count and sperm morphology were examined.

Results: Heparin significantly enhanced sperm motility and normal morphology of sperms but sperm vitality and sperm count did not differ significantly in Hep group in comparison with T/DT group.

Conclusion: Our results suggest that heparin treatment has a protective role on IR-induced testicular injury.

Keywords: Testicular Torsion, Sperm, Heparin

P-48: Electrospun Nanofiber Assemblies for *In Vitro* Three-Dimensional Uterine Tissue Engineering

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Background: The purpose of using this three-dimensional construct is to make a sufficient thickness membrane as a part of the uterine endometrium to protect implantation of the zygote.

Materials and Methods: In this study, Polycaprolactone/Gelatin/Polydimethylsiloxane (PCL/G/PDMS) nanofibers with aligned and random fiber arrangements were used as models to study the growth of uterine endometrial cells. To imitate *in vivo* tissue structure, three-dimensional (3D) cell/micro-nanofiber constructs with cells embedded among micro-nanofiber layers were built via layer-by-layer assembly. This structure showed that aligned nanofibers in the 3D constructs continuously induced cell polarization and promoted and demonstrated the potential of 3D cell/nanofiber construct as a model for proliferation of uterine endometrial cells in a physiologically relevant environment. A layer-by-layer assembly approach was adopted to stack cell-seeded nanofiber mesh (aligned or random) into 3D multilayered constructs. Briefly, aligned and random nanofibers collected on an aluminum foil were evenly seeded with endometrial cells (106 cells/scaffold). After 24 hrs, ten layers of cell-seeded nanofiber meshes were overlaid to each other. Following this approach, one type of 3D cell/nanofiber constructs with either aligned or random nanofibers were formed. To visualize cell morphology inside the 3D cell/nanofiber constructs, at days 7 and 14 after seeding, samples were fixed in 4% glutaraldehyde and cut into 5- μ m thin sections. The sections were then stained with hematoxylin and eosin (H&E) to evaluate the cell distribution. SEM micrographs of the scaffolds were obtained before seeding of the cells.

Results: Histological results showed that the cells homogeneously distributed through the entire constructs, and formed an integrated connection with nanofibers. Cells cultured on 2D random nanofiber meshes exhibited a polygonal morphology. Similar morphology arrangements were also observed with the cells cultured in 3D “sandwich” constructs.

Conclusion: A layer-by-layer approach was taken to assemble cell-seeded nanofiber meshes into 3D constructs with precisely controlled organization of nanofibers for mimicking the isotropy (i.e., stacking random nanofiber layers) of uterus tissue. This 3D culture system allows us to understand nanofiber-induced cellular responses in a physiologically relevant environment and preparation for a proper engineered tissue.

Keywords: Tissue Engineering, Nanofiber, Human Endometrium Cells, Three-Dimensional

P-49: Evaluation of The Effect of Ketorolac on The Rate of Sperm Motility and Survival in Infertile Men with Oligo-Asthenospermia in Culture

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Background: Decreased semen quality is a major factor of male infertility. The primary mechanism of action responsible for ketorolac's anti-inflammatory, antipyretic and analgesic effects is the inhibition of prostaglandin synthesis by competitive blocking of the enzyme cyclooxygenase (COX) 1 and 2. It has been hypothesized that Ketorolac has impact on semen quality and quantity. In this work, we review and reveal the effect of ketorolac on sperm motility and vitality in infertile men suffering from oligoasthenoteratozoospermia *in vitro*.

Materials and Methods: In this study, 40 seminal fluids were obtained from 40 infertile males suffering from oligoasthenoteratozoospermia. After collection by floating method, the samples were randomly placed in ISM1 media in four different groups: the control group without any addition, group number one with 50ug/ml ketorolac, group number two with 100ug/ml ketorolac, and group number three with 200ug/ml ketorolac added to the media. Sperm motility and vitality were examined after 1, 24, 36, and 48 hours. The data was analyzed by SPSS software. A P value of less than 0.05 was considered statistically significant.

Results: The average percentage of motile sperms was measured in control group after the intervals of 1, 24, 36, and 48 hours and a significant reduction of motility ($P < 0.001$) was observed in higher time points compared to the first hour; there was no statistically significant difference ($P = 19.0$) between the four groups. The average percentage of alive sperms was also measured in control group after the intervals of 1, 24, 36, and 48 hours and a significant reduction of vitality ($P < 0.001$) was observed in higher time points compared to the first hour; there was a statistically significant higher decrease in the third group ($P = 0.01$).

Conclusion: Ketorolac increases the sperm vitality in infertile males suffering from Oligo-Asthenospermia only in 200ug/ml dosage but has no effect on sperm motility.

Keywords: Ketorolac, Oligo-Asthenospermia, Sperm

P-50: Ascorbic Acid Attenuates Cognitive Impairment in Ovariectomized Mice

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Background: Menopause is associated with increased memory impairment. Regarding the antioxidant property of ascorbic acid (AA), the present study was designed to evaluate the effects of AA on cognitive function and the level of brain-derived neurotrophic factor (BDNF) in the brain in ovariectomized (OVX) mice.

Materials and Methods: For this purpose, AA (100, 300 and 500 mg/kg/p.o.), was administrated daily in OVX mice for 30 days. Tactile learning was evaluated by novel object recognition task. Also, the levels of serum BDNF were measured.

Results: AA prevented from the deleterious effects of ovariectomy on learning memory (300 and 500 mg/kg). The serum BDNF level was also increased in OVX animals treated with AA (100 and 500 mg/kg).

Conclusion: Collectively, the results of the present study suggest that AA might be an appropriate choice in loss or reduction of estradiol for the amelioration of cognitive impairment.

Keywords: Ovariectomy, Ascorbic Acid, Learning and Memory, BDNF

P-51: Seminiferous Tubules Histoarchitecture Changes in Nandrolone Decanoate-Treated Male Rats

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Background: Nandrolone Decanoate as an anabolic steroid is used for physique- and muscle mass-enhancing purposes by athletes. The misuse of this drug has irreparable consequences such as male infertility.

Materials and Methods: Eighteen rats were randomly categorized into 3 groups; group 1 was served as a control and groups 2 and 3 received 3 and 6 mg/kg of Nandrolone Decanoate weekly through intra-muscular injections, respectively. After 8 weeks, the testicles were harvested and the seminiferous tubules histoarchitecture was analyzed.

Results: The seminiferous tubules epithelium thickness in the Nandrolone Decanoate -receiving groups showed a significant decrease compared to the control group, while seminiferous tubules luminal diameter in the Nandrolone Decanoate -treated rats exhibited a significant increase compared to the control group.

Conclusion: High doses of Nandrolone Decanoate can have negative effects on male reproductive system.

Keywords: Nandrolone Decanoate, Reproductive System, Seminiferous Tubule, Rat

P-52: Effect of Nandrolone Decanoate on Rat Sperm Characteristics

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Background: High doses of nandrolone decanoate can result in decreased testosterone synthesis leading to spermatogenic disorders and male infertility.

Materials and Methods: Eighteen adult male rats were randomly divided into 3 groups; group 1 was served as a control and groups 2 and 3 received 3 and 6 mg/kg of nandrolone decanoate weekly through intra-muscular injections, respectively. After 8 weeks, spermatological parameters were evaluated in all experimental groups.

Results: Nandrolone decanoate administration significantly reduced sperm count, motility and viability compared to control group.

Conclusion: Misuse of nandrolone decanoate can result in spermatological damages causing reproductive disorders.

Keywords: Sperm, Nandrolone Decanoate, Male Rat, Fertility

P-53: Effect of Nandrolone Decanoate on Sertoli Cell and Testicular Tubular Differentiation Indices in Rats

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Background: Misuse of Nandrolone Decanoate, an anabolic steroid, can lead to serious side effects including male infertility.

Materials and Methods: Eighteen rats were randomly assigned into 3 groups; group 1 was served as a control and groups 2 and 3 received 3 and 6 mg/kg of Nandrolone Decanoate weekly through intra-muscular injections, respectively. After 8 weeks, the testicles were harvested and Sertoli cell index (SCI) and tubular differentiation index (TDI) were evaluated.

Results: The SCI and TDI indices showed significant reductions in Nandrolone Decanoate-treated groups compared to the control group.

Conclusion: The use of anabolic steroids such as Nandrolone Decanoate may cause testicular dysfunction.

Keywords: Nandrolone Decanoate, Seminiferous Tubule, Sertoli Cell, Spermatogenesis, Rat

P-54: Optimizing of Mouse Autologous Serum Through Endogenous Granulocyte-Macrophage Colony-Stimulating Factor after Induced Inflammation by Casein and Evaluation of its Effect on Embryo Development

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Background: Research studies on reproductive mechanism of laboratory animals are essential for further advancement of assisted reproductive techniques (ART). One of these studies includes the assessment of the effect of types of sera in culture medium on development of pre-implantation embryos. This study evaluated for the first time the effect of different protein supplements (BSA), mouse serum and mouse serum which treated by casein; on development, ICM, TE and apoptotic cell number and expression of Oct4, Cdx2, Bax, Bcl2 and receptor of GM-CSF (Csf2ra) genes in blastocysts of NMRI strain mouse.

Materials and Methods: Mice were injected IP with 2 ml of 0.2% (wt/vol) solution of casein in mouse tonic phosphate-buffered saline (MTPBS) and at 3 hours after injection blood collected from heart and serum separated. Two pro-nucleus stage embryos from *in vivo* were collected by oviduct flush-

ing. The 2PN's were randomly divided into three groups. 1) culture medium supplemented with 4mg/ml bovine albumin serum (BSA), 2) culture medium supplemented with 10% mouse serum (M), and 3) culture medium supplemented with 10% mouse serum that treated by casein. Then embryos were cultured up to the blastocyst stage. In 4th group embryos were developed in-vivo in to blastocyst stage. The rate of blastocyst development, apoptotic rate and number of Inner cell mass and Trophectoderm of blastocysts were measured, also quantitative expression of Oct4, Cdx-2, Bax, Bcl-2 and GM-CSF receptor (Csf2ra) were performed in these groups, using RNA extraction and Real Time PCR.

Results: Serum GM-CSF levels were measured by ELISA kit 0.348 ng/ml. The difference in the percentages of development to blastocyst stage in culture medium containing BSA, M, treated M serum by casein and in-vivo groups were not significant. There were no significant difference between ICM numbers but TE and total cells of blastocyst were significantly increased in *in vitro* groups. Embryos that cultured with BSA had significantly more apoptotic cells in comparison the other groups. Quantitative PCR analysis showed that the difference in the expression level of Oct4, Cdx2, Bax, Bcl2 and Csf2ra was not significant.

Conclusion: In this study developmental rate was similar to embryos that cultured in 2 ng/ml recombinant GM-CSF even though serum GM-CSF levels didn't increase up to optimized dose (2 ng/ml). Considering the fact that the GM-CSF is endogenous and appropriate developmental rate usage of this serum could be a possible alternative.

Keywords: Culture medium, GM-CSF, Developmental Genes, Apoptosis Genes, Csf2ra

P-55: Seminiferous Tubules Histological Alterations in Oxymetholone-Administered Male Rats

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Background: Oxymetholone, an anabolic steroid, is used extensively among athletes and can affect male fertility.

Materials and Methods: Twenty-one adult male rats were randomly divided into 3 groups; group 1 was served as a control and groups 2 and 3 received 5 and 10 mg/kg/PO of Oxymetholone daily, respectively. After 60 days, the testicles were harvested and the seminiferous tubules histology was examined.

Results: The seminiferous tubules epithelium thickness in the Oxymetholone-administered groups showed a significant decline compared to the control group, whereas seminiferous tubules luminal diameter in the Oxymetholone-received rats exhibited a significant increase in comparison with control group.

Conclusion: High doses of Oxymetholone may have negative impacts on male reproductive system.

Keywords: Oxymetholone, Male Reproductive System, Seminiferous Tubule, Rat, Histology

P-56: Effect of Oxymetholone on Spermatogenic Indices in Rats

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Background: Oxymetholone as an anabolic steroid used extensively among athletes for muscle mass enhancing can lead to testicular malfunctions.

Materials and Methods: Twenty-one adult male rats were randomly categorized into 3 groups; group 1 was served as a control and groups 2 and 3 received 5 and 10 mg/kg/PO of Oxymetholone daily, respectively. After 60 days, the testicles were harvested and spermiation index (SPI) and tubular differentiation index (TDI) were recorded.

Results: The SPI and TDI indices exhibited significant declines in Oxymetholone-administered groups in comparison with control group.

Conclusion: Anabolic steroids abuse can lead to severe spermatogenesis impairment.

Keywords: Seminiferous Tubules, Oxymetholone, Spermatogenesis, Rat

P-57: The Effects of Myo-Inositol on Mitochondrial Function and Oxidative Stress Condition in MII Oocytes of PCOS Model Mice

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Background: Polycystic ovary syndrome (PCOS) is associated with hyperandrogenism, polycystic ovaries, infertility due to ovarian dysfunction and menstrual irregularity and ultimately oocytes with poor quality. The administration of myo-inositol (MYO) was associated with a decreased of serum testosterone, increase insulin sensitivity and improves the oocytes' quality. Inofolic is a dietary supplement for women with PCOS that contains myo-inositol (2 g) and folic acid (200 mcg).

Materials and Methods: Female NMRI mice were treated with a vehicle control or DHEA (6 mg /100 g body weight) or DHEA plus inofolic (0.37 mg /g body weight) for 20 consecutive days. After 20 days, Mature oocysts (MII) were retrieved from isolated ovaries. For inner mitochondrial membrane potential (MMP) or reactive oxygen species (ROS) or reduced glutathione (GSH) staining, denuded MII oocytes were incubated in PBS-PVA containing JC-1, H₂DCFDA and Cell Tracker Blue fluorochromes respectively. After incubation, oocytes were washed and fluorescence was observed using a fluorescence microscope and fluorescence intensity was measured with Image J software.

Results: Significantly GSH and MMP were lower and ROS was higher in DHEA-treated oocytes compared with vehicle-treated. In DHEA+inofolic group, GLUT and MMP were significantly increased in contrast to the DHEA-treated and, On the contrary ROS fell.

Conclusion: It is likely that myo-inositol plays an important role in reducing ROS, increasing the antioxidant capacity, reducing cellular apoptosis and follow by increasing MMP and ultimately improving mitochondrial function in mice MII oo-

cytes of PCOS model.

Keywords: Myoinositol, Polycystic Ovary Syndrome, Reactive Oxygen Species, Reduced Glutathione, Inner Mitochondrial Membrane Potential

P-58: Benzoic Acid-loaded Solid Lipid Nanoparticles Enhances Endometrial Receptivity through Upregulation of LIF and Integrin α V β 3

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Background: Embryo implantation is the crucial step for a successful pregnancy. Diverse factors, including adhesion molecules, growth factors, and cytokines are important for embryo implantation through improving endometrial receptivity. Benzoic acid (BA) is an aromatic carboxylic acid, whose positive effects on endometrial receptivity have been demonstrated, but the poor water solubility and low bioavailability have limited its therapeutic potential. Therefore, employing a nanoparticulate delivery system may enhance BA bioavailability. The present study proposed to develop solid lipid nanoparticles (SLNs) as a delivery system for improving BA effects on endometrial receptivity.

Materials and Methods: BA-loaded SLNs was prepared by hot homogenization technique and the nanoparticles characteristic include size, encapsulation efficiency and morphological behavior was determined by dynamic light scattering technique, ultrafiltration method and Scanning electron microscopy (SEM), respectively. Cytotoxicity of BA and prepared SLNs on endometrial cell line was evaluated by 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Finally endometrial cells were treated with BA and BA-loaded SLNs for 48 h and expression of receptivity related genes evaluated by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Results: The nanoparticles with appropriate characteristics (particle size of 90 nm and Encapsulation Efficiency of 81%) were prepared. BA-loaded SLNs displayed a good stability for 4 weeks of storage at 4-8°C. No apparent cytotoxicity for SLNs and BA was considered which indicating the biocompatibility of the nanocarriers. Expression experiments revealed that BA and BA-loaded SLN upregulate expression of leukemia inhibitory factor (LIF) and Integrin α V β 3. Results also demonstrated that upregulation of LIF and Integrin α V β 3 will intensify when BA is loaded into SLN suggesting capability of SLNs in the more precise delivery of BA into cells than free BA.

Conclusion: The results strengthen our hope that loading BA into SLNs could possibly overcome the therapeutic limitations of BA and make it more effective in enhancing endometrial receptivity.

Keywords: Benzoic Acid, Receptivity, Endometrium, SLN

P-59: Protective Effect of Misoprostol on Ibuprofen-Induced Alteration of Sperm Parameters in Adult Male Mice

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Background: Ibuprofen, a propionic acid derivate is a non-steroidal anti-inflammatory drug (NSAID). In recent years several studies have been published on animal and human clinical experiments with NSAID, pointing to the deleterious effect of prostaglandins (PGs) on spermatogenesis. Misoprostol is a synthetic prostaglandin E1 methyl analogue indicated for the prevention of gastric ulcers induced by NSAIDs. The aim of the present study was to determine the protective effect of misoprostol on ibuprofen-induced alteration of sperm quality and quantity parameters in adult male mice.

Materials and Methods: In this study, 80 adult male mice were divided into 8 groups including: control, ibuprofen treated group which received 6 mg/Kg/day ibuprofen, three misoprostol treated groups which received 1, 10 and 100 µg/Kg/day misoprostol and three ibuprofen+misoprostol treated groups which received 6 g/Kg/day ibuprofen with 1, 10 and 100 µg/Kg/day misoprostol. All treatments were carried out for 40 consecutive days by oral gavage. At the end of experiment, animals were euthanized and their left epididymis was removed and dissected in Ham's F10 and incubated at 37°C. Total count, motility, viability and morphology of sperm as well as trazoospermia (TZI) and sperm DNA fragmentation index (SDFI) were assessed according to the WHO standard methods. Data were statistically analyzed by SPSS using one-way ANOVA test and Tukey's post-hoc.

Results: Results showed that ibuprofen reduced total count, motility and viability of epididymal sperm and increased abnormalities, TZI and SDFI significantly compared to control. It was also indicated that high dose of misoprostol could increase motility and viability of sperm and decreased TZI and SDFI significantly compared to control group. Our results revealed that all three dose of misoprostol could significantly increase total count, motility and viability of sperm and reduce sperm with abnormal morphology, TZI and SDFI compared to ibuprofen treated group in a dose dependent manner.

Conclusion: Based on our results it can be concluded that administration of misoprostol can improve quality and quantity indices of sperm and can be considered as a suitable protective strategy for improvement of male infertility or subfertility due to NSAIDs administration.

Keywords: Misoprostol, Ibuprofen, NSAID, Sperm, Male Infertility

P-60: The Association of Mass and Individual Sperm Motility with Abnormal Sperm Morphology

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Background: Sperm motility is believed to be one of the most important parameters in evaluating the fertilizing ability of ejaculated sperm, and fertilization rates of human oocytes *in vitro* have been shown to correlate closely with sperm motility.

Both sperm motility parameters and percent normal morphology are significant factors in predicting fertilization and pregnancy rates. The purpose of this study was to determine the relationship between the sperm motility and abnormal sperm morphology.

Materials and Methods: Testis samples were obtained from 45 rams. The viability and abnormal morphology parameters of the cauda epididymal sperm were assessed by means of the Eosin-Nigrosin stain method. The viability and sperm abnormalities were assessed by counting 300 sperm cells in a microscope at 1000× magnification, using immersion oil. The cauda epididymal sperm motility was assessed in a light microscopy at 400× magnification at 37°C. A computer-assisted sperm motility analysis (CASA) was used to analyse sperm motility.

Results: According to the statistical analysis, significant correlations were found between coiled principal piece and end piece of tail and mass motility (P<0.01) and mass motility and individual motility (P<0.01). Also, significant positive correlation existed between live sperm and detached head (P<0.01), coiled principal piece and end piece of tail and coiled midpiece of tail (P<0.01), slender head and macro cephalic (P<0.01), pyriform head and twin head (P<0.05).

Conclusion: Observation of individual and mass motility and estimation of the percentage of progressively motile sperm will provide information about sperm membrane integrity, as well as the morphologic integrity of spermatozoa. In conclusion, in this study mass motility correlated significantly with coiled principal piece and end piece of tail and individual motility.

Keywords: Sperm Motility, Abnormal Sperm, Morphology, CASA, Epididymal Sperm

P-61: LIF Can Increase The Expression of The $\alpha\beta 3$ Integrin in Cultured Mouse Blastocysts

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Background: Leukemia Inhibitory Factor, LIF, and integrins play an essential role in the interaction of trophoblast with endometrial cells in the implantation process. It has been determined that LIF regulates the expression of endometrial $\alpha\beta 3$ Integrin, but its role in $\alpha\beta 3$ expression on trophoblast cells is still unclear. Blockage of LIF has a negative effect on the implantation of mouse embryos. The aim of this study was to evaluate the effect of LIF on development of 8-cell mouse embryos into blastocyst stage and also expression of $\alpha\beta 3$ integrin, Bax, and Bcl-2 genes in resulted blastocysts.

Materials and Methods: The 8-cell mouse embryos were obtained from superovulated NMRI mice. The collected embryos were divided into four groups and cultured for 72 hours as follows: group (1): LIF free simple embryo culture; group (2):

simple embryo culture with 1000 U/ml LIF; group (3): embryo co-culture with Ishikawa cells without LIF; group (4): embryo co-culture with Ishikawa cells with 1000 U/ml LIF. Embryo development was recorded every day and Cell counting was performed on the obtained blastocysts. As well expression of $\alpha\beta3$, Bax, and Bcl-2 genes were evaluated in blastocysts of each group using real-time PCR.

Results: The results showed that the percentage of embryos that reached the blastocyst stage, number of cells, embryo survival rates and expression of $\alpha\beta3$ integrin gene were significantly higher in the co-culture groups and simple culture with LIF group than LIF free simple culture group.

Conclusion: According to our findings, it seems that LIF can improve the growth, number of cells and survival of the embryos and Increases the gene expression of $\alpha\beta3$ integrin in single blastocysts which has an important role in the success of implantation. The current results may provide a new approach to increase the implantation rate in infertile women.

Keywords: LIF, Embryo Implantation, $\alpha\beta3$ Integrin, Blastocyst, Trophoblast

P-62: Association between Seminal Prolactin and Sperm HSP90 Transcript Content

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Background: Heat shock proteins (HSPs) are a group of proteins which have ability to protect cells against apoptosis and oxidative stress. To investigate role of seminal prolactin on men fertilizing capacity, this study evaluated correlation between seminal prolactin (PRL) levels with mRNA content of HSP90 in ejaculated sperm.

Materials and Methods: Present study was approved by the institutional review board of Biology Department, Shahid Chamran University of Ahvaz. Sperm parameters were analyzed according to the World Health Organization (WHO) guidelines in men attending an infertility clinic and categorized to normozoospermic and asthnozoospermic groups. Seminal PRL levels assessed via radioimmunoassay method. Real-time PCR was carried out to evaluate mRNA content of HSP90 in ejaculated sperm.

Results: Significant ($P < 0.05$) higher levels of seminal PRL was seen in normozoospermic than asthnozoospermic men. Sperm content of HSP90 transcript was significantly ($P < 0.05$) higher in asthnozoospermic than normozoospermic men. Significant ($r = -0.578$, $P < 0.01$) correlation was seen between levels of seminal PRL and sperm mRNA content of HSP90.

Conclusion: These findings show the significance of seminal PRL in relation to sperm HSP90 mRNA content and show that seminal PRL level has important clinical significance and could be considered as a diagnostic tool in prediction of male infertility.

Keywords: Semen Quality, Oxidative Stress, Asthnozoospermia, Prolactin, HSP90

P-63: Improvement of *In Vitro* Sperm Movement Characteristics in Normozoospermic and Asthnozoospermic Men by Repaglinide

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Background: Human sperm activity must be precisely regulated to achieve natural fertilization. The human sperm motility and hyperactivation induce by the different factors such as intracellular calcium concentration. Repaglinide is one of the current antidiabetic drugs that decrease blood glucose level by inducing the release of insulin in pancreatic islets. Mechanism action of repaglinide is blocked of ATP-sensitive potassium channels and depolarization of b- cell membrane, opening the voltage-gated calcium channels and then increasing in intracellular calcium. In this study; we examined the effect of repaglinide on *in vitro* induction of the human sperm motility and hyperactivated motility.

Materials and Methods: Semen samples were collected from two groups of normozoospermic donors and asthnozoospermic patients that were washed free of seminal plasma and then treated with medium alone (control) and were treated with 100 nM and 1 μ M concentration of repaglinide. After 1 hour of incubation for all treatments, the percentage of sperm motility and sperm hyperactivation were assessed.

Results: Results showed that repaglinide at concentration of 100 nM and 1 μ M caused significantly improvement of *in vitro* induction of sperm motility and hyperactivated motility in both groups, but in normozoospermic 1 μ M concentration of repaglinide has obviously effect on progressive motility and in asthnozoospermic group, highest hyperactivated motility rate was seen in 100 nM concentration of repaglinide in comparison to 1 μ M concentration and control ($p < 0.05$).

Conclusion: In conclusion our results suggest that repaglinide can improve sperm motility and hyperactivity in normozoospermic and asthnozoospermic men.

Keywords: Repaglinide, Motility, Sperm, Hyperactivation

P-64: The Effect of Clomiphene Citrate on Oocyte Quality via Expression of Growth Differentiation Factor-9 in Mice with Polycystic Ovarian Syndrome

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Background: Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women which influences 5 to 26 percent of them during reproductive age. Clinical features consists of menstrual disorders, hirsutism, acne, alopecia and irregular ovulation. Clomiphene citrate is an anti-estrogen combination which is used to increase LH and FSH levels to develop ovulation and improve fertility, can induce ovulation in approximately 80 percent of women with ovulation disorders. Various studies have demonstrated that growth differentiation factor-9 (GDF-9) can be considered as a marker in determining the quality of oocyte. The aim of this study was to determine the expression of GDF-9 gene in the oocytes of PCOS mice treated with clomiphene citrate.

Materials and Methods: In this experimental study, 18 adult female NMRI mice (25-30 g) were studied and divided random-

ly into 3 groups as follows: i) healthy control, ii) PCOS, and iii) treatment groups. The PCOS mice were induced by single intramuscular injection of estradiol valerate. Animals in treatment group were treated with clomiphene citrate for 10 days subsequent the induction of PCOS. Total RNA was extracted from oocytes and the expression of GDF-9 was determined using real time PCR. The level of blood sexual hormones were analyzed using ELISA method.

Results: Our results showed that GDF-9 expression level altered in the group treated with clomiphene citrate compared to the PCOS group which difference was significant at P value of 0.05. FSH and LH hormones level was also improved against the PCOS group.

Conclusion: The Clomiphene citrate treatment can improve the quality of oocytes in PCOS mice. This result could be under consideration of infertility treatment methods.

Keywords: Gene Expression, Clomiphene Citrate, Oocyte, Polycystic Ovarian Syndrome

P-65: The Effect of Pentoxiphyline on Sperm Parameters and Biochemical Factors in Mouse Treated with Dexamethasone

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Background: Infertility in men's crowd is increasing and use a series of medications such as dexamethasone is one of the main reasons for infertility. Pentoxiphyline as a potent antioxidant is able to reduce oxidative stress and the harmful effects of dexamethasone. The aim of this study was to evaluate the preventing effect of Pentoxiphyline on sperm parameters and biochemical factors in in Mouse Treated With Dexamethasone.

Materials and Methods: This study 24 Adult male NMRI mice (35±2 gr.) were divide randomly into 4 groups (n=6): control, Dexamethasone (7mg/kg/day), pentoxiphyline (200mg/kg/day), Dexamethasone + pentoxyphiline. After 7 days of treatment, the left caudal epididymis was cut in the Ham's F10 and the released spermatozoa were used to analyze sperm parameters, biochemical factors and daily sperm production were also measured. Data were analyzed using one way ANOVA and Tukey's test and the means were considered significantly different at P<0.05.

Results: Motility, number, viability, sperm tail length and daily sperm production (P<0.001), Serum testosterone level (P<0.001), TAC (P<0.001) while in the volume of interstitial tissue and MDA level significantly increased in the Dexamethasone group when compared to the control. Co-administration of Dexamethasone and pentoxiphyline didn't significantly compared to control group on the above parameters Co-administration of Dexamethasone and pentoxiphyline didn't significantly compared to control group on the above parameters (P>0/05).

Conclusion: This study showed that simultaneous treatment of pentoxiphyline and dexamethasone can prevent the adverse effects of on sperm parameters, biochemical factors and daily sperm production.

Keywords: Pentoxiphyline, Sperm Parameters, Dexamethasone, Biochemical Factors, Mice

P-66: Effect of Vitamin E on Sperm Parameters and Expression of ODF Gene in Infected Mice with Chlamydia Trachomatis

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Background: Some bacteria example Chlamydia Trachomatis is the most prevalent bacterial cause of sexually transmitted infections in the world and can result in severe genital disease. Over 90 million chlamydial infections are detected annually worldwide and various studies have estimated that there are four to five million new cases of chlamydial infection each year in the USA. The aim of this study was to investigate the harmful effects of Effect of vitamin E on sperm parameters and Expression of ODF gene in infected mice with Chlamydia Trachomatis.

Materials and Methods: 24 Adult rats were divided into four groups: control, infected with C. Trachomatis, infected with C. Trachomatis + Vitamin E, Vitamin E (100mg/kg/day). Treatments were performed till 4weeks. Left caudal epididymis was cut in Ham's F10. Released spermatozoa were used to analyze number, motility and viability of the sperm also the expression of ODF gen was analyzed by real-time PCR.

Results: In present study our results showed treatment of animals with C. Trachomatis significantly decreased of Motility, number, viability, sperm tail length and daily sperm production (P<0.001) and ODF gene expression compared to the control group. While C. Trachomatis +Vitamin E group showed a highly significant increase in sperm parameters and ODF gene expression.

Conclusion: Vitamin E could compensate the adverse effects of C. Trachomatis on sperm parameters in adult mices.

Keywords: Adult Mice, Sperm Parameters, Vitamin E, C. Trachomatis

P-67: Effects of BioLozax-H on The Testis Structure and Pituitary- Gonadal Hormones in Adult Rat following Treatment with Clinical Dose

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Background: The activity of the renin-angiotensin system has an effect on oxidative stress in many tissues, including the testes. Therefore, in this study, the bioLozax-H drug, which has an effect on the spermatogenesis activity and Pituitary- Gonadal Hormones in male rats, is further investigated.

Materials and Methods: Male adult rat (200-250 g) were divided into two groups of sixteen each. Control and bioLozax-H treated group were administered 20 mg/kg bioLozax-H for 45 days, respectively. The animals were sacrificed 45 days after starting treatment. The histological change on germinal epithelium, and Level of hormones was measured by Radioi Immuno Assay (RIA) method. Then, tissues were fixed in Buin's fixative. Sections were cut into 5 µm thicknesses and stained with Hematoxylin and Eosin (H&E). The achieved results were analyzed in SPSS software using t-test and ANOVA.

Results: Count of spermatogenic, Sertoli and leydig cells significantly decreased in the experimental group in comparison with the control and Control groups (P<0.05).also In the experimental group,titer of FSH, LH and testosterone hormone decreased significantly (P<0.05) in comparison with Control.

Conclusion: Regarding physiological role of Sertoli cells dur-

ing spermatogenesis, reduction of FSH hormone may lead to negative effects on the sperm production and reproductive potential of male Rat.

Keywords: Biolozax-H, Sperm Viability, Testis, FSH, LH

P-68: Effect of Metronidazole in Sperm Progressive Motility, DNA Fragmentation and Sperm Quality Parameters in Adult Rat

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Background: Infertility is one of the major problems in the world, sperm progressive motility has been reported to be one of the key factors. Influencing *in vitro* and *in vivo* fertilization rates. There has been an increase in the literature of studies investigated with other semen parameters, however, few reports focused on the relationship between sperm DNA fragmentation and progressive sperm motility.

Materials and Methods: Male adult rat (200-250) gr were divided into two groups of sixteen each. Control and metronidazole treated group were administered 300 mg/kg metronidazole for 90 days, respectively. The animals were sacrificed 90 days after starting treatment. The histological change on germinal epithelium, sperm quality parameters (count and normal morphology), viability and DNA fragmentation on sperm were analyzed by light microscopy, Computer-aided Sperm Analyzer (CASA).

Results: Metronidazole administration significantly decreased parameters of sperm (count and normal morphology) and increased germinal epithelium destruction. The head, mid piece and tail abnormalities of treated group were increased significantly versus control. Higher levels of TUNEL positive cells that were found in treated groups demonstrated the increasing of DNA fragmentation in sperms following metronidazole treatment.

Conclusion: Overall, our data suggest that sperm DNA damage is strongly associated with percentage of motility.

Keywords: Metronidazole, Sperm Motility, Epididymal Sperm, DNA Fragmentation

P-69: Granulosa Cells Morphological Changes in Infertile Women with PCOS Compared to Healthy Subjects Undergoing IVF

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Background: During follicular development, intraovarian paracrine signals coordinate granulosa cells (GCs) proliferation, theca cell differentiation, and oocyte maturation. In women with polycystic ovary syndrome (PCOS), ovarian hyperandrogenism and altered intrafollicular paracrine signaling perturb follicular survival, growth, and selection, causing accumulation of small antral follicles within the periphery of the ovary. Morphological changes of (GCs) have not been thoroughly investigated during this syndrome.

Materials and Methods: The study was performed on ovarian GCs of 220 women who referred for IVF-ET. The PCOS group

consisted of 120 PCOS patients, and 100 women with healthy ovaries were considered as the normal group. The syndrome was diagnosed by a gynecologist according to Rotterdam criteria (Rotterdam ESHRE/ASRM, 2004). After washing and trypsinization with 0.25% trypsin/EDTA solution, isolated GCs were resuspended in DMEM/F12 culture medium, and counted by a hemocytometer. Trypan blue 0.4% was used to determine the viable and total cell counts. GCs were seeded in 6-well plates (2×10^6 cells/well) and cultivated in 3 mL DMEM/F12 supplemented with 5% Fetal Bovine Serum, 1% L-Glutamine, and 1% Penicillin/ Streptomycin for 48 hours. In order to evaluate the purity, morphology, and growth rate of GCs, cell culture plates photography was done, and images were analyzed by ImageJ software.

Results: Typical morphology of normal GCs (N-GCs) was spherical. However, spherical and non-spherical cells were observed in both groups, but the ratio of spherical to non-spherical PCO-GCs was significantly ($P < 0.0001$) lower than that N-GCs (0.36 ± 0.02 versus 7.34 ± 0.38). Photographs analysis showed that the mean value of the diameter of spherical PCO-GCs were significantly ($P < 0.0001$) smaller than N-GCs (6.022 ± 0.054 versus $10.21 \pm 0.058 \mu\text{m}$). Trypan blue exclusion test showed significant difference in viable GCs percent between PCOS patients and normal women ($72.93 \pm 2.12\%$ versus $83.36 \pm 2.42\%$).

Conclusion: The current study findings suggest that metabolic changes in GCs of women with PCOS undergoing IVF-ET correlate with significant morphological abnormalities and decreased viability. Due to bidirectional communication between GCs with oocyte, the quality of the oocyte, consequently, the quality of the embryo are adversely affected.

Keywords: Morphology, Viability, Granulosa Cells, PCOS

P-70: Study The Protective Effect of Kombucha on Reducing The Adverse Effects of Silver Nanoparticles on The Indices Oxidative Stress (FRAP, MDA) and Serum Testosterone Hormones NMRI Mice

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Background: Today, nanotechnology is one of the most relevant topics in the world of science. Among nanoparticles, silver nanoparticles are widely used in modern technology, especially in medicine. Due to their small size, these particles pass through the cell membranes and blood testis barrier and even lead to oxidative stress in the male reproductive system. Kombucha is a traditional fermentation drink with a strong antioxidant properties that evaluates the anti-oxidant activity of the Kombucha by performing DPPH and ABTS tests. The aim of this study was to investigate the protective effect of as a potent Kombucha antioxidant against the effects silver nanoparticle on blood serum testosterone and the level of lipid peroxidation in mice.

Materials and Methods: Adult NMRI mice were randomly divided into 4 groups (n=6), control, Silver nanoparticles (500 mg/kg/day), Kombucha extract (9 ml/kg/day) and Silver nanoparticles + Kombucha extract, and treated for 35 days. Blood Samples Were taken from the heart. Serum Samples Were collected and biochemical evaluations used. Kombucha was prepared industrially, then the amount of polyphenols and DPPH and ABTS radicals and its carbohydrates, as well as glucuronic acid (by HPLC), were measured. The results were analyzed by

one-way ANOVA and Tukey's test and the means were considered significantly different at $P < 0.05$

Results: There was a significant decrease in testosterone levels in the serum of treated rats with silver nanoparticles ($p < 0.05$) and there was a significant increase in antioxidant capacity. Moreover, malondialdehyde level in this group was significantly higher than that in the control group. ($P < 0.05$). The above parameters in the nanoparticle group + kambuca were largely compensated for the silver nanoparticle group.

Conclusion: The Kombucha extract was able to protect against the seminal vesicle damage caused by silver nanoparticles by reducing the oxidative stress.

Keywords: Silver Nanoparticles, Kombucha, Testosterone, Oxidative Stress

P-71: Mancozeb Induced Cell Death in Sertoli-Germ Cells Co-Culture

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Background: Exposure to many substances such as pesticides have detrimental effects on health and are considered to contribute substantially to most diseases. They mediate some of toxic effects by regulation/induction of apoptosis, which ultimately lead to impaired fertility. Mancozeb (MZB) is a fungicide routinely used to protect field crops against fungal diseases. It has been shown to produce hepatotoxicity, neural disorders, thyroid glands and reproductive dysfunctions. We investigated whether MZB induces cell death in Sertoli-germ cells.

Materials and Methods: Experiments were carried out in Sertoli-germ cells from mice. Testes were decapsulated and cells were obtained by mechanical and enzymatic digestion. Sertoli-germ cells were seeded in complete DMEM/F12 supplemented with FBS. Cells were treated with the appropriate concentrations of MZB (1.5, 2.5 μM) for 3 hours. For assaying apoptosis, cells harvested, washed, and stained with Annexin V and PI. They were incubated at room temperature for 15 minutes in dark. Cell fluorescence was acquired by flow cytometer system

Results: Flow cytometric analysis showed that MZB caused apoptotic cell death after treatment with 1.5, 2.5 MZB (significant difference: $P < 0.001$, compared with control).

Conclusion: In summary, result of the current study suggests that MZB can induce cell death which may eventually affect the production of sperms.

Keywords: Mancozeb, Sertoli-Germ Cells, Apoptosis

P-72: Regulatory Effect of Granulocyte-Colony Stimulating Factor on Natriuretic Peptide Precursor Type C during Pre-Ovulatory Period and Successful Implantation of Embryos from Mating in NMRI Mice

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Background: Natriuretic peptide precursor type C (NPPC) is expressed by ovarian follicles. This factor participating in the maintenance of oocyte meiotic arrest at the diplotene stage of prophase I for a long time. G-CSF (Granulocyte-colony stimulating factor) is one of the hematopoietic growth factors and produce in developing follicles, fetal and reproductive tissues. G-CSF has recently been shown to be involved in oocyte maturation and ovulation. In this study we determined whether or not G-CSF plays a role in the regulation of NPPC in resumption of oocyte meiosis and also its effect on fetuses.

Materials and Methods: Immature female NMRI mice were randomly assigned to control and treatment groups were injected i.p. with 10 IU of PMSG (Pregnant Mare's Serum Gonadotropin) to stimulate follicle development. Treatment group were received G-CSF (50 μg / kg i.p.), at the time of PMSG administration, while the control group had the same volume of normal saline instead of G-CSF at the same time. 48 hours post-PMSG administration Pre-ovulatory follicles were collected for quantitative real-time PCR analyses. Also, the above agents were injected into mature mice and 48h after injecting PMSG, in order to stimulate ovulation in mature mice injected i.p. with 10 IU hCG (Human chorionic gonadotropin). On day 16 post coitus, the mature female mice of both groups were sacrificed for withdrawing their fetuses to determine their specification.

Results: The expression levels of NPPC have a significant decrease ($P < 0.05$) in the treatment group compared to the control group. In other hand the weight of fetus in treatment group (0.771 ± 0.028) were significantly more than that of control group (0.667 ± 0.026) ($P < 0.05$). Also no significant changes were found between follicle count, fetal number, CRL (Crown rump length), implantation and absorbed embryo.

Conclusion: GCSF by reducing the expression of the NPPC gene may increase fetal growth.

Keywords: G-CSF, NPPC, Gene Expression, Fetus, Mice

P-73: Effect of Polyvinyl Alcohol on The Motility, Morphology and Viability of Human Sperm after Thawing

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Background: Sperm cryopreservation has been used widely since the 1970s to store sperm in patients undergoing cancer therapy and play a vital role in treating couples with infertility. However, Cryopreservation lead to induce different in cellular organelles which result in reduction of sperm quality. Cryoprotective agents can minimize cryopreservation injury. Small concentrations of the synthetic polymer polyvinyl alcohol (PVA)

were found to inhibit the formation of ice during cryopreservation. PVA clearly shows potent ice recrystallization inhibition (IRI) activity with arrest of ice crystal growth similar to antifreeze proteins. Therefore, the purpose of this study was to evaluate the cryoprotective effects of PVA on human sperm during cryopreservation process.

Materials and Methods: Semen samples were collected from twenty normospermic men and divided into two equal parts to be diluted with two concentrations of (0.01 % and control). Semen samples were diluted with a glycerol egg yolk citrate (GEYC) based freezing medium. Then diluted semen was cooled and packed into straws. The straws were frozen in steam of liquid nitrogen (LN2) and then preserved in the LN2 and stored until thawed and used for evaluation. After thawing, motility, viability and morphology of thawed sperm were assessed. **Results:** Results showed that 0.01 % PVA improved the percentage of total motility, progressive motility and viability of human compare to control samples. Morphology was not affected by adding PVA to freezing media.

Conclusion: It can be concluded that supplementation of freezing media with PVA at concentration of 0.01%, can increase the efficiency of cryopreservation of human sperm.

Keywords: Cryopreservation, Polyvinyl Alcohol, Sperm Parameters

P-74: α -Linolenic Acid Promotes *In Vitro* Maturation and Quality of Mouse Oocytes with Polycystic Ovarian Syndrome

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Background: The maternally syntheses of mRNAs and proteins produced during oocyte growth and maturation. Therefore, the oocyte quality reflected in the stages of embryo development. In the meanwhile, polycystic ovary syndrome (PCOS) as a reproductive hormonal disorder has oocytes with poor quality under in-vitro fertilization (IVF) cycles. In this regard, a significant improvement in oocyte survival and maturation rates will have a great potential for increasing the efficiency of IVF. The α -linolenic acid (omega-3) plays an important role in reproductive physiology, affecting oocyte quality. Therefore, this study was planned to investigate beneficial effect of α -linolenic acid (omega-3) in in-vitro oocyte maturation and oocyte quality from PCOS mice.

Materials and Methods: Female NMRI mice (25-30 day-old) were divided into two groups: i) Non-PCOS, and ii) PCOS groups. PCOS mouse model induced with 4 mg/kg estradiol valerate dissolved in 0.2mg Sesame oil once a day. After 60 days, the immature follicles were collected from PCOS ovaries, and cultured in the α -MEM medium (minimum essential medium: α -MEM) supplemented with 10% bovine serum albumin (BSA) and different concentrations of α -linolenic acid (ALA) (0 [control], 50, and 100 μ M). The in-vitro maturation of PCOS oocytes in the different doses of ALA were evaluated and compared with control and non-PCOS groups. Also, the expression of TFAM gene in mature oocytes was investigated by Quantitative Real Time PCR (QRT-PCR).

Results: In this study, the TFAM gene expression was increased during in-vitro maturation of oocytes. TFAM gene was identified by QRT-PCR in all oocyte. However, the TFAM gene expression significantly increased in the MII oocytes supplement-

ed with 50 μ M ALA (95% \pm 2.1% vs. 84% \pm 2.1%, P=0.005), whereas the gene expression of TFAM in the oocytes treated with higher dose (100 μ M of ALA) significantly decreased. Also, the rate of in-vitro maturation of germinal vesicle oocytes treated with 50 μ M ALA significantly improved in comparison to control group (61/79: 77.2% vs. 59/95: 62.1%).

Conclusion: In this study, the TFAM gene expression was increased during in-vitro maturation of oocytes. TFAM gene was identified by QRT-PCR in all oocyte. However, the TFAM gene expression significantly increased in the MII oocytes supplemented with 50 μ M ALA (95% \pm 2.1% vs. 84% \pm 2.1%, P=0.005), whereas the gene expression of TFAM in the oocytes treated with higher dose (100 μ M of ALA) significantly decreased. Also, the rate of in-vitro maturation of germinal vesicle oocytes treated with 50 μ M ALA significantly improved in comparison to control group (61/79: 77.2% vs. 59/95: 62.1%).

Keywords: PCOS, IVM, Germinal Vesicle, α -Linolenic Acid, TFAM Gene

P-75: The Interfering Effects of IVF and Embryo Vitrification at 2-Cell Stage Upon miR-29a/29b Expressions in Mouse Blastocysts

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Background: Embryo vitrification is an important approach in assisted reproductive techniques (ART), which has improved clinical outcomes. miRNAs are a class of short single-stranded noncoding endogenous RNAs in eukaryotic cells that regulate epigenetic expressions of numerous genes at the posttranscriptional or translational levels. The present study assesses the effects of *in vitro* fertilization (IVF) and vitrification on miR-29a/29b expressions after IVF and vitrification.

Materials and Methods: Expression of miR-29a/29b in mouse blastocysts from control, IVF and vitrification groups was investigated by qRT-PCR.

Results: The levels of miR-29a/29b upregulated in the experimental groups as compared with the control group.

Conclusion: The results of this study have suggested that vitrification at 2-cell stage causes disruption in epigenetic mechanisms in blastocysts.

Keywords: IVF, miR-29a/29b, Blastocyst

P-76: The Role of Mediating Cognitive Fusion in The Relationship between Rumination and Depression in Infertile Women

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Background: One of the important areas of health of the infertile persons is a psychological aspect that requires special attention and prevention of disorders such as depression in them. "Cognitive fusion" refers to the human tendency to become entangled with thoughts as a result of strong belief in their literal content. The aim of this study was to investigate the role of mediating cognitive fusion in the relationship between rumination and depression in infertile women.

Materials and Methods: The sample consisted of 300 infertile women referred to the Royan Institute, a referral infertility clinic in Tehran, the capital of Iran. The sampling method was convenient. Four main questionnaires were used including demographic questionnaire, a patient's health questionnaire (PHQ-9), a cognitive fusion checklist (CFQ) and a rumination questionnaire (RRS-10). Data were analyzed with SPSS software. Statistics including Pearson correlation coefficient and path analysis were used to test the research hypotheses.

Results: The results of this study showed that there was a significant positive correlation between cognitive rumination and cognitive fusion ($P < 0.001$, $r = 0.611$), rumination and depression ($P = 0.001$, $r = 0.336$) and cognitive fusion and depression ($P < 0.001$ and $r = .5881$). Conceptual model of research and standardized coefficients.

Conclusion: In general, it can be concluded that cognitive fusion and rumination are factors affecting depression in infertile women. Therefore presence of psychologists and psychiatrists in diagnostic and treatment centers of infertility is necessary in order to identify the risk factors and to educate the correct ways of dealing with feelings of inadequacy and depression through their expertise.

Keywords: Cognitive Fusion, Rumination, Depression, Infertility

P-77: Direct Effect of Controlled Osmotic Stress on Nuclear Maturation of Bovine Oocytes

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Background: Recent studies have indicated that a well-defined and properly applied stress treatment of oocytes may induce general their adaptation, improve survival, and *in vitro* development. The current study was designed to understand the effect of controlled and sublethal osmotic stress on nuclear *in vitro* maturation of bovine oocytes as a first step toward improving tolerance to various *in vitro* procedures such as cryopreservation, enucleation and SCNT.

Materials and Methods: Bovine immature aspirated oocytes from abattoir-derived ovaries were initially cultured in isosmotic IVM medium (bicarbonate -TCM 199 supplemented with 10% FCS and 0.1IU/ml FSH) (osmolality was 280-285mOsm) for 1h adaptation. After that, the oocytes of treatment group were exposed to hyperosmotic medium (500-510mOsm IVM medium contained sorbitol) for 4h and then transferred to IVM medium. All of the maturation procedure was carried out at 38.5°C with a 5% CO₂/air atmosphere for 22-24h. Eventually nuclear maturation of oocytes was evaluated with 5µg/ml H33342 for 5min under an epifluorescent microscope.

Results: The results showed that exposure of bovine oocytes to high osmolality had not any effect on nuclear maturation po-

tential of osmotic stress-treated oocytes (12.05% MI stage and 86.14% MII stage versus 11.36% MI stage and 84.42% MII stage for control and treatment groups, respectively)($P > 0.05$).

Conclusion: Treatment with sublethal doses of high osmotic stress couldn't induce any detrimental effect on *in vitro* maturation ability of bovine oocytes and oocytes could tolerate hyperosmotic stress for up to 4h. In this study osmotic stress was carried out in the first quarter of bovine oocyte maturation period wherein the transcription potential of oocyte is still active. Accordingly, this controlled osmotic stress can induce efficient changes in the gene expression programs by intracellular signaling networks and may outline a completely new strategy in mammalian embryology.

Keywords: Osmotic Stress, Nuclear Maturation, Bovine, Oocytes

P-78: Extended Incubation of Mouse Spermatozoa Affects the Preimplantation Embryonic Development

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Background: Sperm quality is a determinant factor on the outcome of assisted reproduction and is influenced by many factors, such as time and temperature during *in-vitro* incubation of sperm. This study investigated the effects of long-term *in vitro* sperm incubation at room temperature (RT) on Sperm quality parameters and early embryonic development.

Materials and Methods: Mouse sperm samples were divided into 2 groups (fresh and incubated at RT for 24 hours). Sperm parameters were assessed according to WHO guidelines. DNA Fragmentation index (%DFI) was evaluated by SCSA. Mouse sperm were microinjected into mouse oocytes. Fertilization rate and embryo development were evaluated.

Results: The percentage of progressive motility significantly decreased in samples incubated at RT compared with fresh group ($P < 0.05$). The percentage of DFI was not significantly altered in samples incubated at RT compared with fresh group ($P > 0.05$). The morula and blastocyst rate significantly decreased after 24 hours incubation at RT ($P < 0.05$).

Conclusion: The result of this study demonstrated that extended sperm incubation affects preimplantation embryonic development.

Keywords: Sperm Incubation, Embryo Development, DNA Fragmentation

P-79: Evaluation of N-acetylcysteine (NAC) Effect on In Vitro Culture of Immature Mouse Testis Following Vitrification

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Background: Cryopreservation of testicular tissue in order to preserving spermatogonial stem cells (SSCs) is a suitable method can be provided to prepubertal boys at the risk of infertility due to cancer treatments. Cryopreserved tissue can be transplanted to the individual or cultivated in the laboratory. Due to the culture time, tissue damage is high, so optimizing the culture condition is essential. In this study, the effects of NAC presence in the culture medium of vitrified mouse testicular tissue will be investigated and cell viability will be evaluated.

Materials and Methods: Testis tissue were harvested from sacrificed immature NMRI male mice and vitrified (freezing medium: DMSO, Ethylene Glycol, DMEM, 20% FBS) After warming, testis tissues were cultured *in vitro* for 7 days on agar gel in a medium composed of RPMI, NaHCO₃ and 10% KSOR. Culture medium were supplemented by different dosages of NAC (0, 5, 10, 20, 25, 37.5, 50 mmol/L). Following 7 days of culture, cell viability were evaluated by Flow cytometry method.

Results: Our results showed that cell survival At the end of the culture period was 54% at a NAC concentration of 50 mmol/L, 43% at 37.5 mmol/L, 39% at 25 mmol/L, 33% at 20 mmol/L, 22% at 10 mmol/L and 29% at 5 mmol/L. Cell survival was significantly higher in a group cultured without NAC compared to the groups cultured in presence of NAC ($P < 0.005$).

Conclusion: Although, our results showed that the used concentrations of NAC wasn't effective enough to suppress cell death in *in vitro* culture of vitrified testes, Previous researches established the ability of NAC to inhibit apoptosis in testicular germ cells *in vitro*. Thus, it seems that higher concentrations of this antioxidant should be tested.

Keywords: Cryopreservation, Testicular Tissue, NAC, Cell Viability

P-80: Protective Effect of Misoprostol on Ibuprofen-Induced Alteration of Testes Stereological Structure in Adult Male Mice

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Background: Misoprostol, a prostaglandin E1 analog, is used clinically during treatment of duodenal and gastric ulcers with non-steroidal anti-inflammatory drugs such as ibuprofen to inhibit acid secretion and stimulate bicarbonate and mucus secretion. It is well known that long time administration of ibuprofen can reduce sperm parameter and testosterone level but there is no study about effect of ibuprofen on structure of testis. This study was conducted to evaluate the structure of testis after long time administration of ibuprofen and protective effect of misoprostol on ibuprofen-induced alterations in testis tissue of adult male mice.

Materials and Methods: In this study, 80 adult male mice were divided into 8 groups including: control, ibuprofen treated group which received 6 mg/Kg/day ibuprofen, three misoprostol treated groups which received 1, 10 and 100 µg/Kg/day mis-

oprostol and three ibuprofen+misoprostol treated groups which received 6 mg/Kg/day ibuprofen with 1, 10 and 100 µg/Kg/day misoprostol. All treatments were carried out for 40 consecutive days by oral gavage. At the end of experiment, animals were euthanized and their left testis were removed and fixed in 10% neutral buffered formalin. Tissues were processed by standard paraffin embedding and serially sectioned at 20 µm thickness. Twenty to twenty-five selected sections by systematic random sampling were stained with H&E and total volume of testis, germinal epithelium, interstitial tissue, and total numbers of spermatogenic, Sertoli and Leydig cells were estimated by point counting method based on Cavalieri's principle and optical disector technique using by stereo-investigator software. Finally, one-way ANOVA and Tukey's post-hoc were performed for data analysis.

Results: Results showed that ibuprofen reduced total volume of germinal epithelium and total number of all spermatogenic cells, Sertoli cells and Leydig cells and also increased interstitial tissue significantly compared to control group. Our results indicated that misoprostol could inhibit the ibuprofen-induced alterations in a dose dependent manner. Stereological studies revealed that misoprostol increased total volume of germinal epithelium of seminiferous tubules and total number of spermatogenic cells, Sertoli and Leydig cells as well as decreased total volume of interstitial tissue significantly compared to ibuprofen treated group.

Conclusion: Based on our results it can be concluded that administration of misoprostol can improve structure of testis after ibuprofen administration and can be considered as a suitable protective strategy for improvement of male infertility or subfertility due to NSAIDs administration.

Keywords: Misoprostol, Prostaglandin E, Ibuprofen, Testis, Stereology

P-81: The Effect of Sperm DNA Fragmentation on Parameters of Sperm, Blastocyst Formation, Embryo Quality and Pregnancy Rate in Assisted Reproductive Technique(ICSI)

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Background: The aim of the present study was to investigate the correlation between sperm DNA fragmentation standard laboratory and parameters of human semen. We have also examined an impact of sperm DNA damage on embryo quality and Blastocyst Formation after ICSI procedures.

Materials and Methods: Semen samples from 50 men have been analyzed as a part of diagnostic semen analysis and fertility evaluation by using conventional microscopic semen analyses. In each sample, the concentration, motility, morphology and vitality of spermatozoa were evaluated. Sperm DNA damage was determined by using Halo sperm test with threshold value of DNA fragmentation index (DFI) at (<15%, 15-20%, >30 %). Embryo quality was evaluated undergoing assisted reproduction technique (ART) procedures at our clinic.

Results: The results of this study indicate that the patient group with DFI ≥ 30 % had significantly lower values of standard semen parameters, Blastocyst Formation and embryo quality than the group of patients with DFI index (<15%, 15-20%). In addition Negative correlations were found between DNA fragmentation and motility ($r = -0.41$, $p < 0.001$), morphology ($r = -0.32$,

$p = 0.001$) and vitality ($r = -0.36$, $p < 0.001$). We also found that in the group of patients with DFI index (<15%,15-20%) the were significantly more embryos of better quality.

Conclusion: In men with poorer semen quality, evaluated by standard semen parameters, a higher proportion of sperm with damaged DNA can also be expected. Higher sperm DNA damage, established by Halos perm test, also had an impact on embryo quality in this group of patients.

Keywords: DNA Fragmentation, Parameters Sperm, Blastocyst Formation

P-82: Effect of Royal Jelly in The Presence of Folic Acid Supplement on NMRI Mouse Sperm Count

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Background: About 40 percent of infertility factors are related to men, which can affect a person's job, body, personality, and mentality. Royal jelly is a protein secretion from the Hypopharynx and the lower jaw glands of the worker bee. Only the food given to it during the lifetime of the queen bee and all the small larvae are fed with royal jelly. Extensive combinations of it, as well as many properties like the antioxidant properties of this material have been proven. That's why we chose this material to measure its effects on the sperm count, as well as in the presence of Folic acid supplementation, which is important in the quality of male fertility.

Materials and Methods: In this study, NMRI mouse weighing approximately 20 grams were used. Royal jelly at doses of 0.1, 0.2 mg/Kg and Folic acid, at doses of 0.08, 0.15 mg/Kg was injected intraperitoneally for 15 days. The cauda epididymis was removed by surgery and transferred to MHRM medium and studied after incubation.

Results: The results showed that the sperm count in the Royal jelly (0.1 mg/kg) and Folic acid groups (0.08 mg/kg) significantly increased compared to the control group. Other groups did not increase significantly.

Conclusion: According to the results of this study, Royal jelly has increased the sperm count, and the use of Folic acid as a supplement to it further increases the sperm count and male fertility. The results of this study can be used in the pharmaceutical industry to produce drugs to improve male fertility.

Keywords: Royal Jelly, Folic Acid, Sperm Count, Sperm Quality

P-83: Increase Expression of IL-1b Transcriptin Cultured-PBMCs

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Background: There is strong evidence that cytokines and growth factors play an important role as local mediators of the actions of steroids on the endometrium to prepare it for implantation. In humans, PBMCs were reported to induce the produc-

tion of several cytokines, inflammatory cytokines such as IL-1b which play a key role in primary immune responses. In this article, the impact of intrauterine administration of PBMCs on the expression of il1-b transcript and role il1-b in embryo implantation and pregnancy rate.

Materials and Methods: Pregnant mice were randomly divided into two groups, including embryo implantation dysfunction (EID) group; EID with PBMCs group. Mouse PBMCs were isolated from whole blood of the non-pregnant female mouse and then cultured 0, 24, or 48 hours *in vitro*. Uterine horns were excised to determine the number of pregnant mice and implantation sites on the day 7.5 postcoitum. mRNA expressions of interleukin-1b (IL-1b) in the cells were examined using the quantitative real-time polymerase chain reaction analysis (real-time PCR).

Results: IL-1b transcript was expressed in mouse cultured-PBMCs. PBMCs significantly increased IL-1b ($P < 0.05$) mRNA level in mouse PBMCs. This result showed that the expression of IL-1b mRNA in mouse PBMCs was transiently increased.

Conclusion: Intrauterine administration of mouse PBMCs derived from unpregnant mice prior to embryo implant have a good influence on endometrial receptivity and embryonic implantation in EID mice.

Keywords: Embryo Implantation Dysfunction, Peripheral Blood Mononuclear Cells, Endometrial Receptivity,il-1b, Mouse

P-84: Supplementation of Beltsville Extender with Plant-Origin Cryoprotectant for Cryopreservation of Rooster Semen

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Background: The avian sperm is susceptible to sever damages during the freeze-thaw process due to high sensitivity to cold shock. Integrity of the rooster sperm membrane should be protected during freeze-thaw process. Extenders play an important role to reduce detrimental effects of freezing on sperm due to their stabilization effects on the sperm plasma membrane. Soybean lecithin is a novel plant-origin cryoprotectant that can protect sperm during freeze- thaw process. This cryoprotectant has not been assess in combination with Beltsville extender for rooster semen freezing. The aim of present study was to assess rooster sperm parameters after cryopreservation in Beltsville extender supplemented with different concentrations of soybean lecithin (SL) compared to Beltsville extender supplement with different concentrations of egg yolk (EY).

Materials and Methods: Semen samples were collected twice a week from ten roosters. Then samples were pooled to eliminate individual difference and subsequently divided into 5 equal parts to be diluted with Beltsville Extenders containing different cryoprotectants as follow: 0.5% SL, 1% SL, 5% EY, 10% EY and 15 % EY. The diluted semen was gradually cooled to 4°C for 3 hours. Then cooled semen was loaded into 0.5-mL straws and they were exposed to liquid nitrogen vapor for 10 min, plunged into liquid nitrogen (LN2), and stored LN2 until thawed and used for evaluation of sperm parameters.

Results: The highest significant percentage of total and pro-

gressive motility were obtained in Beltsville supplemented with 1% SL ($P < 0.05$). 0.5% SL produced the lowest significant percentage of motility and viability ($P < 0.05$). Between extenders containing egg yolk, 15% egg yolk produced the higher motility and viability of thawed sperm compared to 5% and 10% EY.

Conclusion: It seems that replacement of egg yolk by soybean lecithin in Beltsville extender may produce the higher quality of frozen-thawed semen. According to the result, lecithin at concentration of 1%, improves the motility and viability of rooster semen during cryopreservation.

Keywords: Cryopreservation, Phospholipids, Antioxidant

P-85: Effect of Freezing–Thawing Process and Quercetin on Sperm Survival and DNA Integrity in Patients with Asthenospermia

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Background: Sperm cryopreservation is an important component of fertility management and much of its successful application seems to affect the reproductive outcome of assisted reproduction technologies (ART): appropriate use of cryoprotectants before and sperm selection technologies after cryopreservation seem to have the greatest impact on preventing DNA fragmentation, thus improving sperm cryosurvival rates. Quercetin is a flavonoid with high reactive oxygen species (ROS) scavenging and ion chelating activity. We were interested in testing the effect of quercetin, as an antioxidant, in preventing sperm damage during the freeze - thawing process.

Materials and Methods: Spermatozoa from 20 patients with asthenospermia were incubated *in vitro* with 50 μ M quercetin or phosphate-buffered saline as a control for 1 hours. After this incubation period and administration of these compounds during freezing process, sperm progressive motility, sperm morphology, viability and DNA integrity were assessed before and after freezing/thawing process. Sperm motility and count were assessed according to WHO criteria, eosin nigrosin assay to assess sperm viability and the acridan orange assay to assess sperm DNA integrity, and papanicolaou assay to assess sperm morphology. Statistical analysis was performed by the student's t test.

Results: Data showed that supplementation of the cryopreservation medium with quercetin induced a significant improvement in post thaw sperm parameters, compared to those of control, regarding sperm morphology ($P=0.05$), viability ($P=0.001$) and DNA integrity ($P=0.05$).

Conclusion: Quercetin could have protective effect during cryopreservation and improves the quality of cryopreserved human semen but further research is needed to confirm this effect.

Keywords: Quercetin, Cryopreservation, DNA Integrity, Sperm Viability, Asthenospermia

P-86: Effects of L- Carnitine and Fibrin Encapsulation on In Vitro Maturation and Oocyte Developmental Potential Obtained from Transplanted Mouse Ovarian Tissues **Shahi Sadrabadi F**

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Background: Ovarian tissue transplantation is emerging as a powerful approach for preserving fertility for women that are losing ovarian function. In addition, *In vitro* maturation of oocytes retrieved from grafted ovaries may overcome the fertility defects in some cases. The objective of this study was to evaluate the potential of using L- Carnitine (LC) as an antioxidant and fibrin encapsulation to improve developmental potential of oocytes obtained from grafted ovarian tissues.

Materials and Methods: NMRI mice were divided into six groups: Control (non-graft), Transplant (autograft), Saline group (autograft + saline), LC group (autograft + LC), Fibrin group (autograft + fibrin), LC + Fibrin (autograft + LC + Fibrin). 6- weeks- old mice were ovariectomized and left ovaries were transplanted into the back muscle tissue. LC (200 mg/Kg) was injected intraperitoneally one day before surgical operation and repeated until one week after grafting. On surgical day, Tissues were encapsulated in fibrin and transferred into the back muscle. Three weeks later, ovarian grafts were recovered and oocytes were harvested for *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* development (IVD).

Results: Our results indicated that the number of retrieved immature oocytes as well as successful IVM, IVF and IVD in transplanted groups was significantly lower than control group ($P < 0.05$). All transplanted groups contained some oocytes that survived following IVM, IVF and IVD and no significant difference was seen between grafted groups.

Conclusion: Our study demonstrate that LC and fibrin scaffold did not show any negative effect on transplants but could not support further development of oocytes. It seems that usage of scaffold in combination with a growth factor could improve autotransplantation results and more studies are needed in this area.

Keywords: Ovarian Transplantation, L-Carnitine, Fibrin, IVF

P-87: Effect of Melatonin on The Number and Quality of The Egg and The Number of Embryos in The Antagonist Intracytoplasmic Sperm Injection Cycle

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Background: In women with a history of intracytoplasmic sperm injection (ICSI), this study was conducted to investigate the effect of melatonin on the number and quality of the egg and the number of embryos in the antagonist cycle. The primary focus of our present study was to evaluate the influence of Melatonin on oocyte and embryos quality.

Materials and Methods: A group of 100 female infertility clients from Qom Infertility center Acecr were randomly selected and divided into two groups. Group 1 received melatonin 3mg and group 2 received matching placebo capsule. At the same time as the start of gonadotropin drugs, the group1 was given a melatonin capsule at night and continued until the oocyte retrieval day.

Results: According to the findings of the study, the average number of adult oocytes (MII) in group1 was more than group 2 (88.5 vs. 56.4) and this difference was significant. Also, there was a significant difference between the mean number of immature oocytes (MI) in both groups .

Conclusion: Despite the positive effect of melatonin on the number of MII oocytes and the improvement in the quality and number of mature oocytes, Melatonin has no significant effect on the number of embryos during antagonist cycle.

Keywords: Melatonin, Oxidative Stress, Infertility

P-88: Protective Effect of Heparin on Blood Antioxidant Enzyme Activities in Ischaemic-Reperfusion Injury of The Rat Testicle

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Background: Testicular torsion is an urologic emergency that causes testicular damage and lead to reduced fertility or infertility. It appears that the main pathophysiology of testicular torsion is ischemia/reperfusion (I/R) injury of the testis caused by the twisted spermatic cord and its release. Surgical detorsion is currently the only treatment and allows blood reperfusion. These ischemia-reperfusion injuries are associated with over production of reactive oxygen species (ROS). Many antioxidants and free radical scavengers have been proposed in recent years for treatment of testicular torsion-induced male infertility. Heparin is a naturally occurring anticoagulant produced by basophils and mast cells.

Materials and Methods: Eighteen Wistar Albino male rats weighing 250–300 g were divided into three groups: sham (group S, n = 6); torsion/detorsion (group T/DT, n = 6), and heparin pretreatment (group Hep, n = 6). The left testes were rotated 720° clockwise for 2 hours in the rats of the torsion–detorsion group (group T/DT). Rats in the treatment group underwent the same surgical procedure as the torsion–detorsion group but were also given heparin 800 IU/kg (Hep group) by an intraperitoneal route 30 min prior to detorsion. In sham group (group s), the left testes were brought out through the incision and were placed back in the scrotum. After 2 hours of reperfusion 5ml intracardiac blood samples were taken. Blood plasma malondialdehyde (MDA), Glutathione peroxidase (GPx), catalase (CAT) and total antioxidant capacity (TAC) activities, and levels were measured.

Results: Heparin significantly reduced MDA and increased CAT but GPX and TAC did not differ significantly in Hep group in comparison with T/DT group.

Conclusion: Our results suggest that heparin treatment has a protective role on I/R-induced testicular injury.

Keywords: Testicular Torsion, Antioxidant Activity, Heparin, MDA, CAT

P-89: Evaluation of Oxidative Stress and Malondialdehyde Levels after Recombinant FSH (Gonal F) Treatment in Oligozoospermia Infertile Men

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Background: FSH plays an important and essential role in spermatogenesis, maintaining and integrating the DNA sperm. Several studies showed that infertile men with normal semen parameters have low levels of stress oxidative while infertile men with abnormal semen parameters have more stress oxidative level. The aim of this study was to evaluate whether the administration of recombinant FSH (Gonal-f) could improve sperm parameters. The secondary endpoints of this study were to evaluate stress oxidative and Malondialdehyde Levels in oligozoospermia infertile men.

Materials and Methods: In the present study, an interventional study was carried out with a sampling of 50 infertile men (oligozoospermia). The semen samples were examined in accordance to the 2010 World Health Organization criteria. Then before and after treatment with rhFSH (Gonal F) sperm parameters was determined by light microscopy and oxidative stress were measured with flowcytometry assay. Also Malondialdehyde Level was evaluated with TBA assay.

Results: Measurement of sperm parameters (concentration, motility and morphology) in oligozoospermia patients before and after rhFSH treatment was significantly different and improved (P<0.001). Also, the oxidative stress and Malondialdehyde Levels of seminal plasma significantly decreased after rhFSH treatment (P <0.05).

Conclusion: According to the above results, rhFSH treatment has a beneficial effect on sperm parameters in oligozoospermia males and dramatically reduces oxidative stress. Also, increasing Malondialdehyde level could be a negative effect of oxidative stress on sperm quality and performance. In this study, Malondialdehyde Level also showed a significant decrease after treatment.

Keywords: Oxidative Stress level, Malondialdehyde level, FSH Treatment, Oligozoospermia

P-90: Fennel and Cinnamon Combined Extract Improve Spermatogenesis and Protect Testis Tissues in Busulfan Induced Infertile Rats

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Background: Busulfan is one of the common cancer treatment drugs with infertility side effect, fennel and cinnamon are two medicinal plants with fertility enhancement properties. The aim of this study is to investigate the effects of fennel and cinnamon on busulfan induced infertile rats.

Materials and Methods: 40 male wistar rats were divided into 4 groups including: sham group: healthy rats without intervention, control group: Busulfan treated rats, fennel group: busulfan and fennel extract treated, fennel and cinnamon group: busulfan, fennel and cinnamon extract treatment. Testicular tissues were sampled and the testicular physical parameters and spermatogenesis level were evaluated by H & E staining and optical microscopy imaging.

Results: The biggest and the smallest length testis were observed in cinnamon + fennel and fennel groups respectively (P <0.05). The highest and lowest sperms level was observed in the cinnamon + fennel group and fennel group respectively (P <0.001). The total average of reproductive cells in the cinna-

mon + fennel group was the most (104.17) and in control group was in least level .

Conclusion: The combined extract of fennel and cinnamon significantly protect the testicular tissues against infertility effect of busulfan, however the fennel extract alone increased the busulfan effect in rat.

Keywords: Fennel, Infertility, Cinnamon, Rat, Busulfan

P-91: Enrichment of Human Spermatogonial Cells by Culture of Testicular Cell Suspension in Obstructive Azoospermic Patients

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Background: 50 million of men are infertile, and notably, azoospermia comprises 25% of male infertility cases. Therefore, it is of great interest to generate functional gametes for patients with male infertility especially for azoospermia. Spermatogenesis is a complex process regulated by multiple interactions between developing germ cells and the surrounding microenvironment. Production of sperm *in vitro* would not only provide male gametes for azoospermic patients but also offers various methods for the *in vitro* derivation of male germ cells have been developed. So, in the present study, we investigated different methods to proliferation of SSCs in obstructive azoospermic patients.

Materials and Methods: Human testicular samples were obtained from men with obstructive azoospermia. after enzymatic digestion process, cells assigned to following groups: culture of SSCs in the dish without cover (control group), co-culture of SSCs with infertile sertoli cells (I), co-culture of SSCs with fertile sertoli cells (II), culture of SSCs on nanofiber (covered with laminin) (III), culture of testicular cell suspension (IV). Then cells were cultured for two weeks in 34-StemPro medium and evaluated colony formation of human spermatogonial cells and gene specific methylation and quantitative genes expression of pluripotency (Nanog, C-Myc, Oct-4) and specific germ cell (Integrin $\alpha 6$, Integrin $\beta 1$, PLZF) genes in five different culture systems.

Results: We found that the highest number and diameter of colonies and cellular proliferation associated with IV group which were significantly different with control group and other groups, while it was fewest in control group, III, II, I groups respectively. Expression of germ specific genes in IV group were significantly increased ($P \leq 0.05$) and levels of expression of pluripotency genes were significantly decreased in this group ($P \leq 0.05$) compared with other groups. Gene specific methylation pattern of examined genes did not show any changes during culture period in culture systems.

Conclusion: Our findings indicate that testicular cell suspension can reconstruct a microenvironment capable of regulating

proliferation of cell colonies.

Keywords: Spermatogonial Stem Cells (SSCs), Colonization, Culture Systems, Obstructive Azoospermia

P-92: The Hormonal Effect of The Artificial Sweetener (Acesulfame Potassium) on Adult Male Rats

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Background: Acesulfame potassium is a low-calorie artificial sweetener. It is important to know its possible health effects because of high consumption of acesulfame potassium. Therefore, this study aimed to investigate effect of the intraperitoneal administration of acesulfame potassium in pregnancy and after lactation on adult male offspring sexual and gonadotropin hormones.

Materials and Methods: In this study, acesulfame potassium with four different doses, 50 mg, 100 mg, 200 mg and 400 mg/kg body weight was injected intraperitoneally to pregnant rats (wistar) and then to their adult offspring after lactation until 60th. Blood samples were also collected after puberty and the levels of FSH, LH and testosterone were measured by using Elisa method. Also, mother's rats and their offspring were weighted during study. ANOVA was used to compare data between groups.

Results: The mean weight in both treatment groups with concentrations of 50 and 200 mg / kg was significantly higher than that in controls and the other groups. There was no statistically significant difference in LH levels between different groups. Administration of acesulfame potassium significantly reduced FSH levels in neonates who received two different doses of 50 mg and 100 mg/kg BW ($P < 0.01$). Testosterone level was significantly ($P < 0.001$) increased in 50 mg/kg 200 mg/kg and 400 mg/kg as compared with controls.

Conclusion: It seems that the use of acesulfame potassium as an artificial sweetener in food products and its use by pregnant mothers and infants probably have hormonal effects, although there is no evidence that this alteration causes spermatogenesis changes.

Keywords: Acesulfame Potassium, Pregnancy, Testosterone, Male Rat

Female Infertility

P-93: Different Expression of Immune Responses in Patients with Repeated Implantation Failure

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Background: In spite of good clinical endometrial condition and good-quality embryos, implantation failure sometimes occurs. Repeated implantation failure (RIF) is a condition in which the embryos implantation decreases in the endometrium. Maternal immune cells such as Th2 and Treg cells (CD4+) which populate the decidua are specialized not only to develop placenta but also to minimize the chances of embryo to be attacked as a foreign entity which is transplanted. Understanding the role of these decidual T cells, in normal and abnormal implantation has been limited. Therefore, current study aimed to evaluate the expression of Th2-type cytokines such as IL 4, IL 10 and Treg cytokine receptors CCR4, CCR 8 and CD86 gene, in women with RIF.

Materials and Methods: Ten eligible patients with RIF and 10 fertile women as control were allocated to different groups: case group (n=10) and control group (n= 10). Genomic evaluation using qPCR was performed for all pipelled endometrial samples in the luteal phase for both groups.

Results: IL 4, IL 10, CCR4, CCR 8 and CD86 genes expression were detected in endometrial samples of case and control groups. The mean relative expression of these genes was higher in control compared to case group.

Conclusion: The production of the Th2-type cytokine IL-4 and IL-10 and Treg cytokine receptors showed to be important for the induction and the maintenance of semi allograft tolerance. These findings suggested that Th2-type cytokines may inhibit the Th1 responses which promote semi allograft tolerance and therefore may improve implantation success.

Keywords: Repeated Implantation Failure, Th2 Cells, Treg Cells, Endometrial Sample,

P-94: Effect of the Time Interval between The Injection of HCG and The Time of ICSI on Assisted Reproduction Outcomes

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Background: Assisted Reproductive Technology (ART) results depend on many factors which affect follicular development, oocyte maturation, fertilization and subsequently embryo development. One of the factors is the time interval between the injection of hCG and the time of Intracytoplasmic Sperm Injection (ICSI). The aim of this study was to effect of the time interval between the injection of hCG and the time of ICSI on assisted reproduction outcomes.

Materials and Methods: A total of 102 women referred infertility treatment center of besat hospital, sanandaj underwent IVF/ICSI cycles during the study period. The metaphase II oocytes were retrieved after 36 hours of hCG injection. The cycles were divided into 2 groups: in group I, ICSI was done after hours 38-40 from the hCG injection (including 2-4 hours of oo-

cyte incubation). In group II, ICSI was performed after 42—44 hours from the hCG injection (including 6-8 hours of oocyte incubation).

Results: A total of 765 metaphase II oocytes were obtained of which ICSI was performed in 366 oocytes in group I and 380 in group II (P > 0.05). The remaining oocytes were conventionally inseminated (IVF). There was a significant difference between the two groups in the fertilization, pregnancy, and implantation rate, a higher pregnancy and implantation rate was found in group II (P < 0.05).

Conclusion: The present study indicated the time interval between the injection of hCG and the time of ICSI affected on assisted reproduction outcomes.

Keywords: ART, IVF, ICSI, hCG,

P-95: The Impacts of Controlled Ovarian Stimulation (COH) on Autoantibody Levels in Women Undergoing In Vitro Fertilization/Intracytoplasmic Sperm Injection Cycles

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Background: Some evidence indicated that the autoimmune responses are contributed in etiology of female infertility and controlled ovarian stimulation (COH) may have impact on humoral immunity by the time of embryo transfer and concluded that further studies are required. This study was conducted to evaluate the effect of COH with gonadotropins on the serum levels of autoantibodies in the women who underwent *in vitro* fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) cycles and to compare basal levels of these autoantibodies between groups according to history of COH.

Materials and Methods: In this prospective cohort study the volunteered infertile women who underwent IVF/ICSI cycles, were recruited with regard to the inclusion criteria, from October 2014 to March 2016 in the Royan Institute. The COH was performed according to standard long GnRH agonist protocol. The mean levels of the autoantibodies including anti-nuclear (ANA), anti-smooth muscle, anti-ovarian (AOA), anti-mitochondrial, anti β 2-glycoprotein I, anti-parietal cell (PCA) and anti-follicle-stimulating hormone (FSH) antibodies were measured at three time points: on the 3–5 days of the menstrual cycle, one week after starting of COH and on the ovum pick-up (OPU) day. Data analysis was done using independent sample t test and one-way analysis of variance (ANOVA), followed by the Tukey post hoc test.

Results: Of all participants (n = 189), 73 women had history of COH and 116 women did not have such history. The analysis indicated that the autoantibodies changes during COH were similar in both groups. COH has no significant impact on the level of autoantibodies one week after stimulation and on ovum pick-up day. The mean levels of autoantibodies before stimulation were compared among the patients groups according to different cause of infertility. The analysis revealed that patients with unexplained factor had higher mean level of PCA than

those with male factor (35.8 ± 20.9 vs. 8.5 ± 2.6 ; $P=0.02$) and those with PCOS (35.8 ± 20.9 vs. 7.9 ± 4.3 , $P=0.05$).

Conclusion: No significant effect of COH was found on the studied autoantibodies by the time of OPU; however, more researches with specific autoantibodies profiles on this topic require to be undertaken to draw appropriate conclusion.

Keywords: Assisted Reproductive Cycles, Serum Autoantibody Levels, Controlled Ovarian Stimulation

P-96: The Effect of L-Arginin on The Fallopian Tubes and Uterus Histology in The Mouse Model of The Endometriosis

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Background: Infertility in women is one of the most common problems. One of the most important causes of infertility in women is endometriosis. In this disease, the uterine endometrium connects to the peritoneum outside of uterus and leads to inflammation and changes in the structure of the uterus and fallopian tubes. L-Arginine, based on improved metabolism, can be a solution to improve the complications of endometriosis on the uterus and uterine tubes and improve their histology. Therefore, the aim of this study was to investigate the effects of L-arginine on uterus and fallopian tubes in mouse model of endometriosis.

Materials and Methods: In the first stage, induction of endometriosis in mice was done by injection of suspension of endometrium separated from the uterus of donor mice. In the second stage, the control group was divided into four treatments: control without any treatment, control receiving L-arginine intraperitoneally (250 mg/1kg, every day), control receiving danazol intraperitoneally (25 mg/1kg, every other day) and control receiving L-arginine together with danazol. The endometriosis group was divided into four treatments: endometriosis without any treatment, endometriosis receiving L-arginine intraperitoneally (250 mg/1kg, every day), endometriosis receiving danazol intraperitoneally (25 mg/1kg, every other day), and endometriosis receiving L-arginine together with danazol. At the end of the treatment period, the mice were dissected then uterus and fallopian tubes were separated and histologically examined.

Results: study on sections of uterus and fallopian tubes tissue with optical microscope showed that induction of endometriosis causes inflammation in the uterus and fallopian tubes, increasing epithelial cell length, increasing cell proliferation, decreasing myometrium layer thickness, dilating of the blood vessel, increasing glands and their secretions, reducing the cilia of the epithelial cells in fallopian tubes, adhesion and obstruction of the fallopian tube. Microscopic examination showed that L-arginine improves some of the symptoms of endometriosis, include decreasing cell proliferation and length of endometrial cells, reducing the secretion of the glands, and reducing adhesion. Also Injection of danazol improved symptoms of endometriosis, include reducing cell proliferation, decreasing secretions and adhesions in fallopian tubes and the reducing obstruction of fallopian tubes.

Conclusion: It can be suggested that L-arginine and danazol can reduce the complications of endometriosis, and simultaneous injection of L-arginine and danazol greatly reduces the effects of endometriosis on the uterus and fallopian tubes.

Keywords: Endometriosis, L-Arginine, Uterus, Fallopian Tubes, Mouse

P-97: Sitaformin Reduces The Serum Level of Anti-Mullerian Hormone More Significantly than Metformin in Infertile PCOS Patients

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Background: Women with Polycystic Ovary Syndrome (PCOS) have a large number of small follicles in the pre-antral and antral stages and their AMH serum concentrations is higher than normal women. So Anti-Mullerian hormone (AMH) may have a role in disordered folliculogenesis in women with PCOS. Today, blood glucose lowering and type 2 diabetes drugs, especially metformin, are widely used in the treatment of PCOS(3). Since it has been suggested that insulin play an important role in the pathophysiology of PCOS, Some studies have focused to investigate the serum level of AMH during treatment with metformin in patients with PCOS. Sitagliptin, as an oral antihyperglycemic agent, is used in the treatment of diabetes mellitus type 2, and recently is considered as an antihyperglycemic agent in the treatment of type 2 diabetes and clinical investigations of PCOS. The aim of this study was to compare the effects of metformin and sitaformin (a combination product abstained from sitagliptin and metformin) on serum levels of AMH in the PCOS patients suffering from infertility.

Materials and Methods: In this clinical trial, 60 infertile PCOS patients were selected based on the Rotterdam criteria and candidate for IVF/ICSI. Then they divided into 3 groups (n=20) including metformin (500 mg of metformin/ two times per day) group, sitaformin (50/500 mg of sitaformin/ two times per day) group and placebo group. All patients were undergoing treatment with antagonist GnRH protocol. In all groups, medications were administrated from the day 3 of the previous cycle to the egg pancreas. Data was analyzed statistically using one-way ANOVA and Tukey's test and the means difference was consider significantly different at $P<0.05$.

Results: A significant decrease in the Serum level of AMH was found in the metformin and sitaformin groups when compared to the placebo-treated group ($p\leq 0.03$). Meanwhile this parameter significantly decreased in the sitaformin group compared to the metformin group ($p=0.04$).

Conclusion: The results of this clinical study showed that sitaformin is more effective than metformin in reducing the serum level of AMH in women with infertility due to PCOS. Therefore it seems that sitaformin has therapeutic efficiency for treatment of PCOS.

Keywords: Polycystic Ovary Syndrome, Anti-Mullerian Hormone, Metformin, Sitagliptin, Sitaformin

P-98: Effects of Assisted Reproductive Techniques on Human Placental and Neonatal Weight

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Background: Use of assisted reproductive techniques (ARTs)

has increased throughout the world and numbers of babies were born as a result of ARTs has reached approximately 5 million. Several studies have shown higher maternal/perinatal complications and adverse outcomes in pregnancy and childbirth in ARTs compared with natural pregnancies. These outcomes may be related to the reproductive technology or associated with maternal factors who undergone ARTs, which needs to be elucidated.

Materials and Methods: In this study, we have compared maternal pre-pregnancy BMI, maternal blood pressure (BP), neonatal birth weight (NBW), placental weight (PW) and their ratio (NBW/PW) in patients who conceived naturally (C) (n=20) or conceived after transfer of fresh ICSI embryos (frICSI) (n=17) or frozen ICSI embryos (fICSI) (n=18). All the babies in all groups were delivered by cesarean surgery.

Results: Our results have shown that there is no significant difference in maternal pre-pregnancy BMI, Maternal BP and NBW between groups (P>0.05). However the PW in control group was significantly lower than frICSI and fICSI groups (541.56 ± 8.47gr, 662.93 ± 20.41gr and 724.16 ± 15.67, respectively) (P<0.05). In addition the ratio of NBW/PW in control group was significantly higher than frICSI and fICSI groups (6.04 ± 0.10, 4.74 ± 0.12 and 4.46 ± 0.04, respectively) (P<0.05).

Conclusion: Our results showed that embryo and maternal conditions can affect placentation.

Keywords: ART, Placenta, Embryo, Maternal, ICSI

P-99: Expression of 11β-Hydroxysteroid Dehydrogenase Type 1 and 2 (11BHSD1, 2) in Subcutaneous Adipose Tissue of Pregnant Women with PCOS and Non-PCOS Pregnant Women

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disease, occurring in 2.2–10% of adolescence and childbearing age women. Clinical and/or biochemical hyperandrogenism is an important feature of PCOS. Adipose tissue is not only an energy storage organ, but also the largest endocrine organ, playing an important role in energy balance, reproductive function maintenance and steroid storage and metabolism. The increasing epidemic of PCOS has led to a great deal of interest in the function of adipose tissue (AT) and dysfunction of AT has been involved in the pathogenesis of PCOS. The enzyme 11BHSD1 catalyzes the regeneration of active cortisol from inert cortisone while 11BHSD2 participates in inactivating cortisol to cortisone. Although attention has focused on local sources of cortisol and, in particular, on the AT expression of 11BHSD-1, very limited information exists on expression of 11BHSD-1 and 11BHSD2 in AT of PCOS women. Thus our objective was to assess mRNA abundance of 11BHSD1 and 11BHSD2 in abdominal subcutaneous AT of pregnant women with PCOS and also non-PCOS pregnant women at delivery day.

Materials and Methods: In this case control study, signed informed consent was obtained from all subjects, abdominal

subcutaneous AT samples (3-4 g) from 13 pregnant women with PCOS and 13 pregnant non-PCOS women were collected during cesarean section. Samples were stored at liquid nitrogen (-196 °C) until analysis. 11BHSD1, 2 genes expression were measured by real time qPCR technique.

Results: The level of mRNA expression of 11BHSD1 and 11BHSD2 did not differ significantly between two groups (P>0.05). The level of mRNA expression of 11BHSD1 was lower in PCOS subjects and 11BHSD2 expression level in PCOS group was higher. Also, no significant differences were found with respect to age and body mass index before pregnancy and at delivery day among non-PCOS and PCOS AT.

Conclusion: Based on our data it can be hypothesized that lower expression of 11BHSD1 lead to less conversion of cortisol to cortisone in PCOS that may amplify cortisone accumulation in AT of PCOS than non-PCOS women. Further studies are warranted to replicate these findings as well as investigate gene expression profiles in larger sample size.

Keywords: PCOS, Adipose Tissue, Glucocorticoids

P-100: Diagnostic Chlamydia Trachomatis by PCR in Women with Frequent Abortions in Chalus (North of Iran)

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Background: Chlamydia trachomatis (C. trachomatis) is a sexually transmitted disease, considered as one of the most common causes of bacterial infections. This study aimed at scrutinizing the Chlamydia trachomatis screening tests using vaginal samples. The relationship between Chlamydia trachomatis infection and abortion was also sought.

Materials and Methods: In this case-control study which was conducted in Gynecology Clinic of Razi Hospital of Chalus, Iran from August 2017 to January 2018, a total of 50 vaginal swabs were collected. Detection of C. trachomatis DNA was performed from vaginal swabs by amplification of MOMP gene. Independent T-test and Chi-square were used for comparison of quantitative and qualitative variables, respectively. P<0.05 was considered significant.

Results: Examining the endocervical swabs of women revealed that the prevalence of C. trachomatis was obtained 5(10%). The prevalence of Chlamydia trachomatis infection in woman with abortion and in women with over 3 pregnancies was 10%. There were significant differences between chlamydia infection and duration of sexual activity. No significant difference was discovered between the presence of this infection and vaginal abnormalities.

Conclusion: Those women with history of spontaneous abortion should be examined for presence of Chlamydia trachomatis infection and, necessary measures should be taken to avoid further miscarriages.

Keywords: Diagnostic, Chlamydia Trachomatis, PCR, Frequent Abortions

P-101: Proteome Profiling of Early-Stage Serous Ovarian Cancer

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Background: Serous carcinoma, the subtype of ovarian cancer has the highest occurrence and mortality in women. Proteomic profiling using mass spectrometry (MS) has been used to detect biomarkers in obtained tissue from patients with ovarian cancer. Thus, this study aimed at analyzing the interactome (protein-protein interaction (PPI)) and (MS) data to inspect PPI networks in patients with early-stage serous ovarian cancer.

Materials and Methods: For proteome profiling in early stage serous ovarian cancer, 2DE and mass spectrometry were used. Differentially expressed proteins which had been determined in early-stage serous ovarian cancer and experimental group separately were integrated with PPI data to construct the Query-Query PPI (QQPPI) networks.

Results: 6 Hub-bottlenecks proteins with significant centrality values, based on centrality parameters of the network (Degree and between), were found including Transgelin (TAGLN), Keratin (KRT14), Single peptide match to actin, cytoplasmic I(ACTB), apolipoprotein A-I (APOA1), Peroxiredoxin-2 (PRDX2), and Haptoglobin (HP).

Conclusion: This study showed these six proteins were introduced as hub-bottleneck protein. It can be concluded that regulation of gene expression can have a critical role in the pathology of early-stage serous ovarian cancer.

Keywords: Proteome Profiling, Early Stage, Serous Ovarian Cancer

P-102: Recurrent Pregnancy Loss and Infertility Due to A New Mutation in an Iranian Family

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Background: Recurrent pregnancy loss (RPL) is a multifactorial disorder and defined as two or more consecutive miscarriages that affects up to 5% of clinical pregnancies. Several factors are associated with RPL, however, in 35–60% of miscarriages the etiology remains unexplained. In recent years, the advent of high throughput technologies, such as next generation sequencing (NGS), have provided new capabilities for discovery of too small genetic changes in hereditary diseases such as RPL. In this study, we used whole-exome sequencing approach (WES) to find pathogenic variants in an Iranian family with RPL history.

Materials and Methods: After finding an affected family, clinical investigations were done. Genomic DNA was extract-

ed from blood samples and qualified and quantified DNA was subjected to WES. DNA sequencing was done by HiSeq 2000 from Illumina®, and the reads were aligned to the human reference genome (hg19). After variant calling and filtering, the variations that scored as 'tolerated' and 'benign' were excluded. And at the end, a candidate variant was confirmed by sanger sequencing method in family members.

Results: Clinical investigations such as thrombophilia's, uterine anatomy, hormonal /endocrine disorders, immunologic factors, infections, karyotyping and Microarray-based comparative genomic hybridization (array CGH) in the proband were normal. A new missense mutation was identified in 100% analyzed subjects with RPL history.

Conclusion: In conclusion, to our knowledge, this is the first study investigating genetic variations in RPL through a family-based exome sequencing which found a new mutation in 3 out of 3 (100%) RPL patients. Our results expand our knowledge on the involvement of a new gene in human pregnancy complications. Indeed, our findings confirm that WES is a useful alternative approach to reach a genetic diagnosis in patients.

Keywords: Recurrent Pregnancy Loss, Next Generation Sequencing, Whole Exome Sequencing

P-103: Association between Altered Titers of Antiphospholipid Antibodies and Recurrent Implantation Failure

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Background: Recurrent implantation failure (RIF) is failure to achieve clinical pregnancy after transfer of three good-quality embryos in a minimum of three fresh/frozen cycles in a woman under the age of 40 years. Immunological causes of RIF are of controversial aspects of implantation, mainly because antiphospholipid antibodies (aPL) are an important cause of recurrent pregnancy loss. So, the emerging role of aPL on implantation and pregnancy complications led us to investigate these antibodies in RIF patients.

Materials and Methods: For this respect a pilot study was performed on 30 RIF women referred to Royan Institute and 10 healthy women with at least one child (control group). RIF patients had no immunological disease. Consent was obtained from all women according local ethical approval then, blood samples were collected from them (blood collection of RIF patients was through ART procedure). Quantitative detection of IgG/IgM antibodies against cardiolipin, B2GPI, phosphatidyl ethanolamin and phosphatidyl-serine plus native human prothrombin was conducted using ELISA immunoassay. Also lupus anti-coagulant test was performed on all samples.

Results: The results showed that titers of antibodies against cardiolipin, B2GPI and phosphatidyl ethanolamin were higher

in RIF patients vs. controls. Titer of anti phosphatidylserine-prothrombin antibody in RIF women was significantly higher than healthy women. Results of lupus anti-coagulant test were negative for all participants.

Conclusion: These findings showed association between altered levels of antiphospholipid antibodies titers with recurrent implantation failure and women infertility.

Keywords: RIF, Antiphospholipid Antibodies, ELISA

P-104: Duration of Luteal Phase Support in Frozen Embryo Transfer Cycles: A Randomized, Controlled Phase III Trial

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Background: Luteal phase support for frozen embryo transfer (FET) cycles is essential for maintaining conceptus. The luteal phase is supported with different dose and duration of estradiol (E2) and progesterone (P) until 8-12 week of pregnancy. E2 has teratogenic effects on fetus such as low birth weight and urogenital abnormalities, moreover an increased risk of thromboembolism for pregnant woman. We aimed to evaluate the consequences and ongoing pregnancy rate after interrupting E2 at a time to identify gestational sac with heart beat while P continued until twelve gestational weeks in women undergone FET cycle.

Materials and Methods: A total of 291 patients were eligible for recruitment (30 pregnant women in each arm) with surplus embryos who undergone FET. Endometrial prepared with standard long GnRH protocol and for luteal phase support patients received 6mg oral E2 and intramuscularly P 100 mg/d and embryos on day 2-3 were transferred. Control group continued E2 until 12 week of pregnancy, while for cases after identify gestational sac with heart beat (the 6 week of pregnancy) by vaginal ultrasonography, E2 will discontinued. P will remain use until twelfth gestational week in both groups. The E2 and P of maternal serum were assessment at the 12 weeks of gestational.

Results: Thirteen patients not consent to participate and 200 patients who agreed to participate were not randomized because they did not achieve pregnancy or carry out all randomized criteria. Sixteen patients were therefore randomized. There were no significant differences in the spontaneous abortion (0/30[0%] vs. 2/30[6.7%]) and blighted (1/30[3.3%] vs. 3/30[10%]), between the study and control (P>0.05). The mean serum E2 concentration (3994.85 ± 2072.74 and 3184.88 ± 1566.61 pg/mL, respectively) and P (57.56 ± 7.27 and 55.56 ± 10.38 ng/mL, respectively) at 12 weeks of gestational were not different between case and control group (P>0.05).

Conclusion: There was no significant complication following interrupt of E2 at 6 gestational weeks, because placenta is a major source for product of E2 and P. Our knowledge about side effects of hormonal treatment in first trimester is unclear which makes this important.

Keywords: Estradiol, Frozen Embryo Transfer, Luteal Phase Support, Ongoing Pregnancy Rate, Progesterone

P-105: Optimal Method of Control Ovarian Stimulation in Patients with Polycystic Ovarian Syndrome; A Randomized

Controlled Trial

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Background: Currently, optimal method of ovarian stimulation in patients with polycystic ovarian syndrome is unknown. The present research aims to study the efficiency of minimal ovarian stimulation method in the treatment of infertile patients with polycystic ovarian syndrome and also the effect of gonadotropin type on treatment cycles with antagonist GnRH.

Materials and Methods: A total of 120 eligible patients were randomly allocated into four groups of ovarian stimulation to IVF/ICSI: minimal ovarian stimulation with recombinant FSH, minimal ovarian stimulation with Human menopausal gonadotropin, mild ovarian stimulation with recombinant FSH and mild ovarian stimulation with Human menopausal gonadotropin. IVF/ICSI outcomes were compared between groups. Data analysis included t test, ANOVA and χ^2 .

Results: Demographic characteristics and hormonal profiles of the patients did not differ between groups. The statistical analysis showed that there were significant differences between groups regarding the stimulation duration (P < 0.000), number of retrieved oocytes (P = 0.000), number of obtained embryos (P = 0.001) and also cancellation (P = 0.01) and OHSS risk (P = 0.008). There were no statistically significant differences in the fertilization rate (P = 0.815) and implantation rate (P = 0.654) between the groups.

Conclusion: On the basis of present results the minimal ovarian stimulation with human menopausal gonadotropin is optimal method of control ovarian stimulation in patients with polycystic ovarian syndrome; however, larger randomized clinical trials are required.

Keywords: Polycystic Ovarian Syndrome, Minimal Ovarian Stimulation, Mild Ovarian Stimulation

P-106: Computational Analysis of Association Pattern Among Patients with Mullerian Mixed Tumor and Serous Ovarian Cancer

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Background: Ovarian cancer is the fifth most deadly cancer in women, and is usually diagnosed too late. malignant mixed

Mullerian tumour (MMMT) of the uterine is an extremely rare and aggressive malignancy. The majority of these tumors arise in the uterus, they can also occur in ovaries, fallopian tubes, and cervix. These two diseases cause problems in the fertility and often is requires to ovary preservation. The purpose of this study is to finding the common and important genes as a therapeutic target.

Materials and Methods: All genes involve in mullerian mixed tumor and serous ovarian cancer were obtained from Genecard and disease databases. Then Common genes of these two diseases were identified by venn diagram. The network was founded by string web server and Cytoscape software (version 3.6.1), also main component of the network was analyzed with parameters including degree, betweenness, and closeness.

Results: Output of genecards database shown 312 genes in mullerian mixed tumor and 2048 genes in serous ovarian cancer is differential expressed. 202 genes were common between mullerian mixed tumor and serous ovarian cancer. IGF1, LEP, GHRL, BRCA1, LIF, CGA, GHR, SHBG, GATA6 are genes with high degree among the 202 genes. Nine crucial were analyzed by GO analysis, in which via molecular function among them "hormone activity" (GO:0005179, adj.P value: 1.26e-6) was disclosed as top category, Also Biological Process (GO:0048639) and Cellular Component (GO:0000931) were identified.

Conclusion: The analyses of these common genes showed that there are crucial in cancer progression. Therefore, these genes and pathways involved in it can be considered as an appropriate target for cancer control and treatment. And so will be helpful in controlling infertility.

Keywords: Mullerian Tumor, Ovarian Cancer, Infertility, Genecards, Cytoscape

P-107: Investigation of The Level of Insulin-Like Factor 3 in Fetal Amniotic Fluid in Second Trimester in Pregnant Women Referring to Treatment Clinic in Ardabil Province

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Background: By increasing the age of marriage and the gestational age, the probability of infertility and the formation of embryos with genetic problems and the birth of newborns with congenital anomalies also increases. In such circumstances, the use of diagnostic methods for the health of the fetus during pregnancy is emphasized to prevent the risk of the birth of children with abnormalities of the nervous system, and in particular Down syndrome. One of these diagnostic methods is amniocentesis, which is done before birth, and usually between 15 to 25 weeks of gestation, to examine genetic data and to detect abnormalities in the birth and sex of the fetus. Different factors can be titrated and diagnosed by amniocentesis, including amniocentesis and human insulin-like factor-3 levels, and testosterone levels in amniotic fluid in male and female embryos and analysis. The relationship between this factor and testicular loss, The sex of the fetus, testosterone and other hormones.

Materials and Methods: Of pregnant women who were candidates for amniocentesis, 109 pregnant women entered writing with written consent. Sampling of amniotic fluid was carried out under the supervision of a prenatal practitioner and samples were transferred to the lab in refrigerated conditions to be analyzed and evaluated.

Results: Data analysis revealed that there is a significant rela-

tionship between maternal weight and age, and the amount of testosterone in the fetus. As the age and weight of the mother increase testosterone levels, there is a direct relationship between testosterone and insulin-like factor- 3.

Conclusion: Testosterone is one of the most important steroid hormones in the human body, and its production begins in the mother's womb before the second month of the embryo, which plays a role in determining the sex of the fetus, and the main source of its secretion in the male body is testicular. There is a relationship between this hormone and the insulin-like factor-3. The insulin-like factor plays an important role in the decline of the testicles in the fetus, so timely diagnosis and treatment of these disorders is important.

Keywords: Amniocentesis, Insulin- Like Factor- 3 (INSL3), Testicular Depression, Testosterone (T)

P-108: Evaluation the Effect of Anti-thyroid Antibody on Intracytoplasmic Sperm Injection in Infertile Euthyroid Patients

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Background: Thyroid gland disorders in adults are often caused by autoimmune processes that trigger excessive thyroid hormones or damage to the gland and reduce the production of thyroid hormones. Antithyroid peroxidase antibody and anti-thyroglobin antibody, also called anti-microsomal antibody, are found in Hashimoto's thyroiditis, Graves' disease, postpartum thyroiditis, and chronic pain-free thyroiditis. Contradictory reports on the effect of anti-thyroid antibodies in cycles IVF is there. Considering the controversial studies on the effect of anti-thyroid antibodies in IVF-ET candidates in order to investigate the presence of this antibody in HIV patients in the Motahari Hospital, this study was designed to investigate the association and effect of ATA in IVF cycles.

Materials and Methods: : In the procedure, patients with IVF-COH who were candidates for IVF-COH were screened for serum TSH and anti-TPO levels. Blood samples were taken at -70 ° C and AntiTPO was evaluated in all samples. TSH and T4 levels have been used to evaluate the thyroid gland activity. Then, the patients were followed up and ICSI results were evaluated in two groups. After extraction of the dominant follicle, the injected HCG injection of 36-34 units of ascribed and ICSI follicles was performed and finally, ICSI and the number of pregnancy and abortion were evaluated in both groups.

Results: In this study, 119 female candidates for IVF-ET were evaluated. The mean \pm standard deviation of the age of the women was 32.24 ± 5.63 years (the lowest and highest were 21 and 44 years, respectively). We divided the subjects into two groups as the first positive group in terms of TPO antibody and the second group negative TPO antibody. Of the 119 women examined, 100 patients (84%) were negative for TPO and 16 (16%) were positive for this antibody. The mean number of oocytes in the first group was 8.54 ± 5.7 and 8.54 ± 16.4 in the first group (P value = 51.5). . The mean number of embryos in women with positive TPO was higher than those with negative TPO (Pvalue = 0.21). The frequency of positive β HCG in the first group (31.6) and in the second group (19) was 19 (P value 0.22). The side effect of OHSS was in the first group (15.8) and in the second group (8) 8. (0/70 = P value). The frequency of

abortion in the first group (40) 2 and in the second group (52.6) was 10. (1 = Pvalue)

Conclusion: In this study We compared the prevalence of positive anti-thyroid antibodies, the number of oocytes, the number of embryos derived from cycles, the frequency of positive IVF, and the side effects of β HCG, including excessive ovarian hyperstimulation syndrome, frequency of abortion, and multiplicity of frequency in these two groups. Considering the fact that there is no significant difference between the existing cases, further studies are needed on the importance of the anti-thyroid antibody response in IVF cyclist candidates.

Keywords: ICSI, Anti TPO, Infertility, IVF

P-109: The Impact of Intrauterine Human Chorionic Gonadotropin Injection on Live Birth Rate after Vitrified-Warmed Embryo Transfer: A Randomized Clinical Trial

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Background: On the basis of recent review articles, there was no evidence that miscarriage was influenced by intra-cavity human chorionic gonadotropin (IC-hCG) administration, irrespective of embryo stage at transfer or dose of IC-hCG and well-designed multicenter trials are required to provide robust evidence. The present study was conducted to assess the influence IC-hCG infusion before embryo transfer on the clinical pregnancy and live birth rates after vitrified-warmed embryo transfer (ET) in programmed cycles.

Materials and Methods: This study was a single-blind randomized clinical trial for eligible patients underwent frozen ET cycles with long-term hormonal GnRH agonist protocol for endometrial preparation. Immediately prior to ET, the women were randomly divided into three groups. In the experimental group, 7-10 minutes before embryo transfer, 500 IU of hCG with a 40 μ L of culture medium was injected into the uterus. In the first control (sham) group, 7-10 minutes before ET just 40 μ L of culture medium intrauterine was infused. In the second control group, no intervention was done. The pregnancy outcomes were compared in the three groups by using appropriate statistical tests.

Results: Finally 180 patients allocated into three groups. There was no significant difference in terms of patients' characteristics among three groups. No significant difference was found in terms of clinical pregnancy among three groups. The miscarriage rate in control group (0%) was significantly lower than those of in the sham and hCG groups (9.8% and $P=0.01$, 6.6% and $P=0.04$, respectively). Also, live birth rate (39.3%) in control group was significantly higher than those of in the sham and hCG groups (16.4% and $P=0.005$, 23% and $P=0.051$, respectively).

Conclusion: It was found that intrauterine injection of 500 IU hCG before vitrified-warmed ET at cleavage stage has no beneficial effect on pregnancy outcome and is not suggested. (NCT02355925)

Keywords: Intrauterine Human Chorionic Gonadotropin, Live Birth Rate, Vitrified-Warmed Embryo Transfer

P-110: Prediction of Gestational Diabetes Mellitus in Women Conceived by Assisted Reproductive Technology: Body Mass Index and Fasting Glucose Cut Points

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Background: Despite several studies in general population, there is no direct evidence regarding the cut-off levels of pre-pregnancy body mass index (BMI) and first trimester fasting glucose (FG) to predict the risk of developing GDM in infertile women as a high risk population. Accordingly, the present study was designed to evaluate the predictive value of BMI and FG to predict GDM risk, and then to determine the cut-points of BMI, FBS and the combination of two biomarkers (BMI+ FG) for diagnosis of at risk pregnant women conceived by ART to target clinical surveillance in a more effective manner.

Materials and Methods: In this case-control study, 270 singleton pregnant women consisted of 135 (GDM) and 135 (non-GDM) who conceived using ART were assessed. The diagnosis of GDM was confirmed by a one-step glucose tolerance test (O-GTT) using 75 g oral glucose. BMI was classified base on World Health Organization (WHO) criteria. The association between BMI, FG, and BMI+FG with the risk of GDM development was determined by logistic regression and adjusted for confounding factors. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the value of BMI, FG, and BMI+FG for the prediction of GDM.

Results: There were significant differences between GDM and non-GDM groups in terms of maternal age, BMI, family history of diabetes, and history of polycystic ovary syndrome ($P<0.05$). The overweight and obese women had 3.27, and 5.14 folds increase in the odds of developing GDM, respectively. There was a 17% increase in the risk of developing GDM with each 1 mg/dl increase in FG level. The cut points 84.5 mg/dl for FG (with 72.9% sensitivity, 74.4% specificity), 25.4 kg/m² for BMI (with 68.9% sensitivity, 62.8% specificity), and 111.2 for BMI+FG (with 70.7% sensitivity, 80.6% specificity) was detected.

Conclusion: On the basis of present results, since the combination of BMI and FG is associated with a better prediction value; the early screening and high-quality prenatal care should be recommended in women undergone ART with the co-occurrence of the pre-pregnancy BMI (≥ 25.4 kg/m²) and high FG (≥ 84.5 mg/dl) in the first-trimester of the pregnancy.

Keywords: Gestational Diabetes Mellitus, Assisted Reproductive Technology, Fasting Blood Glucose, Body Mass Index

P-111: The Effect of L-Carnitine on The Ovarian Histology

in Mouse Model of The Endometriosis

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Background: Endometriosis is a common disease in women that is characterized by the presence and growth of endometrial tissue, having blood vessels in abnormal locations outside the uterine cavity, which is closely related to infertility and causes complications and abnormalities in the ovary. Improving endometriosis can help improve fertility. L-carnitine, based on the improvement of metabolism and antioxidant and anti-inflammatory effects, can be a solution to improve the complications of endometriosis on the ovary and improve histology. The purpose of this study is to investigate the effect of L-carnitine on the ovarian histology in mouse model of endometriosis

Materials and Methods: At the first stage, induction of endometriosis in mice was done by injection of suspension of endometrium separated from the uterus of donor mice. In the second stage, the control group was divided into four treatments: control without any treatment, control receiving L-carnitine intraperitoneally (250mg/1kg, every day), control receiving danazol intraperitoneally (25mg/1kg, every other day) and control receiving concurrently L-carnitine and danazol. The endometriosis group was divided into four treatments: endometriosis without any treatment, endometriosis receiving L-carnitine intraperitoneally (250mg/1kg, every day), endometriosis receiving danazol intraperitoneally (25mg/1kg, every other day), and endometriosis receiving concurrently L-carnitine and danazol. At the end of the treatment period, the mice were dissected then ovaries separated and histologically examined.

Results: Induction of endometriosis causes problems in ovary include reducing the number of folliculogenesis, decreasing the number of different follicles and their quality, decreasing the quality and health of the ovum, increasing the number of cysts and bleeding in the ovary and adhesion of the ovary to the tissues and surrounding pelvic organs. Treatment with L-carnitine lead to reducing the number of ovarian cysts, increasing the number of types of follicles especially the antral follicles and graph, reducing ovarian hemorrhage. Also, treatment with danazol reduceing the number of ovarian cysts, increasing total follicles, and reducing the amount of bleeding. Co-administration of danazol and L-carnitine also improved the condition of the ovary, as well as increasing follicular growth and ovulation in the endometriosis ovary.

Conclusion: Therefore, it can be concluded that danazol and L-carnitine can improve the condition of the ovary compared to the endometriosis group, which can improve the complications of the disease.

Keywords: Ovary, Endometriosis, L-Carnitine, Mouse

P-112: Prevalence of Adenomyosis in Infertile Women Using Transvaginal Ultrasound in A Single-Center Tertiary Referral Clinic

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Background: This study was designed to use ultrasound imaging to determine adenomyosis prevalence in infertile women.

Materials and Methods: This cross-sectional screening study included 963 eligible infertile women referring for the first time to the infertility clinic of Royan Institute between 2014 and 2017. The patients were referred for transvaginal ultrasound prior to initiating the treatment. Women who had vaginismus excluded from the study. Adenomyosis was diagnosed in presence of enlarged globular uterine body, asymmetric uterine wall thickening, heterogeneous myometrial echotexture, small myometrial cysts/subendometrial cysts, Echogenic nodules or linear striations radiating. Patients were divided into groups of women with adenomyosis and without adenomyosis.

Results: The overall prevalence of adenomyosis was found to be 17.1%, of which 53.4% were severe adenomyosis. The mean age of participants was 29.9 ± 5.4 years (range 18 to 45 years). The prevalence of patients had concomitant fibroids and ovarian endometrioma were 21.8 % and 8.5 % respectively. The mean age was significantly higher in the adenomyosis group than in the non-adenomyosis group (32.1 ± 5.1 vs. 29.5 ± 5.3 years, $P > 0.001$). There were significant differences between groups in regards to cause of infertility and infertility type ($P = 0.004$ and $P < 0.001$ respectively). The presence of adenomyosis has been shown to be associated to ovarian endometrioma [8.5 % vs. 1.4 %; $P < 0.001$]. The results of the multivariate logistic regression analysis showed that the strongest predictors of adenomyosis were age (OR = 1.05, 95% CI = 1.01-1.09), cause of infertility (OR = 1.01, 95% CI = 1.03-1.23) and ovarian endometrioma (OR = 3.36, 95% CI = 1.74-6.50).

Conclusion: Adenomyosis seems to be a clinical condition with a high prevalence that may affect the reproductive outcomes.

Keywords: Prevalence, Adenomyosis, Infertility, Ultrasound Diagnosis

P-113: Drug Discovery for Treatment of Pregnancy Complications with APS

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Background: Antiphospholipid antibody syndrome (APS or APLS), is an autoimmune disease, caused by antiphospholipid antibodies. APS is associated with pregnancy complications,

including preeclampsia, thrombosis, fetal growth restriction, fetal loss and recurrent implantation failure. β 2-glycoprotein I (β 2GPI) is the major antigen for antiphospholipid antibodies and associated with thrombosis. β 2GPI is a glycoprotein with five repeated domains. β 2GPI exists in different conformations, an open J-shaped conformation, a circular conformation and a S-shape conformation. The fifth domain of J-shaped conformation binds to negatively charged phospholipids and causes platelet aggregation and blood clotting reactions.

Materials and Methods: Purpose of this study was selection of the best compounds for treatment of APS. For this purpose, bioactive compounds of herbals that were involved in preventing blood coagulation and platelet aggregation, drugs that are prescribed during pregnancy complications and all of the FDA drugs were selected. Based on these compounds, a library was created for studying in silico. Then we applied drug repurposing strategy using virtual screening and molecular dynamics for drug Discovery.

Results: In this study, it was shown that the J-shaped of β 2GPI was transformed into a s-shaped during the 50 ns. Docking result of drugs that are prescribed during pregnancy against β 2GPI's fifth domain showed that Heparin had a higher ΔG than other drugs that are prescribed during pregnancy. The results shown that folate and warfarin had the highest ΔG after heparin. Eight FDA drugs and Thirteen bioactive compounds had maximum binding negative free-energy change (ΔG) with β 2GPI. FDA drugs that were involved in the inhibition of platelet aggregation had been considered for molecular dynamics (MD) studies. Also the bioactive compounds that had maximum negative ΔG of interactions with β 2GPI's fifth domain were selected for more accurate studies using MD. Therefore, vorapaxar and antrafenine as FDA drugs and lonchocarpic acid, oleracein C and procyanidin as bioactive compounds were selected for MD studies. Lonchocarpic acid as a bioactive compound of portulaca oleracea had maximum binding negative ΔG with β 2GPI. Based on MD results, lonchocarpic acid complex was more stable than other complexes.

Conclusion: Vorapaxar, antrafenine and bioactive compounds of portulaca oleracea might be useful candidates for treatment of antiphospholipid antibody syndrome.

Keywords: Drug Repurposing, Virtual Screening, Molecular Dynamics, Beta-2-Glycoprotein I, Antiphospholipid Antibody Syndrome

P-114: Ulmus Minor Bark Hydro-Alcoholic Extract Ameliorates Histological Parameters and Testosterone Level in an Experimental Model of PCO Rats

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Background: Polycystic ovary syndrome (PCOS) is a common and multifactorial disease associated with female factor infertility. Ulmus minor bark (UMB) is one of the medicinal plants used in Persian folklore as a fertility enhancer. In the current study, we aimed to elucidate the effect of UMB hydro-alcoholic extract on histological parameters and testosterone condition in

an experimental model of PCOS rats.

Materials and Methods: Thirty female rats were randomly divided into five groups: (1) control, (2) vehicle, (3) PCOS/50 mg [6 mg/kg dehydroepiandrosterone (DHEA) + 50 mg/kg UMB hydro-alcoholic extract], (4) PCOS/150 mg (6 mg/kg DHEA + 150 mg/kg UMB hydro-alcoholic extract), and (5) PCOS (6mg/kg DHEA). All interventions were performed for 21 days. Afterwards, stereological analysis was done for determination of ovarian volume and follicle number. The serum level of testosterone was measured by ELISA kit.

Results: UMB hydro-alcoholic extract improved the total number of the corpus luteum in the treatment groups when compared to the PCOS group ($P<0.05$). PCOS/150 mg and PCOS/50 mg groups showed significantly lower total number of the primordial, primary, and secondary follicles as well as testosterone level compared to the PCOS group ($P<0.05$). The total number of antral follicles and volume of ovary did not differ significantly between groups.

Conclusion: UMB extract may be an effective and good alternative in improving PCOS histological and testosterone disturbances although further studies are warranted to confirm the safety of UMB plant in human.

Keywords: Ovary, PCOS, Stereology, Ulmus minor Bark (UMB), Testosterone

P-115: Vitamin C Restores Ovarian Follicular Reservation in a Mouse Model of Aging

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Background: Aging is a complex biological process that involves the depletion of germ cells and loss of ovarian follicles. The maternal age is related with infertility and reduction of ovarian follicles reservation. The aim of this study evaluation of the effect of vitamin C on restores ovarian follicular reservation in a mouse model of aging.

Materials and Methods: In this study, 24 adult mice (28–30 g), divided into four experimental groups: (1) control, (2) vehicle (DMSO 2%) (10 μ l) (3) busulfan and (4) busulfan+ SCs (1 \times 10⁴ cells/ μ L). SCs were isolated from 4-week-old mouse testis and after using anesthetics, 10 μ l of SCs suspension (1 \times 10⁴ cells/ μ l) was injected over 3–5 min, into each testis and subsequently, sperm samples were collected from the tail of the epididymis. Afterward, the animals were euthanized and testis samples were taken for histopathology experiments, and RNA extraction, in order to examine the expression of c-kit, STRA8 and PCNA genes.

Results: Our data showed that SCs transplantation could notably increase the total sperm count and the number of testicular cells, such as spermatogonia, primary spermatocyte, round spermatid, SCs and Leydig cells, compared to the control, DMSO and busulfan groups. Furthermore, the result showed that expression of c-kit and STRA8 were significantly decreased in busulfan and busulfan/SCs groups, at 8 weeks after the last injection ($P<0.001$), but no significant decrease was found for PCNA, compared to the control and DMSO groups ($P<0.05$).

Conclusion: These findings suggest that SCs transplantation may be beneficial as a practical approach for therapeutic strategies in reproductive and regenerative medicine. We further highlight the essential applications that might provide a mechanism for correcting fertility in males, suffering from cell deformity.

Keywords: Vitamin C, Ovary, Stereology, Aging, Mouse

P-116: Fetal Reduction to Twins or A Singleton Pregnancy and Postpartum Complication

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Background: Multiple pregnancies are high-risk pregnancies and usually were complicated by, pre-eclampsia low birth weight, preterm delivery and augmented perinatal morbidity and mortality. The trans abdominal fetal reduction technique under ultrasound-guided is a practical method for the prevention of preterm delivery and medical and obstetric complication associated with multiple pregnancy concluded from ART treatment

Materials and Methods: Multiple pregnancy reduction was done in 65 cases of ART pregnancies (IVF) and one quintuplet, two quadruplet resulting from intrauterine insemination (IUI) and 4 twins using tran abdominal procedure with guiding ultrasonography, others were triplet and reduced to twins or singleton pregnancy, then at every age of termination we considered postpartum hemorrhage and complications until 6 weeks.

Results: Of the 65 fetal reduction surgeries, 60 (92.30%) were done between the eleven and fourteen weeks of gestation, five (7.6%) between fourteen and eighteen weeks of gestation. fifty-eight patients (89.23%) underwent reduction from triplets to twins, two from quadruplet to twins, and one from quintuplet to twin pregnancy. One of triplet to twin was failed and aborted but others were successfully performed. After delivery all at cesarean section, one case return with bleeding 6 weeks later and had parts of reduced fetus were detected. Three of 64 (4.68%) cases had sever postpartum hemorrhage and one case had reoperation due to retained tissue (of reduced missed fetuses).

Conclusion: We should pay attention high to risk of postpartum hemorrhage and retained tissue after fetal reduction as a complication even at cesarean section.

Keywords: Fetal Reduction, Assisted Reproductive Technology, Post Partum Hemorrhage

P-117: Comparison of two Ovarian Stimulation Protocols on Developmental Competence of Ovum Pick Up Derived Jersey Oocytes

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Background: This experiment was the first study in the world conducted to evaluate the effect (s) of two protocols of ovar-

ian stimulation using FSH, prior to ovum pick up (OPU), on quantity and quality of retrieved oocytes and their subsequent developmental competence.

Materials and Methods: The Jersey heifers (n=9) after estrous synchronization were randomly assigned into three experimental groups: I) heifers receiving 225 mg FSH in two equal doses for one day (1-Day) II) heifers receiving 225 mg FSH in four decreasing doses for two days (2-Day), and III) heifers receiving no FSH treatment (control).

Results: There were no significant differences between groups in quantity and quality of recovered COCs per OPU session. The cleavage rate was significantly higher in 2-Day group compared to the other groups (83.6 ± 2.8 vs. 67.9 ± 3.3 and 74.4 ± 2.8 ; $P=0.001$). The blastocyst rate in 2-Day group on day 7 (44.8 ± 4.1 vs. 22.7 ± 3.4 and 33.7 ± 3.4 ; $P=0.002$) and day 8 (47.3 ± 4.3 vs. 26.7 ± 3.4 and 36.1 ± 3.4 ; $P=0.001$) was significantly greater compared to other groups. No significant difference in transferable embryos was observed among groups.

Conclusion: In conclusion, two days of ovarian stimulation, in comparison to one day stimulation, increased the quality of recovered COCs which was evidenced by their higher developmental competence.

Keywords: Ovum Pick Up, Jersey, Embryo, Development

P-118: The Most Frequent Genotypes of Human Papillomavirus in Women From Rasht-Iran

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Background: Cervical cancer is one the most frequent cancer among sexually active women. Human papillomavirus (HPV) is the most important cause of cervical cancer. More than eighty subtypes have been identified which can be subdivided to high risk and low risk on the basis of tumorigenicity. There are many studies which implicated that the prevalence of HPV subtypes is related to age and ethnics. The aim of the present study was to evaluate the distribution of HPV in women from Rasht-Iran.

Materials and Methods: The present cross sectional study involved two thousand women who referred to Jam pathobiology and medical genetic laboratory Rasht-Iran from 2016 to 2018. Two hundred seventy-six patients were identified as HPV positive. Following DNA extraction according to the manufacturer instructions, multiplex polymerase chain reaction (PCR) was performed based on specific primers of low and high risk HPV. Finally, under UV light electrophoresis, PCR products were evaluated. Patients were grouped based on age: <20 (n=3), 20-39 (n=225), 40-59 (n=37), 60-79 (n=2).

Results: Of total women, 13.8% were positive for HPV subtypes which 6.5% was high risk. The patient's age ranged from 18 to 61 with average of 32.5 ± 7.3 . Two hundred and sixty-seven (94%) of collected specimen were cervical Pap smear and 6% were other genital samples. The most frequent genotype among HPV positive patients were HPV6 (27%) and HPV16 (11%). Eighty four (30.5%) of all patients were infected with multiple HPV subtypes. Women aged 20-50 years had the highest frequency of HPV subtypes (n=256).

Conclusion: HPV6 and HPV16 were the most frequent low and high risk HPV. The prevalence of HPV in women aged 20-

50 years was the highest. The identification of ethnic and age related HPV subtypes help clinicians to develop therapeutic and preventive plans and vaccination.

Keywords: Human Papilloma Virus, Cervical Cancer, Prevalence, Genotypes

P-119: The Assessment of Sufficient and Insufficient Vitamin D in Treatment of Infertile PCOS Patients

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Background: Vitamin D deficiency is common in women with polycystic ovary syndrome (PCOS). 67–85% of women with PCOS having serum concentrations of 25-hydroxy vitamin D (25OHD) <20 ng/ml. Some evidence is suggested that vitamin D deficiency has a role in incidence of PCOS patients. The aim of this study is to assess the effect of sufficient and insufficient levels of vitamin D on treatment of PCOS infertile patients.

Materials and Methods: In this clinical trial, 60 PCOS infertile patients referred to Dr.rasekh clinic that randomly divided in case and control groups. Each of these two groups were randomly divided into case and control groups (15 participants in each group). Data collection was performed via questionnaires by midwives and statistical analysis by SPSS 21.

Results: There was a significant relationship between follicular size and use of vitamin D (P<0.05). Increased endometrial thickness and reduced BMI was detected In using vitamin D groups. The overall pregnancy rate was twenty (66.7%) that was related to using vitamin D groups.

Conclusion: Vitamin D has a positive effect on the treatment of PCOS patients. Although response to the treatment is better in patients with insufficient vitamin D levels, but prescription of this vitamin in patients with sufficient vitamin D level is also effective. According to rare side effects related to this vitamin, its low cost and toxicity, we recommend to add 1000 units of vitamin D3 daily to drug regimen of infertile PCOS patients.

Keywords: Vitamin D, PCOS, Infertility, Pregnancy Rate

P-120: PCOS Infertile Patients' Treatment with Optimal Results and Decrease the Rate of OHSS

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies affecting women in the reproductive age group, and is one of the most common causes of ovarian hyperstimulation syndrome(OHSS) in infertile patients. The aim of this study was decreased OHSS due to induction of ovulation with clomiphene citrate and then letrozole in comparison of letrozole and cabergolin and an optimal pregnancy rate.

Materials and Methods: This is prospective clinical research from Dr.Rasekh infertility clinic,jahrom city, Iran. 64 infertile

PCOS women were selected with 47 months infertility. The average age of them is 27.3 years (STD=5). The patients were divided into two groups: Group A; 36patients (40%); initially tablet clomiphene citrate (from day 3 of menstrual cycle),then the second drug Letrozole was started from day 8 to 11 menstrual cycle. Group B; 28 patients (31.1 %); initially tablet Letrozole (from day 3 of menstrual cycle),then the second drug cabergolin started from day 8 to 11 menstrual cycle. Pregnancy rate in group A, 8 (22%) and in group B; 6(21%). The patients were monitored for ovulation by transvaginal ultrasonographic folliculometry, with measurement of number and size of the follicles, as well as endometrial thickness. Human chorionic gonadotrophin (HCG) was injected intramuscularly when at least one mature follicle 18-22 mm diameter. Data was analyzed with SPSS version 21.

Results: The rate of OHSS was similar in both groups nearly zero (P<0.05). This means that both methods of treatment can be prevented the creation of OHSS. Pregnancy rate in both groups was almost the same (P<0.05).

Conclusion: We recommend for the prevention of OHSS that is a serious complication of PCOS infertile women treatment which one of the two mentioned methods is to be used. Whilst in both methods has been favorite fertility rate. The goal of treatment is minimal side effects with optimal result.

Keywords: OHSS, PCOS, Clomiphene, Letrozole, Cabergolin

P-121: The Effect of L-Carnitine on The Uterine Tubes and Uterus Histology in The Mouse Model of The Endometriosis

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Background: Endometriosis is a female condition that causes complications in the reproductive system, especially in the uterine and uterine tubes, and has a direct relationship with infertility. So improving endometriosis improves fertility. L-carnitine, based on improved metabolism and antioxidant role, can be a solution to improve the complications of endometriosis on the uterus and uterine tubes and improve their histology. The target of this study was to investigate the effects of L-carnitine on uterus and uterine tubes histology in mouse model of endometriosis.

Materials and Methods: At the first stage, induction of endometriosis in mice was done by injection of suspension of endometrium separated from the uterus of donor mice. In the second stage, the control group was divided into four treatments: control without any treatment, control receiving L-carnitine intraperitoneally (250 mg/1kg, every day), control receiving danazol intraperitoneally (25 mg/1kg, every other day) and control receiving concurrently L- carnitine and danazol. The endometriosis group was divided into four treatments: endometriosis without any treatment, endometriosis receiving L- carnitine intraperitoneally (250 mg/1kg, every day), endometriosis receiving danazol intraperitoneally (25 mg/1kg, every other day), and endometriosis receiving concurrently L-carnitine and danazol. At the end of the treatment period, the mice were dissected then uterus and uterine tubes were separated and histologically examined.

Results: The results showed that inducing endometriosis caused serious problems in uterus and uterine tubes such as severe inflammation, fibrosis, increasing proliferation and secretion

of epithelial cells, dilating of blood vessels, decreasing cilia in uterine tubes, increasing of foamy discharge in the lumen of uterine tubes and the closure of the channels. Microscopic examination showed that L-carnitine improves some of the complications of endometriosis, such as decreasing cell proliferation of endometrial cells and their secretion, reducing inflammation and fibrosis. Also Injection of danazol improved of endometriosis, such as reducing cell proliferation, decreasing secretions and adhesions in uterine tubes and the channels were almost open. Simultaneous administration of L-carnitine and danazol more reduced the complications of endometriosis, and the animal's estrus cycle was more regularized.

Conclusion: Since endometriosis has a bad effects on the uterus and uterine tubes and this has a direct relationship with infertility, we can increase the fertility rate by reducing the harmful effects of endometriosis on the uterus and uterine tubes. Therefore, it can be concluded that treatment with danazol and L-carnitine improving the condition of the uterus and uterine tubes compared to the without any treatment

Keywords: Endometriosis, L-Carnitine, Uterus, Uterine Tubes, Mouse

P-122: Effect of Low Glycemic Diet on Overweight Women with and Without Polycystic Ovary Syndrome

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Background: Some evidence suggested that obese women with PCOS in comparison with controls had more difficulties in weight loss. We aimed to compare the effects of the hypocaloric low glycemic diet (LGI) on anthropometric variables and insulin resistance in women with and without PCOS.

Materials and Methods: Out of 108 women who invited for the purpose of the present study, 62 participants (PCOS= 28, non PCOS= 34) completed the study. The diagnosis of PCOS was based on Rotterdam criteria. We frequently matched our control subjects with PCOS cases based on age and BMI. For both groups energy restricted LGI diet was designed to cause a weight loss of at least 0.5 kg per week. To assess food compliance, a 3 day dietary food records (2 week days and one weekend day) was completed twice a month. Anthropometric and metabolic measurements were assessed and analyzed at zero point, 12 weeks and 24 weeks after intervention.

Results: Both groups tolerated the dietary intervention and no adverse effects were reported. At zero point and during intervention, physical activity levels in both groups did not differ significantly. Weight loss was similar in cases and controls (PCOS= -8.53 ± 3.77 , non PCOS= -8.12 ± 3.79 , $P < 0.001$). Until week 24, waist circumference was reduced for 7% with no difference between the two groups ($P > 0.05$). In both groups, no significant reductions in glucose were observed. With regard to insulin there was a significant reduction within both groups when compared to baseline in PCOS (14.25 ± 1.17 mu/L vs. 9.45 ± 1.27 mu/L, $P = 0.001$) and non PCOS (13.41 ± 1.2 mu/L vs. 9.64 ± 0.78 mu/L, $P = 0.001$), although the insulin change did not differ significantly between the two groups ($P = 0.59$). Reduction in HOMA (PCOS= -0.88 ± 0.05 , non PCOS= -0.79 ± 0.1 , $P <$

0.001) were reported which showed no difference between the two groups ($P > 0.05$).

Conclusion: The energy restricted LGI diet induces equivalent beneficial effect on anthropometric and metabolic variables in women with or without PCOS.

Keywords: Polycystic Ovary Syndrome, Low glycemic index (LGI) diet, Weight reduction, Insulin

P-123: The Effect of Myo-Inositol Supplementation on Oocyte Quality In PCOS Women Undergoing ART Cycles

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Background: Polycystic ovary syndrome (PCOS) is the most common cause of infertility, hormonal and metabolic disorder, 5%–10% of women in reproductive age. Some evidence has shown that these disorders cause the disturbance in steroidogenesis pathway. Inofolic is a combination of myo-inositol and folic acid, which improves pathological conditions associated with PCOS and improves fertility. Recently many authors have investigated whether supplementation with inofolic, improved the oocytes' quality and increase the number of oocytes collected after ovarian stimulation in patients with PCOS undergoing *in vitro* fertilization. But so far, no study has examined the signaling pathway of inofolic on steroidogenesis and folliculogenesis. The aim of this study was to evaluate the effects of inofolic on oocyte quality, fertilization rate, embryo quality and steroidogenesis signaling pathway in cumulus cells of PCOS women undergoing ART cycles

Materials and Methods: We studied 60 women undergoing IVF using intracytoplasmic sperm injection (ICSI), 40 of them were PCOS and 20 women were non-pco. After randomization, 20 women received 4 g myo-inositol combined with 400 mg folic acid. and 20 women received 400 mg folic acid alone as placebo. 20 women was non-pcos (control group). The treatment was made for 12 weeks, before starting the antagonist cycle until the day of ovum pick up. Oocytes and embryos quality were assessed according to (ESHRE) guidelines. The gene expression FSHR, CYP11A1, CYP19A1, ER, StAR, 3β-HSD2 in cumulus cells were analyzed using real-time RT-PCR.

Results: The percentage of metaphase II oocyte, fertilization rate and embryo quality significantly improved in the study group which received inofolic ($P < 0.05$). Furthermore, the gene expression of FSHR, CYP11A1, CYP19A1, ER, StAR, 3β-HSD2 were significantly higher in the study group ($P < 0.05$) and the gene expression of LHR was significantly lower in the study group.

Conclusion: The findings of our study provides some new molecular evidence about the possible mechanism of Myo-Inositol effect on the fertilization rate, quality of oocytes and embryos

in PCOS women undergoing ART cycles. This might be related to modification of steroidogenesis pathway in cumulus cells.

Keywords: Myo-Inositol, Infertility, Polycystic Ovary Syndrome
P-124: Circulating Insulin-Like Growth Factor Binding Protein-5 Levels and Promoter Variation Are Associated with *In Vitro* Fertilization and Embryo Transfer Outcome (IVF-ET) in An Iranian Population

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Background: Infertility is a global social and economic concern. Successful implantation requires an appropriate interaction between the embryo and the endometrium. *In vitro* fertilization (IVF) outcome depends on the completion of three successive steps: ovarian stimulation, embryo quality and most importantly, embryo implantation in the endometrium. Insulin-like growth factor (IGF) was shown to improve the clinical pregnancy rate. IGF is recognized as a potent growth factor because of its ability to promote the proliferation endometrial and trophoblastic cells and embryo implantation. The IGF axis includes two homologues IGFs (IGF-1 and -2), at least six high affinity IGF binding proteins (IGFBPs) including IGFBP-1 to IGFBP-6, and two membrane bound IGF receptors (IGFR1 and 2). IGFs in circulation and other physiological fluids are associated with IGFBPs. The aim of this study was to examine the association of IGFBP-5 circulation levels and gene polymorphisms with IVF-ET outcome in an Iranian population.

Materials and Methods: In this case-control study, 100 infertile women who had unsuccessful IVF (IVF-) history and 110 infertile women who had successful IVF outcome (IVF+) as controls were included. The samples were collected from Mehr fertility institute, Rasht, Iran. Genomic DNA was extracted from peripheral blood. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR). The circulating concentration of IGFBP-5 was measured by enzyme-linked immunosorbent assay (ELISA).

Results: Statistical analysis was performed using the χ^2 test and the Med Calc version 12.1.4. The results from allele and genotype frequencies showed that there are significant differences between (IVF+) and (IVF-) groups. Furthermore, we showed that there is a significant change in serum IGFBP-5 levels in IVF- as compared to IVF+ ($P < 0.001$).

Conclusion: It is thus concluded that IGFBP-5 serum levels and promoter polymorphism are associated with IVF-ET outcome in our population. Further studies are needed to confirm the role of IGFBP-5 in IVF-ET outcome.

Keywords: IGFBP-5, Genetic Polymorphism, Serum, IVF-ET Outcome

Genetics

P-125: Association Study of HSP90 Gene Expression and Total Antioxidant Capacity (TAC) with Sperm Motility in Infertile Patient

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Background: Heart shock proteins are a group of proteins that play an important role in fertility ability and important many studies verified that balance disturbance between antioxidants and free radicals has a distractive effect on sperm competence that aim of this study was to evaluate the correlation between sperm motility, hsp90gen expression and total antioxidant value in infertile patterns.

Materials and Methods: The study was performed on 50 infertile and 10 fertile individuals according to WHO criteria. Briefly after sample collection, sperm motility, total antioxidant values and gene expression were evaluated.

Results: Progressive sperm mobility progressive sperm motility rate decreased significantly in infertile group (24.33%, 17.25%) as compared to control group (42.22%, 31.55%). percentage of inactive sperm rose dramatically in infertile group (56.87%) in comparison with fertile group (26.23%) significant decrease of TAC was observed in fertile patients there wasn't any observable differences between two experimental group gene expression.

Conclusion: It seems that sperm motility is associated with total antioxidant, but independent from hsp90 gene expression.

Keywords: HSP90, Antioxidant, Sperm

P-126: Evaluation of Thrombophilic Factors in 230 Iranian Patients Affected with Recurrent Spontaneous Abortion

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Background: One of the most common complications of reproduction is RSA (recurrent spontaneous abortion). Based on previous researches, genetic abnormalities are one of the most common causes of RSA. It has been confirmed by many studies that there is a relationship between inherited thrombophilia and miscarriage and other adverse pregnancy outcomes. Thrombophilia can be congenital or acquired. The most important types of congenital thrombophilia are those that arise as a result of over activity of coagulation factors and associated with variation in additional genes including F2, F5, F5R2, MTHFR (Methylenetetrahydrofolatereductase), F13, FGB (Fibrinogen-Beta) and EPCR (Endothelial protein C receptor).

Materials and Methods: In this study, we have investigated some important polymorphisms (F5 Leiden (G1691A), F5R2 (4070A>G or H1299R), F2 prothrombin (G20210A), F13 (V34L), FGB (455G>A), MTHFR (C677T and A1298C) and EPCR (4600A>G and 4678C>G)) using PCR-Sequencing method, in 230 patients with at least two idiopathic spontaneous abortions.

Results: According to our results, MTHFR and EPCR are two of the most important factors that have the most association with RSA. In MTHFR (A1298C) the frequency of genotypes AA, AC and CC in controls group were respectively 67%, 28.5% and 4.5% and in samples group were respectively 38.3%, 49.5% and 12.5%. In MTHFR (C677T) the frequency of genotypes CC, CT and TT in controls group were respectively 63.5%, 31% and 5.5% and in samples group were respectively 72%, 37% and 15%. In EPCR (4600A>G) the frequency of genotypes AA, AG and GG in controls group were respectively

89%, 10.3% and 0.7% and in samples group were respectively 60.5%, 34.5% and 5%. In EPCR (4678C>G) the frequency of genotypes CC, CG and GG in controls group were respectively 27.5%, 56% and 16.5% and in samples group were respectively 15%, 63% and 22%.

Conclusion: There are many studies that these polymorphisms are responsible for increasing the risk of eradication of recurrent abortions, while there are other reports that eliminate the association between these polymorphisms and recurrence. This difference can be due to differences in the criteria for choosing patients and the control group. The present results of this study confirm the association between RSA and investigated variants in FV Leiden, F2, FGB, MTHFR, and EPCR, while we did not find this association for variant in F5R213. In addition, the variant in F13 seem to be a protective factor against RSA.

Keywords: RSA, Thrombophilic, MTHFR, EPCR,

P-127: Elevated Gene Expression in Tubal Pregnancy and Its Relation to Ectopic Implantation

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Background: Establishment of viable pregnancy requires embryo implantation and placentation. Ectopic pregnancy (EP) occur outside of the uterine cavity with around 98% implanting in the fallopian tube. Among the effective genes involved in the receptive endometrium and appropriate implantation procedure FGF2, CSF1, HBEGF, MUC1 and VEGF-A are of considerable importance. This study aims to investigate mRNA expression of above mentioned genes in fallopian tubes (ampulla) and endometrium of women who had EP compared with fallopian tubes and endometrium of pseudo-pregnant women.

Materials and Methods: This case-control study consisted of ampulla and endometrium of EP patients who underwent salpingectomy (n=5) and five women overtaken pseudo-pregnant fallopian tubes and endometrial tissue that underwent hysterectomy. Prior to tubal sampling, each control woman received an injection of human chorionic gonadotropin (HCG) to produce a state of pseudo-pregnancy. All samples collected in window of implantation phase. Ampulla sections are separated slightly away from trophoblastic implantation site. Fallopian tubes and endometrial tissue from both groups were procured, and after the RNA extraction and cDNA synthesis, mRNA expressions were analyses in endometrial tissue and ampulla region of the fallopian tube by qRT-PCR. Data were analyzed by SPSS 21 software, and $P \leq 0.05$ was considered as significant.

Results: Our results showed altered expressions of these genes in endometrial tissue and ampulla region in both groups. The expression of MUC1, HBEGF, and FGF2 genes in endometrial and ampulla was significantly higher in the case group than

control ($P \leq 0.05$). The expression of VEGFA and CSF genes was significantly higher in the endometrium of the EP whereas was significantly higher in ampulla of the control group compare to patients ($P \leq 0.05$).

Conclusion: Different expression of selected genes in the WOI of endometrium and ampulla of tubal pregnancy might have a role in the pathogenesis of ectopic embryo implantation, especially due to critical function of these in appropriate implantation process. This data suggests a fallopian tube probably predisposing implantation and non-receptive endometrium in EP.

Keywords: Tubal Pregnancy, Gene Expression, Heparin-binding EGF-like growth factor, Colony Stimulating Factor 1, Fallopian Tube

P-128: Preimplantation Genetic Screening Efficacy on Pregnancy Outcome in Recurrent Gestational Trophoblastic Disease

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Background: Gestational trophoblastic disease (GTD) characterized by abnormally proliferating trophoblastic tissues. Genetically, the presence of excess paternal genome and maternal chromosome loss resulted in 46XX embryo (androgenic hydatidiform mole). Recurrent GTD are defined by the occurrence of at least two GTD pregnancies in the same patient. Intracytoplasmic sperm injection (ICSI) could be prevent of recurrent GTD through ensure fertilization by a single sperm. Also, ICSI with preimplantation genetic screening (PGS) assurances that only one spermatozoa enters the oocyte and identifies the 46XY embryos can be prevented from recurrent GTD. We aimed to evaluate the pregnancy outcome following ICSI and ICSI/PGS in recurrent GTD.

Materials and Methods: In this retrospective study, we recruited all couple who referred to Royan Institute causes infertility complaint with GTD history during 2010 to 2015 years. GTD confirmed by serial β hCG titer, ultrasonography and histopathology assessment of the evacuated uterine contents. The ICSI procedure was routinely performed in order to prevent dispermia. The fluorescent in situ hybridization (FISH) probes were specific for chromosomes 13, 16, 21, X and Y.

Results: In this study, we analyzed all patients with GTD history that included total 56 cycles. ICSI and ICSI/PGS was 32 cycles (57.1%) and 24 cycles (42.9%), respectively. We analyzed between women with only one GTD history who underwent ICSI cycles (72.1%) and who was ≥ 2 GTD history that performed ICSI/PGS cycles (27.9%). In all groups, the total dose of received gonadotropins, total oocyte retrieved numbers, the number of MII and the embryos in the ICSI/PGS group were significantly higher than the ICSI group ($P \leq 0.05$). Pregnancy outcome was shown, GTD not observed in all groups, however, ongoing pregnancy for ICSI and ICSI/PGS in both compared group was 20%-20.6% and 16.5%-20.8%, respectively, which was almost the same ($P > 0.05$).

Conclusion: Despite receiving a high dose of gonadotropins in ICSI/PGS cycles and achieving more embryos, the success rate of pregnancy in both ICSI and ICSI/PGS groups is approximately the same, and both of these methods can be effective in preventing GTD pregnancy by considering that ICSI/PGS is an expensive method.

Keywords: Gestational Trophoblastic Disease (GTD), Recurrent GTD, Intracytoplasmic Sperm Injection (ICSI), Preimplantation Genetic Screening (PGS),

P-129: Decreased Expression of BCKDK Gene Related to Branched Chain Amino Acid Metabolism in Subcutaneous Adipose Tissue of Pregnant Women with PCOS

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Background: Access gene expression of enzyme involved in the metabolism of branched-chain amino acids in subcutaneous adipose tissue in pregnant women with PCOS and its comparison with pregnant women without PCOS

Materials and Methods: In this case control study, biopsies of subcutaneous abdominal fat during cesarean section was performed from 12 pregnant women with PCOS and 6 pregnant women without PCOS. Samples were then washed, cut and snap-frozen immediately. Branched chain ketoacid dehydrogenase kinase (BCKDK) gene expression of subcutaneous tissue samples was measured by real time PCR.

Results: The level of mRNA expression of BCKDK in subcutaneous adipose tissue was decreased in pregnant women with PCOS in comparison with pregnant women without PCOS ($P < 0.05$).

Conclusion: The results suggest that alteration in gene expression of BCKDK in BCAAs metabolism of white adipose tissue can play a dynamic key role in pathology of PCOS which warrants further studies.

Keywords: Polycystic Ovary Syndrome (PCOS), BCKDK, Subcutaneous Abdominal Fat

P-130: Investigating The Expression of Mir34c in Testis Tissue of Cholestatic Male Rats

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Background: Cholestasis is a pathophysiological condition that is occurred due to various reasons such as gallstone, malig-

nancy or genetic factors. Histological studies have been shown that cholestasis reduces proliferation of spermatogenic cells, causes apoptosis, and prevents the maturation of germ cells, in men. microRNAs (miRNAs) are non-coding sequences that have several regulatory roles in cell. Several evidences have been suggested the role of miRNAs during spermatogenesis and their importance in male fertility. miR34c has a specific and high expression in adult male testis. Mir34C and its related genes, are important mediator of self-renewal and differentiation of germ cells and have been considered as new targets in fertility problems.

Materials and Methods: Eight adult male wistar rats were divided into two groups named as control and cholestatic (n=4 each). To induce obstructive cholestasis their common bile duct was closed by surgery and the expression of miR34C in testis tissue of cholestatic and control rats, was assessed by quantitative real time PCR (qRT-PCR) technique. First, the primer sequence for mir34c and U6 was designed and then, the total RNA was extracted from the tissues and converted to cDNA. The synthesized cDNA was used to carry out the reverse transcription reaction and the relative expression of mir34C was determined using $R=2^{-(\Delta\Delta CT)}$ formula.

Results: The results of qRT-PCR showed that the expression of miR34c in the cholestasis group decreased by compared to the control group. This result showed that cholestasis reduced the expression of miR34C in testicular tissue. According to statistical analysis, this difference was significant.

Conclusion: Previous studies demonstrated that expression of miR34c is disrupted in pathological conditions of testis such as oligospermia and azoospermia and may be led to interfere with self-renewal and differentiation of germ cells. Our results also indicated the reduced expression of miR34c during cholestasis. Reducing miR34c expression in cholestasis animals may suggest that cholestasis has been able to disturb the expression of miR34C in the testis tissue and possibly through this change leads to disruption of the spermatogenesis process.

Keywords: Cholestasis, Infertility, Testis, microRNA

P-131: Increased gene expression of CRT2 in Ectopic and Eutopic Endometrium of Women with Endometriosis

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Background: Endometriosis is an estrogen-dependent disease in women of reproductive age and characterized by the growth of endometrial-like tissues outside the uterine cavity. Aberrant expression of enzymes involved in the biosynthesis of estro-

gen, steroidogenic acute regulatory protein (StAR) and aromatase, have been reported in the endometrium of women with endometriosis. Prostaglandin E2 (PGE2) is a potent inducer of StAR and aromatase expression which impresses its effect via activation of protein kinase A (PKA) and nuclear translocation of cAMP response element binding protein (CREB)-regulated transcription coactivator 2 (CRTC2). PKA phosphorylates CREB which allows it to bind to a CRE site on the promoter regions of target genes. In parallel with it, translocation of CRTC2 enhanced its association with CREB to form a transcription complex. CRTC2 belongs to the CREB-regulated transcription coactivator (CRTC) family and enhances CREB transcriptional activity by associating with the leucine zipper DNA-binding region of it. It has been reported that CRTC2 up-regulated the aromatase expression. In addition, CREB/CRTC2 complex directly regulates the expression of a number of critical genes involved in cellular proliferation and apoptosis. Previously, we showed overexpression of CREB in ectopic endometrial tissues of women with endometriosis. The aim of this study was to investigate the gene expression profile of CRTC2 in endometrial tissues of women with endometriosis in comparison to controls.

Materials and Methods: In this study, 10 normal women (who had no evidence of endometriosis through laparoscopy) and 10 women with endometriosis were enrolled. Ectopic biopsies from endometriosis women were obtained through laparoscopy while control endometrial samples (as a control group) and eutopic samples were collected via pipelle. After endometrial tissues collection, RNA extraction, and cDNA synthesis were done. Real-time PCR technique used for investigating the gene expression profile of CRTC2. Gene expression data were analyzed using one way ANOVA.

Results: Gene expression level of CRTC2 was higher in ectopic and eutopic endometrium of women with endometriosis in comparison to control group.

Conclusion: The increased gene expression of CRTC2 in ectopic and eutopic endometrium of women with endometriosis may contribute to the pathogenesis of endometriosis through its regulatory role on target gene expression.

Keywords: Endometriosis, CREB, CRTC2, eutopic, ectopic

P-132: Protective Effect of Vitamin E on Expression of P53 Gene and FSH/LH Levels in Infected Rats with *Candida albicans*

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Background: Various microbial agents with different pathologic mechanisms may affect sperm function by infecting the urogenital tract at different sites. The aim of this study was to investigate the harmful effects of *C. albicans* on Expression of P53 gene and Equal amounts of total protein of P53, Serum levels of Follicle Stimulating Hormone (FSH) and luteinizing hormone (LH) Levels in infected Rats with *C. albicans*.

Materials and Methods: The treatment of 24 Adult rats was performed in four groups: control, infected with *C. albicans*, infected with *C. albicans* + Vitamin E, Vitamin E (100mg/kg/day) for 5 weeks. The expression of P53 gene was analysed by real-time PCR and expression of p53 gene, also Equal amounts of total protein of P53 was analysed by Western Blot and Serum levels FSH and LH were determined with the enzyme-linked immunosorbent assay method.

Results: In the present study no significant difference was observed in the serum level of FSH *C. albicans*-exposed rats. Conversely, our results showed significantly decreased of LH and mRNA and protein expression during treatment of animals with *C. albicans* compared to the control group. While *C. albicans* + Vitamin E group showed a highly significant increase in LH and mRNA expression but no significant difference of protein expression compared to *C. albicans* group.

Conclusion: Vitamin E could compensate the adverse effects of *C. albicans* on Serum level of LH, mRNA and protein expression in adult rats.

Keywords: P53, Vitamin E, FSH, LH, *C. albicans*

P-133: A Novel Balanced Reciprocal Translocation and A Heteromorphism in A Patient with Recurrent Pregnancy Loss: A Case Report

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Background: Recurrent pregnancy loss (RPL) as one of the most common complications of pregnancy is responsible for significant emotional distress to the couple desiring to conceive, and defined as three or more consecutive pregnancy losses. 2% to 4% of RPL is associated with a parental balanced structural chromosome rearrangement, generally balanced reciprocal translocations (ROBs). Reciprocal translocations are usually an exchange of material between nonhomologous chromosomes with an estimation of incidence range from about 1 in 500 to 1 in 625 human newborns. Meanwhile the heteromorphism of chromosome 9 is the most common structural chromosomal variant and it is not thought to be of any functional importance, which often considers as a normal variation in structural polymorphisms, nevertheless there are some studies which claim that there is an association between heteromorphism of chromosome 9 and some pregnancy complications.

Materials and Methods: The study is including an Iranian Azeri couple referred to the Dr. Mohaddes Medical Genetics Laboratory (Tabriz, Iran), having a history of RPL in the first trimester. After genetic counseling, conventional chromosomal analysis of parents was done to ascertain the role of chromosomal abnormalities through the G-banded karyotyping technique.

Results: Clinical and hormonal profile of the couple revealed normal phenotypes and the ultrasound scan of the female showed normal uterus and ovaries. Chromosomal analysis of the couple revealed a normal 46, XY karyotype for the male spouse, and a unique balanced reciprocal translocation 46, XX, t(9;16) (q34.2;q23.2) and heteromorphism of chromosome 9 (9qh+) in the female partner.

Conclusion: In the present case; a unique balanced reciprocal translocation as an original investigation and a heteromorphism of chromosome 9 in a female with the history of recurrent pregnancy loss is reported. Due to this investigation and similar studies, it seems reasonable that conventional cytogenetic analysis should be suggested for couples with the history of

recurrent miscarriage in order to estimate whether they have chromosomal rearrangements.

Keywords: Recurrent pregnancy loss, Balanced reciprocal translocation, Heteromorphism, Chromosome 9, G-banded karyotype

P-134: Effect of Cholestasis on Claudine-11 Gene Expression in Testis of Male Wistar Rats

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Background: Cholestasis is a liver disease that results in the destruction of testicular tissue. One of the most important factors in maintaining the testicular tissue integrity is the blood-testis barrier (BTB). The Claudine-11 gene is expressed in sertoli cells and plays important role in the blood-testis barrier construction. The purpose of this study was to investigate the effect of cholestasis syndrome on BTB and the expression of Claudine 11 in male rats.

Materials and Methods: In this study, 12 male rats were divided into control and cholestasis groups. To induce cholestasis, the bile duct was closed during surgery. The testicular barrier permeability was evaluated after 3 weeks using Evans Blue's staining. Claudine 11 gene expression was analyzed using real time PCR method and the data were analyzed by one-way variance using SPSS software.

Results: The results of this study indicated that cholestasis syndrome leads to a change in the permeability of BTB, so that the permeability of BTB increased almost more than 2 times in the cholestasis group compared to the control group. The results of gene expression analysis also showed that the relative expression of Claudine 11 gene in testis tissue of cholestasis was significantly lower than healthy animals.

Conclusion: Cholestasis is one of the diseases that can lead to fertility problems in men. The present study showed that cholestasis can damage the testicular tissue by destroying the blood-testis barrier and altering the expression of genes involved in intercellular connections.

Keywords: Cholestasis, Claudine 11, Blood-Testis Barrier, Fertility

P-135: A Molecular Approach in A Case Series of 46, XY Gonadal Dysgenesis Patients.

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Background: 46,XY Gonadal dysgenesis (46,XY GD) is a rare congenital disorder with no apparent incidence, thus current knowledge about age and clinical presentation at diagnosis is sparse. 46,XY GD results from alteration in genes involved in testis differentiation and thereby affect the development of the internal and external genital organs. In most of the cases, the underlying molecular mechanism remains elusive. The aim of this study is to identify the genetic causes of 46,XY GD patients.

Materials and Methods: A retrospective study was performed of patients with female phenotype and 46,XY karyotype. They were assessed preoperatively with ultrasonography, hormonal examination of the gonadotropins FSH and LH as well as testosterone, and histopathological reports. We performed sequence analyses of the SRY, NR5A1, and ZFP281 genes also deletion in SOX9 promoter will be done by qPCR

Results: The series consisted of 12 patients, all with female gender and non-palpable gonads. Ambiguous or female external genitalia, primary amenorrhea, absence or disorder of Mullerian structure, delayed puberty and infertility are important manifestations of these patients. We analyzed the clinical investigations; all of the patients during puberty had a strong rise of FSH, LH levels, and testosterone levels were reduced. Patients had a gonad or gonads that were described as "small" or "infantal". We did not observe mutation in the SRY gene likewise, specific variation in NR5A1 and ZFP281 were not reported.

Conclusion: We describe the first Iranian 46,XY GD patients. The presentation of gonadal dysgenesis is during pubertal years. Follow-up laboratory investigations showed decreased testosterone levels, despite elevated gonadotropin levels, indicating gonadal dysfunction. We conclude that while SRY is an essential transcription factor of testicle development, there are many genes involved in sexual development. Further clinical studies are required for better molecular diagnosis.

Keywords: 46, XY Gonadal Dysgenesis, Testis Development, SRY

P-136: Investigation of Serum Extracellular Vesicles Derived microRNAs in Endometriosis

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Background: Recent studies offer a non-invasive method for detection of endometriosis based on extracellular vesicle miRNAs as potential biomarkers. However no reliable biomarker has been introduced yet. Level expression of miRNAs change in progression of endometriosis.

Materials and Methods: PubMed search using micro RNA

and endometriosis in title/abstract or Mesh terms was used to find out previously reported candidates following by bioinformatics analysis based on mirbase, miRTarBase, enrichr, mirnamap. Twenty women with histologically proven severe endometriosis and 20 endometriosis-free were enrolled in this study to check existence of potential candidate miRNA in extracellular vesicle. All samples collect in morning.

Results: Six miRNAs including miR-451a, miR-199a, miR-100, miR-148a, miR-154 and miR-23b based on literature review (114 article between 2007 and 2018) and bioinformatics analysis were selected and their expression level in extracted EVs between samples showed some differences.

Conclusion: Golden standard method for diagnosis is laparoscopy that is an invasive and costly method. In addition, sampling of ovaries in endometrioma can decrease reserve oocytes and lead to subfertility or infertility. Hence use of a non-invasive method such as biomarker is suggested. Significant alteration in serum extracellular vesicles derived candidate microRNAs can consider them as a potentially biomarker for detection of endometriosis.

Keywords: Endometriosis, microRNA, extracellular vesicle, Biomarker, Bioinformatics analysis

P-137: Effect of Curcumin and Nanocurcumin on The Expression Level of Prominent Developmental Gene (TIMP-1) in Endometrial Tissue Culture of Women with Polycystic Ovary Syndrome.

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Background: Poly Cystic Ovary Syndrome (PCOS) is a common endocrinological disorder. This syndrome affects 5–7% of women at their reproductive ages. It considered as a complex disorder with interaction of genetic and environmental factors. This syndrome is associated with: insulin resistance, hyperandrogenemia, chronic inflammation and oxidative stress. Endometrium undergoes a complex series of organized changes in each menstrual cycle. It is receptive in a short period of time which is known as window of implantation (WOI). There is increasing evidence of dysregulated expression of markers of uterine receptivity in endometrium of women with PCOS. TIMP-1 has showed crucial role in reproductive organs and its decreasing level has been detected in follicular fluid of women with PCOS.

Materials and Methods: In this study, endometrial biopsy of a

woman with PCOS has been cultured *in vitro* and after the second passage, the cells were exposed to 4 different treatments: curcumin, nanocurcumin (DNC), carrier (Sham), DMSO (Sham), paralleled to untreated group. By MTT assay the best concentration of curcumin and nanocurcumin and carrier were chosen. After 48 hours of treatment each 5 group's cells were snap frizzed separately and were kept in -70 °C. After cDNA synthesis, the quantification of TIMP-1 expression evaluated by real-time PCR.

Results: Our data demonstrated that the treated cells showed significant decrease in TIMP-1 expression for both curcumin (P value = 0.004) and nanocurcumin (P value = 0.05) compared to untreated group.

Conclusion: This primary data indicates that curcumin and nanocurcumin can influence on gene expression profile of endometrial cells of a woman with polycystic ovary syndrome. The data needs further investigation.

Keywords: PCOS, Timp-1, Implantation, Curcumin, Nanocurcumin

P-138: Genetic Investigation of Patients with 46,XX Testicular Disorder of Sex Development

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Background: 46,XX Testicular Disorder of sex development (46,XX Testicular DSD) is a rare disease with 1/60000 birth incidence. These patients are characterized by 46,XX karyotype, testicular gonads, male external genitalia, male gender identification, hyper gonadotropic hypogonadism, and azoospermia. Some of these patients can be diagnosed at birth or early life because of penile abnormalities, but in some cases, there is no evidence of abnormalities in their genitals, therefore they are referred to the clinic due to puberty disorders or infertility. Most of these patients do not have any molecular diagnosis.

Materials and Methods: In the collaboration of Royan institute with Endocrinology and Metabolism Research Center of Tehran University, 326 patients referred due to infertility, or genital abnormalities, have been nominated for this study. Karyotype has been performed on their peripheral blood to investigate chromosomal abnormalities. Patients with 46,XX karyotype and male phenotype underwent PCR based SRY gene analysis to determine the presence of this gene in their genome. In order to analysis genetic variations, upstream region of SOX9, and SOX3, and exon 4 of NR5A1, and RSPO1 were selected.

Results: 66 patients had 46,XX karyotype with female phenotype, and 46,XX SRY-Negative has been observed in 5 patients.

Genetic variations on the candidate genes were not observed in the patients' samples.

Conclusion: This study indicated that there is a large number of patients with unknown etiology which their samples should undergo Whole Exome Sequencing for better understanding of their genomic status.

Keywords: 46,XX Testicular DSD, Disorders of Sex Development, Ambiguous Genitalia, Sex Reversal,

P-139: Curcumin and Dendrosomal Nanocurcumin Effects on Androgen Receptor Gene Expression in Endometrial Tissue Culture of Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common gynecological disorder, affecting 5–7% of women of reproductive age, and characterized by oligo-/anovulation, polycystic ovaries morphology and elevated circulating concentrations of androgens and/or signs of hyperandrogenism. It is well established that the cellular actions of androgens are mediated via its binding to and activation of androgen receptor (AR). PCOS endometrium overexpresses AR. In fact, many of the consequences of hyperandrogenism such as hirsutism, acne, hyperinsulinemia as a result of insulin resistance, and implantation failure are due to the elevated expression of AR in endometrium of PCOS women. However, several studies have shown that curcumin (a phenolic curcuminoid derivative from *Curcuma longa*) has beneficial effects on PCOS induced rats. Our aim was to determine the effects of curcumin and dendrosomal nanocurcumin (DNC, nanoformulation of curcumin), in endometrial tissue culture of women with PCOS.

Materials and Methods: Endometrial biopsy was taken from a PCOS woman. After stromal cell culture, MTT assay was exploited to measure their viability against curcumin, DNC, DMSO and nanocarrier (DMSO and nanocarrier as the SHAM groups) treatment. Cells were separately subjected to these substances for 48 hrs. Afterward, RNA extraction, DNA synthesis and real-time PCR were applied to examine and analyze the mRNA expression level of the AR gene.

Results: Our data revealed that curcumin treatment causes to a significant decrease in mRNA expression level of the AR gene in all treated cells compared to control group.

Conclusion: This primary finding implies the dynamic role of curcumin in gene expression pattern of endometrial cells in women with PCOS; although we're investigating these treatments with more sample number to get more reliable

results.

Keywords: Polycystic ovary syndrome, Androgen receptor, Hyperandrogenism, Curcumin, Nanocurcumin

Ethics and Health

P-140: Defense Mechanisms, Self-Esteem and Marital Adjustment in Infertile Couples Based on The Cause of Infertility

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Background: Infertility could have a negative impact on various aspects of women's lives. The defense mechanisms are mental processes in the structure of the personality which has an impact on psychological coping mechanisms. Self-esteem and marital adjustment play key role in infertile women's mental-social health. So, the aim of this study was to investigate the role of defense mechanisms, self-esteem and marital adjustment based on the cause of infertility.

Materials and Methods: In this cross-sectional study, 400 infertile couples referred to infertility center in Tehran, Iran. Data gathering tool in this study was Demographic questionnaire, Revised Dyadic Adjustment Scale (RDAS), Rosenberg Self-Esteem Scale and Defense Styles Questionnaire (DSQ). Data were analyzed (SPSS 18 software) using paired t-test for comparison between the groups according to the cause of infertility using regression analyses and Kruskal Wallis tests.

Results: The results of this study showed that marital adjustment was significantly higher in women with unknown factor than in other groups ($P < 0.07$). Also in couples with female factor, male factor, and both factor, husbands appeared significantly higher mean score for marital adjustment in comparison with wives ($P < 0.07$). When the cause of infertility was unknown, the women expressed greater self-esteem compared to other groups ($P < 0.029$). In men with female factor, self-esteem was significantly higher than other groups ($P < 0.049$). In couples, husbands with female and male factor showed more self-esteem than their wives ($P < 0.001$, $P < 0.008$, respectively). In defense mechanisms, there was no difference between women, men, and couples (women with husbands). Self-esteem has a significant positive correlation with marital adjustment and mature defense mechanism and also has a significant negative correlation with the immature and neurotic defense mechanisms ($P < 0.05$).

Conclusion: The findings of this study showed that women had higher levels of vulnerability than their husbands even when they were not the cause of infertility. Perhaps becoming pregnant is a feminine process and associated with feminine identity and these women are responsible for this matter. Also, there was

a relation between defense mechanisms, self-esteem, and marital adjustment in infertile couples.

Keywords: Defense Mechanism, Infertility, Marital Adjustment, Self-Esteem

P-141: Knowledge of Endometriosis in Girl's Students and Their Mothers in High Schools

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Background: Endometriosis (the presence of endometrial-like tissue outside the uterus) is a chronic disease associated with pelvic pain and subfertility. Prevalence rates in the general population are unknown, because a definitive diagnosis is established only at laparoscopy. The lack of strong information about the impact of endometriosis world-wide led us to initiate this study. The aim of this study is about a Survey on Knowledge of Endometriosis in High School Girl Students and Their Mothers in High Schools in Tehran.

Materials and Methods: This cross sectional study was carried out on 1580 high school girl's student and 480 their mothers in Tehran, capital of Iran from August to October 2017. We considered Tehran as 5 geographic regions (north, south, east, west and center) and selected schools from each of these regions randomly. Students and their mothers answered a self-administered questionnaire about knowledge of endometriosis separately. Statistical analyses were carried out with R version 3.2.1. Main analyses were multilevel analyses.

Results: In this study, the average age of students and their mothers were 16.97 ± 0.84 (Mean \pm SD) and 45.19 ± 5.02 , respectively. The results of this study showed that only 7 students (0.5%) and 83 mothers (17%) had knowledge about endometriosis. All girls got their information about endometriosis from their mothers and study. The results of this study showed that knowledge of student about endometriosis was directly and significantly related to mother's knowledge about endometriosis ($P < 0.001$).

Conclusion: Findings of this study indicated that the information of students and their mothers about endometriosis was low. However, it can be necessity of providing health instructions, especially genital health and proper nutrition before and after puberty, also correcting false beliefs in mothers and girls through classes and educational programs (brochures or Internet) are essential.

Keywords: Girls students, Knowledge, Endometriosis, ,

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Background: Nowadays almost 186 million people deal with infertility. Recruitment, development and monitoring of multiple follicles in woman play crucial roles in Assisted Reproductive Technology (ART). Concordant with traditionally follow ups such as Follicular Stimulation Hormone (FSH) and Anti-Müllerian Hormone (AMH) measurement, antral follicles count (AFC) even achieves the best predictive value in ovarian response to gonadotropin stimulation. Since there were some controversies in researches, we aim to compare predictive value of Two-dimensional Ultrasound and Three-dimensional Ultrasound in follicular tracking in ART cycles.

Materials and Methods: This is a study which compares value of Two-dimensional and Three-dimensional Ultrasound in follicular tracking in ART. Eligible articles had extracted according to inclusion and exclusion criteria in reputable online library (Pubmed, Science Direct, Googlescholar, Iran Medex).

Results: There are huge amounts of study which compare and analyze the advantages application of Three-dimensional Ultrasound versus Two-dimensional Ultrasound. Since both 2D and 3D ultrasound are used for count the numerous follicles in each ovary, the techniques will be tiring and the reliability and validity of counts may be uncertain. But in general the reliability and validity of Three-dimensional Ultrasound are much significant. The most studies are in agreement with reduction of time for AFC with Three-dimensional application. The better images quality, exact ovarian volume and easier storage for further monitoring are other advantages.

Conclusion: Three-dimensional Ultrasound can be best modality of choice for determination of exact AFC, ovarian volume and stroma with a significant reduction in time without increasing patient discomfort. Since it is not cost effective that three-dimensional Ultrasound applied for all patients, so poor responder cases and those who had low ovarian reserves can take advantage of it.

Keywords: Two-Dimensional Ultrasound, Three-Dimensional Ultrasound, Follicles, ART

Reproductive Imaging

P-142: Three-Dimensional Ultrasound and Two-Dimensional Ultrasound Application for Antral Follicles Count in Assisted Reproduction Technology

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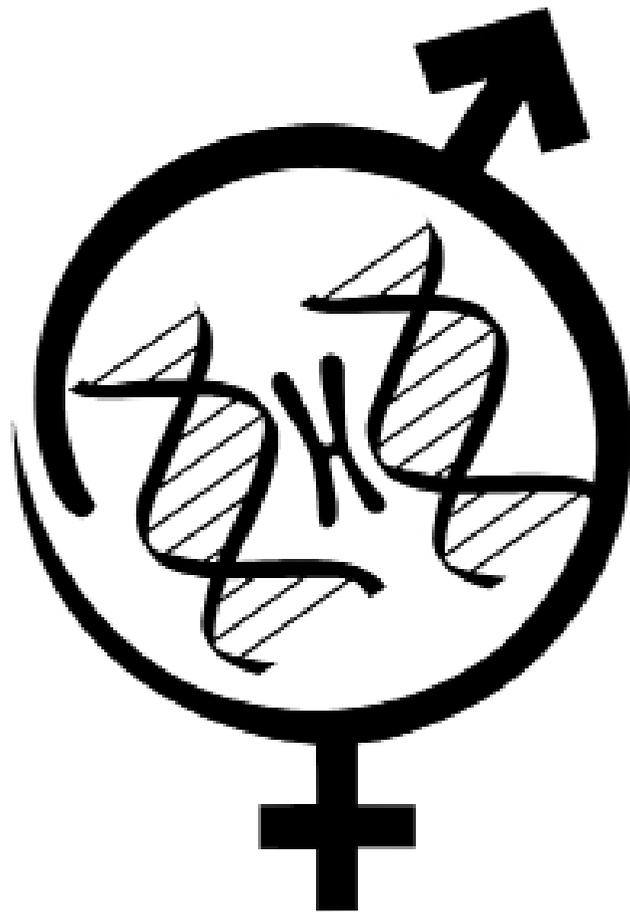
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Abstracts of
Royan International Twin Congress

14th Seminar on Nursing and Midwifery
28-30 August 2019



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Invited Speakers

I_{nm}-1: Preservation Female Fertility and Supportive Role of Midwife and Nurse

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Nowadays, due to wonderful development in prognosis, diagnosis, and treatment of different kind of disease that affect ovarian function and ovulation quality and quantity such as cancers, endometriosis, autoimmune disease, and some genetic conditions; fertility preservation demands are receiving raising consideration in the world. Besides, some women wishing to childbearing in older ages because of social matters, so they would be fertility preservation candidate too.

Since decision making process in fertility preservation is not simple for women, the most of them need to a comprehensive support. In this process, the role of infertility midwives and nurses for holistic care and support, is undeniable and continually developing and even changing to meet the needs of these women. These midwives and nurses should help to women for finding and knowing their needs and gain insight into themselves and all aspect of their problems and treatment process. Regardless fertility preservation cause, midwives and nurses should have a responsibility to understand following topics:

- There is a requirement for the development counselling knowledge and skills.
- Fertility preservation and its process, indication and contraindications, potential benefits and risks.
- Women support needs in mental, psychosocial, marital, financial, physical, informational, and spiritual areas.
- Ethical and legal issues especially in women who has been diagnosed with cancer.
- Cultural and religious considerations associated with fertility conversation in each context.
- The best timeframe referral consideration for treatment and/or storage.
- And, the importance of interdisciplinary fertility preservation team work.

Midwives and nurses can play a key role in women fertility preservation, if they understand the reality of possible choices in present time and available ones in future. Based on these facts, they can supports women to manage their fertility conditions.

I_{nm}-2: Ovarian Somatic Stem Cells The Role of Epigenetic on Infertility

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Infertility is a multifactorial disorder which could be caused by male and female factors. There are some approaches to recognize the mechanisms of infertility, one of them is epigenetic. Epigenetics defines as heritable and reversible forms of gene activity and expression without any DNA sequence modification. Recently, the presence of aberrant DNA methylation of imprinted genes and reproduction-related genes might be helpful to define unexplained infertility. Male and female germinal cells due to the possible transgenerational effects related to

epigenetic modifications, undergo differentiation into matured sperm and oocyte cells. Epigenetic modifications could affect reproduction by (1) gametogenesis failure; (2) embryogenesis defect; (3) outcome of assisted reproduction technique (ART) protocols and (4), long-term effects on offspring lifetime.

Keywords: Infertility, Epigenetics, Gametogenesis, Embryogenesis, Transgenerational Effect

I_{nm}-3: Preservation Female Fertility and Supportive Rule of Midwife and Nurse

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Fertility preservation is a current option and increase delayed child bearing in cancer patients and females who hope to become mothers in advanced age. Cytotoxic drugs and radiotherapy are used to treat malignant and non-malignant systemic disease and the sequel of these therapies is ovarian failure. Ovarian fertility preservation introduces an important health issue by increasing survival rate after these treatments. A non-medical reason (social) of egg freezing is another issue to overwhelmed ovarian aging and decreased ovarian reservation. After introduction of new and effective methods of cryopreservations such as vitrification; different procedures and proposed to preserve fertility including; gamete, embryo and tissue freezing. Egg freezing is a process after ovarian stimulation and is followed by transvaginal oocyte pick up proceeded by freed viable eggs. Social egg freezing does not guarantee a successful pregnancy or live birth.

We should inform our patients about different aspects of view of our procedure and all dangers to putting them in stimulation and freezing and survival- rate of oocyte after vitrification and thawing.

I_{nm}-4: Premature Ovarian Insufficiency

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Women with premature ovarian insufficiency (POI) experience menopause before 40 years of age. This condition affects approximately 1% of women and is associated with multiple health problems such as infertility, osteoporosis, cardiovascular disease and decreased well-being. POI is considered a multifactorial heterogeneous condition for which the exact underlying causes have been identified and associations with autoimmune disease and environmental factors have been described. Extremely low success rates have been reported with various infertility interventions in women with POI. To date little information is available concerning the reproductive characteristics of women diagnosed with POI during their preceding reproductive life.

I_{nm}-5: The Impact of Lifestyle Changes on Fertility Improvement

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This evidence-based review focuses on the impact of potentially modifiable, non-communicable lifestyle factors on reproductive performance in the general population and the infertile population undergoing assisted reproductive technology (ART) treatment. These factors include female age, weight, smoking, diet, exercise, psychological stress, caffeine consumption, alcohol consumption and exposure to environmental pollutants.

The impact of lifestyle on reproductive performance may vary depending on individual aetiology and circumstances.

In males, smoking negatively affects sperm production, motility and morphology and is associated with an increased risk of DNA damage (Zenzes et al. , 1999 ; Kunzle et al. , 2003). In the female, the constituents of cigarette smoke may affect the follicular microenvironment and alter hormone levels in the luteal phase.

The above evidence associated with age, smoking and weight shows that there is strong substantiation of an adverse association between these lifestyle factors and the risk of impaired fertility.

The above evidence associated with age, smoking and weight shows that there is strong substantiation of an adverse association between these lifestyle factors and the risk of impaired fertility.

If there is a clear impact on fertility, such as with smoking and alcohol, cessation should be advised. Similarly, weight loss should be recommended if the BMI is in the overweight and obese category, and weight gain should be recommended for an underweight BMI. Antioxidants seem to improve semen parameters in men, but the effect on female fertility is less clear. If conflicting evidence exists, such as with caffeine consumption or exercise, moderation should be emphasized. Finally, the diagnosis of infertility and subsequent fertility treatments are stressful for both partners. The psychological aspects should not be ignored and methods such as yoga and cognitive behavioral therapy may be beneficial.

Melatonin is naturally produced by the body during sleep. Any artificial light, including device screen light can affect melatonin production. Sleep decreases cortisol levels which can lower testosterone.

Men can improve their fertility by eliminating tight fitting clothes, long bike rides and hot tubs, and avoiding holding a laptop in their lap because this can increase scrotal temperature and reduce sperm production.

simple lifestyle changes that can help improve your chances of conception. The goal is to create the healthiest environment for conception and pregnancy.

I_{nm}-6: Epigenetic of Infertility

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Infertility is a heterozygous disease that involves almost 22% of couples of reproductive age. The contribution of male and female is almost the same, and a lower percentage about 10% is related to cases with an unknown cause. The main causes of infertility are genetics and epigenetics.

Stress affects the health of a person including fertility in different ways. Oxidative stress accumulates harmful free radicals in the gonads and induces negative effects like DNA breaks and point mutations on gametogenesis. Also, Sympathic adrenal system (SAS) and hypothalamus pituitary adrenal (HPA) axes are two pathways that cause physiological changes due to stress. Oxidative stress leads methylation changes that alter the level of DNA density in embryonic gonads and its consequence gene expression.

Lifestyle factors such as smoking, alcohol consumption, and poor nutrition can lead to infertility, development of human diseases, and pregnancy complications.

It seems that by changes in lifestyle, overall health including reproductive health of each individual can be increased and the diseases that environmental factors have high impact on them could be prevented.

I_{nm}-7: The Role of Epigenetic On Infertility

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The term “Epigenetics” denotes reversible patterns of gene expression that do not include alterations to the DNA sequence. Epigenetic alterations are responsible for chromatin structure stability, genome integrity, modulation of tissue-specific gene expression and embryonic development. One notable feature in epigenetics is that all epigenetic modifications are “reversible” and “dynamic”. So these reversible alterations can cause to a selective utilization of genome information, through activation/inactivation of the functional genes during gametogenesis and embryogenesis which ultimately leads to a successful fertility.

While the global epigenetic patterns remain precisely stable through somatic cell divisions (the phenomenon termed as “Epigenetic Memory”), during gametogenesis and initial embryo development, these patterns have widespread variations and are highly susceptible to the influence of environmental and *in vitro* conditions. Thus, parallel to studying the epigenetic aspects of male/female infertility, identification of epigenetic markers related to gamete and embryo quality sounds critical for optimization of new protocols that increase the efficacy of different Assisted Reproductive Techniques (ARTs); although genetic/epigenetic backgrounds of the couples referring to ART centers are of major objectives needs to be considered as well.

With the brief aforementioned background, making more and more allowance for the role of epigenetic on infertility will help us to develop new diagnostic and therapeutic tools for improving the efficiency and safety of fertility.

Keywords: Epigenetics, Infertility, ART

I_{nm}-8: What is Telemedicine?

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Telemedicine is known as a tool that makes healthcare more accessible, cost-effective, and that increases patient engagement. The World Health Organization (WHO) refers to telemedicine as “healing from a distance“. It is the use of telecommunications technology and information technologies to provide remote clinical services to patients. Physicians and patients can share information in real time from one computer screen to another. And they can even see and capture readings from medical devices at a faraway location

What is Telemedicine Healthcare

Telemedicine is simply defined as, “the remote delivery of healthcare services“. There are 3 common types of telemedicine, which include but not limited to:

- Interactive Medicine
- Store and Forward
- Remote Patient Monitoring

How is it Conducted?

Telemedicine is conducted in a number of ways. The most basic is just a simple video call (like you normally do with family and friends), however most countries required secured HIPAA compliant video conference tool, so telemedicine company such as VSee also provides this kind of secure and simple to use solution for providers.

Top 8 Telemedicine Benefits

- Increase Revenue
- See More Patients
- Convenience
- Cost Saving
- Get a quick 2nd opinion
- Patients love it
- Improved healthcare quality
- More Intelligent Solution

The Role of Telehealth in Medical Tourism

Telehealth, also known as telemedicine, is the remote provision of health care services enabled by technology. A continuum of successful telehealth applications has been demonstrated over the last twenty years, ranging from the transmission of digital photographs and patient histories for diagnostic consultation, to remote monitoring of physiologic data for chronic disease management, to interactive patient physical examination using medical video endoscopes and ultrasound over high-definition videoconferencing links. The common tie among these varied applications is that technology is used to improve access to health care services independent of geography.

Telehealth can improve quality, efficiency and customer service in medical tourism applications by better coordination of care between providers in patients’ home and foreign countries, enhanced preoperative and postoperative care, and optimizing patient and family member travel. This article describes the basic principles and applications of telehealth and explores the potential roles and challenges of telehealth in medical tourism.

Oral Presentation

O_{nm}-1: General Health in Women with Polycystic Ovary Syndrome: Effect of *Salvia Officinalis*

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Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age that has many psychological complications such as anxiety, depression and mood disorders. *Salvia officinalis* (*S. officinalis*) is one of the most famous herbal drugs that have potential effects on the central nervous system, cognitive performance, and mood. This study was designed to determine the effects of oral *S. officinalis* extract on mental health parameters in women with PCOS.

Material and Methods: In the current randomized, Triple-blind, placebo-controlled trial, 60 PCOS patients were randomized to take either 330mg oral *S. officinalis* extract (n=30) or placebo (n=30) for 8 weeks. We used General health questionnaire version 28 (GHQ -28) for assessment of mental health once before and once immediately after the intervention. All statistical analyses performed by using the SPSS software.

Results: After 8 weeks oral *S. officinalis* extract intake resulted in a significant improvement in general health questionnaire total scores (P = 0.001), mental health status and increase in the number of people with a normal general health score (score below 24) (P = 0.031). In addition, the scores of GHQ-28 subscales in physical health (P = 0.001), anxiety (P = 0.001) and depression (P = 0.027) have been improved, but the scores of social performance did not change significantly (P= 0.495).

Conclusion: Our study demonstrated that oral *S. officinalis* extract use for 8 weeks among patients with PCOS had favorable effects on mental health status.

Keyword: Polycystic Ovary Syndrome, Phytoestrogens, Mental Health, *Salvia Officinalis*

O_{nm}-2: The Social Construction of Infertility among Iranian Infertile Women

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Background: Infertility is considered an important phenomenon in couples' life. Infertility and its treatment process influence all aspects of the individual's life. This study aimed to explain the psycho-social process of the social construction of infertility among Iranian infertile women.

Materials and Methods: This was a qualitative study using a grounded theory approach. The study setting was the Vali-e-Asr Fertility Health Research Center and Avicenna Infertility Center in Tehran. The sampling started purposefully and it was

continued theoretically. The data collection was performed by using 36 semi-structured interviews, observation and field notes with 27 women who suffered from primary and secondary infertility having no living children. The method suggested by Strauss and Corbin was used for data analysis.

Results: Results indicate that 'Concerns over life instability' and 'being judged by others' were the participants' most important preoccupation. Attempts to stabilize life and get rid of being judged by others were key aspects of the social construction of infertility and the main strategies for resolving their preoccupation. This core concept explained the basic psychological-social process of infertility in relation to axial codes. Couple's interactions, family and society's judgment performance, and the effect and control of treatment process over the life cycle were causal conditions of concerns, which initiated the process of stabilizing life and getting rid of being judged by others. Personal beliefs and motivations for childbearing and the psychosocial consequences of infertility (as the context) created conditions under which the participants went through this process. The characteristics of infertile women and the existence of social supporters against its absence were considered to be the intervening conditions facilitated or limited this process. Hope for treatment interventions against its damages, couples' closer relationships against the threat to life instabilities, relieving or adapting to Psychological stress against its increase and spiritual growth against spiritual challenges were the consequences of the process.

Conclusion: The results of the study show that various interactive factors affect the social construction of infertility among infertile women who are focused on the central concept of attempts to stabilize life and get rid of being judged by others. Therefore, in order to achieve this goal, infertile women should be empowered by effective coping strategies.

Keywords: Social Construction, Infertility, Infertile Women, Grounded Theory

O_{nm}-3: Translation and Validity of Infertility Stigma Scale in Infertile Women

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Background: In a society women with infertility background, even when embryo transfer to the uterus express it as the best feeling and Sense of peace in the world and the existence of the child will lead to strength the marital relationship. The privation of child is a stigma that a person will experience by herself, her relatives and the community. Therefore, it is necessary to have a valid, durable and applicable tools in order to measure the experienced stigma in infertile women who may endanger their mental health. Due to the lack of a comprehensive questionnaire to measure infertility in society, this study aimed to "translate and validate infertile stigma women's infertility measure".

Materials and Methods: In this methodological study, initially the translation of the Infertility Inventory questionnaire which was designed by John Lei, first translated in to Persian, and then back translated by specialists. The infertility stigma scale (ISS)

consists of 4 subcategories: self-devaluation (7 items), social with drawal (5 items), public stigma(9 items) and family stigma(6 items). In the next step, the validity of the questionnaire was done through face validity, content validity (content validity index and content validity ratio), construct validity (confirmatory factor analysis and exploratory factor analysis. Internal consistency and test-retest methods were used to estimate the reliability of the scale. The participants in this study were 350 referring infertile women to a selected infertility center affiliated to Iran University of Medical Sciences in Tehran. The Sampling was done available in 4 months. Data analysis was performed using SPSS software version 16 and Liserl software version 8.8.

Results: The 27- items ISS were been into the validation phase after the translation of the questionnaire. It was performed after determining the content validity ratio and content validity index, a confirmatory factor analysis. The confirmatory factor analysis did not fit well; thus, five factors were identified by exploratory factor analysis and the some items position was transferred in the factors. After consultation with the research team, the name of the factor change to self- devaluation(7 item), public stigma(7 item),self-stigma (4 item),social stigma(4) and family stigma(5 item).The Cronbach's alpha coefficient for the instrument was found to be 0.96, and the external reliability, as evaluated by the test-retest method and the infraclass correlation, was (ICC = 0.93).

Conclusion: The results of validation showed that this questionnaire with 5 domains and 27 phrases was a reliable and valid scales for investigating infertility stigma in infertile women in Iran

Keywords: Infertile Women, Translation, Scale, Psychometrics, Stigma

O_{nm}-4: Preconception Counseling in Couples with Infertility

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Background: Preconception conditions are a major contributor to pregnancy health. Education and counseling at this time will lead to the success of treatment and the outcome of pregnancy in infertile couples. The purpose of this study was to determine the pre-pregnancy education and counseling of infertile couples.

Materials and Methods: This cross-sectional study was carried out in 268 couples from Isfahan Infertility Centers using records of the case and completing the questionnaire by interview method. Sampling method was census. In this study evaluated component of standard preconception counseling (Lifestyle, nutrition, sexual health, unauthorized materials and social counseling) and specific preconception counseling for infertile couples (Failure, follow up and complications of treatment). The results are analyzed using descriptive, analytical statistics and SPSS software.

Results: The results showed that most couples did not receive any counseling regarding lifestyle, nutrition, sexual health or social psycho social issues. The highest percentage of coun-

seling provided was related to follow up and treatment complications. The highest rates of counseling were related to the unknown causes of infertility. (more than 75%). The lowest percentage of counseling was sexual counseling.

Conclusion: The findings of this study showed that there is a major defect in infertility couples' pre-pregnancy education and counseling. Given the importance of pregnancy and its success in this group, it is necessary to pay more attention to pre-pregnancy counseling. This leads to more successful treatment. Training and sensitization of providers, preparation of standard forms, brochures, educational pamphlets, virtual education and sensitization of clients in this field are useful.

Keywords: Preconception, Infertility, Education

O_{nm}-5: The Effect of Cognitive Behavioral Therapy on Sexual Function in Infertile Women: A Randomized Controlled Clinical Trial

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Background: Infertility and its consequences can have effects on various aspects of a couple's life and also bring about serious problems in their marital relationships. The purpose of this study was to investigate the effect of cognitive behavioral therapy (CBT) on sexual function in infertile women.

Materials and Methods: The participants included a total number of 52 infertile women randomly assigned to intervention and control groups. Moreover, a group-based six-session sexual counseling using a CBT approach was also performed for the intervention group but the controls received routine care and education. Female sexual functions of the participants in both groups were then assessed via Female Sexual Function Index (FSFI) at 3 times. The findings revealed no statistically significant difference in the baseline FSFI mean scores of both groups.

Results: A significant difference was also reported for FSFI mean scores in the intervention group (29.35 ± 2.71) in comparison with those in the control group (25.84 ± 2.52) ($P < 0.001$) one month after intervention with an effect size of 0.32. The mean scores of all the sexual function domains including sexual desire ($P < 0.001$), arousal ($P < 0.001$), lubrication ($P < 0.001$), orgasm ($P < 0.001$), satisfaction ($P < 0.001$), and pain ($P < 0.001$)

Conclusion: Among the participants in the intervention group had also significantly increased following CBT implementation. The results of this study could be used to promote sexual health status in infertile women.

Keywords: Cognitive Behavioral Therapy, Sexual Counseling, Female Infertility, Mental Health Services

O_{nm}-6: Exploring The Problems and Concerns the Infertile reast Cancer Patients' Spouses: A Qualitative Study

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Background: Breast cancer diagnosis has a negative effect on patient's spouse. On the other hand, breast cancer and infertility also cause difficult conditions in the family. Women in the family have two complementary roles: feminine and motherhood. Disruption in each of the roles causes disturbance. If there is a problem with infertility and breast cancer is added, more physical and psychological problems will be imposed on the woman and family, especially patient's spouse. In women with infertility, after breast cancer, there is a possibility of impaired family and sexual functioning of couples due to changes in role, emotional stress, physical changes, and so on. The aim of this qualitative study was to explore the spouses' problems after their women breast cancer diagnosis, in Iran.

Materials and Methods: Thirty participants were recruited from Motamed breast clinic in Tehran. In-depth interviews each lasting approximately one hour per participant were scheduled and conducted in a private room. The interviews intended to motivate the participants to reflect on their problems since their women's breast cancer diagnosis. The interviews were recorded and were transcribed to elucidate the major themes encountered in the interviews.

Results: The mean age of spouses was 50.7, (SD = 6./97), 25 of them (83%) were up to diploma educated, and 16 of them (53%) were self-employee. Overall, two major them were emerged from the analysis these were: 1) physical and psychological problems (sexual, fatigue, anger, feeling of guilt, sadness) and 2) Concerns (worries about metastasizing and about their health and death and their wives fear of disclosure, bad event, financial problem).

Conclusion: It seems special attention is necessary for women with history of infertility and breast cancer. For the infertile women with breast cancer, a review of the history of hormonal treatments is very important. Because some of their spouses are involved in infertility treatments for breast cancer. Infertility treatment is effective in developing breast cancer For breast cancer infertile patients, it is important to study the history of infertility treatment and to address the psychological problems of the spouses as the most influential people in starting and continuing treatment. According to the results of this study, the main problems of spouses were fatigue, feelings of guilt, anger, lack of control and fear of metastasis. Therefore, it is recommended that by conducting more extensive studies and accurate examination of the individual and family problems of the patient's wife and beneficial interventions, it would be helpful to reduce the quality of life and to improve their quality of life.

Keywords: Breast Cancer, Infertility, Qualitative Study, Spouses' Problem, Iran

Poster Presentation

P_{nm}-1: Ethical Consideration in Infertility Treatment Process: A Non-Evasion Dilemma

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Background: Now day, infertility treatment knowledge and technology are growing rapidly. Although this is very encouraging matter for infertile couple; but in some cases, facing ethical and religious issues caused these treatments as a double-edged blade.

Materials and Methods: This is a review about Ethical considerations in infertility treatment.

Results: Reviews of literatures shows some general ethical considerations before starting infertility treatments: 1. Attention to the physical and mental health and economic power of couples to create a safe environment for the child in the future. 2. Although as the World Health Organization recommendation, the maximum age for maternity is 49 years, Parents who decide to have a child on the verge of middle age may not have enough power to play the role of parent correctly. 3. All infertility treatments are along with many complications, such as repeated ovulation stimulation, over-stimulation of ovarian syndrome, sperm injections into ovules and maternal and fetal complications, Failure to accept infertility and insistence on treatment, denial of problems, especially fear of losing the foundation of the family in the assumption of remarriage, So, some limitations in the treatment of infertility may be suitable, One of these limitations can be the assurance in Failure results. One of the obvious examples for infertility treatment in vain is the continuity of the treatment cycle at the old ages. It is true that the treatment is a patient's right, but the doctors' commitment is not to harm the patients. 4. Providing services to applicants with gender disruptions will be challenged, as the child will not be in the same family as the normal family, and in the future they may be faced with many risks. 5. Conscious Consent involves providing sufficient information, examining couple decision-making capacity, voluntary decision-making, especially for the donor's couple. 6. Evaluation and screening of embryo donors and recipients 7. Confidentiality of information about donors, recipients and child from the donation. If a child lives with the knowledge of his/her biological parents from the beginning, he/her may never be sure of the parents who raised him/her (the official law of donation is the unknown of the parties.)

Conclusion: Clinicians should pay attention to the ethical considerations mentioned above in the treatment centers. We should note that according to the definition of "right", the therapist cannot prohibit anyone from childbirth. But in case of treatment, careful consideration should be given to the child's benefit and treatment seekers.

Keywords: Ethical Considerations, Fertility, Infertility Treatment

P_{nm}-2: Primary Ovarian Insufficiency and ART

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Background: Primary ovarian insufficiency (POI) is a disease with gradual onset, in which the function, activity and follicles of the ovaries diminish before the age of 40. The prevalence is 1%. The disease is due to various causes such as autoimmune diseases, chemotherapy, radiotherapy, genetic disorder and Turner syndrome.

Materials and Methods: A narrative review was performed within articles published a "PubMed", "Elsevier", "SID" and original text books to reach the aim.

Results: Clinical signs in primary ovarian insufficiency is amenorrhea before the age of 40, hot flashes, anxiety, depression are fatigue. Primary ovarian insufficiency can be detected by using laboratory criteria and ultrasound techniques. Laboratory Criteria are Increased serum Follicle stimulating hormone (FSH) levels above 40 mIU / ml and decreased serum Anti-Mullerian Hormone (AMH) levels. women with ovarian failure have no antral follicles and size of ovaries are less than normal. It must be considered ultrasonography, specially vaginal sonography in detecting the follicles has major role.

Conclusion: A women with primary ovarian insufficiency (POI) have less chance for pregnancy and they most not waste the time.

Keywords: Primary Ovarian Insufficiency, Assisted Reproductive Technology, Infertility

P_{nm}-3: Quality of Life in Women with Polycystic Ovary Syndrome: A Literature Review

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Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder characterized by hyperandrogenism and chronic anovulation. PCOS is one of the leading causes of infertility and manifests with acne, hirsutism, and obesity. PCOS has been shown to cause a reduction in quality of life. The aim of the study was to evaluate the effect of polycystic ovary syndrome on quality of life.

Materials and Methods: Searching performed in some databases like PubMed, google scholar, Proquest, Scopus, Springer and Science Direct. 21 full-text articles in English from 2000 until 2019 were found which their topic was similar to our topic. Main outcome measures were Short Form-36 Health Survey (SF-36), concerning socio-demographic conditions (age, marital status, education, occupational activity, type of work, profession, physical activity) and clinical information of PCOS. Major clinical PCOS features including excessive body hair (hirsutism score), acne, and menstrual cycle disturbances.

Results: Quality of life parameters for women with polycystic ovary syndrome were lower than for the controls in the aspect of: general health, limitations due to physical health, limitations due to emotional problems, social functioning, energy/fatigue and emotional wellbeing. Moderate and severe hirsutism negatively correlated with some quality of life parameters in the study group: general health, limitations due to emotional problems, social functioning, and emotional well-being. Moreover, a negative association was found between Quality of life and menstrual disturbance.

Conclusion: Polycystic ovary syndrome decreases quality of

life with respect to: general health, limitations due to physical health and emotional problems, social functioning, energy/fatigue, as well as in the emotional and psychological sphere among women. A negative effect of hirsutism severity on quality of life. Awareness and knowledge of healthcare professionals regarding the quality of life in women should be increased.

Keywords: Quality of life, SF-36, Polycystic Ovary Syndrome

P_{nm}-4: The Effect of Stress Management Based on Cognitive-Behavioral Method on Infertility Related Stress in Infertile Couples

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Background: Infertility, have an emotional and stressful nature, is one of the life crisis for men and women. Different methods have been used to reduce infertility related stress and thus improve treatment in infertile couples. The purpose of this study was to investigate the effect of stress management based on cognitive behavioral method on infertility related stress in infertile couples.

Materials and Methods: In this randomized clinical trial study, 20 infertile couples who referred to the Mahdieh Hospital from April 15 to May 15, 2017, were attending. Sampling was done randomly and assigned to two experimental group (10 couples) and control group (10 couples). Data were collected using demographic questionnaire, Fertility Problem Questionnaire (infertility-related stress). Data were analyzed by descriptive and analytical statistics (Generalized Equation Estimation Model (GEE)).

Results: The mean score of total stress associated with infertility in the control group was significantly higher than the intervention group ($P < 0.001$). Also, the mean scores of subscale of social concern, sexual concern, and rejection of childfree lifestyle in the control group were significantly higher than the intervention group ($P = 0.001$, $P = 0.01$, $P = 0.006$).

Conclusion: The implementation of stress management based on cognitive behavioral and infertility counseling is an effective method for controlling the infertility-related stress in infertile couples. It is suggested that this effective counseling method be put into the care and support package of infertile couples to reduce the consequences of infertility by reducing and controlling their stress.

Keywords: Cognitive Behavioral Therapy, Infertility, Stress, Couples

P_{nm}-5: Evaluation Effect of Curcumin in Treatment of Polycystic Ovary Syndrome

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Background: Polycystic ovarian syndrome (PCOS) is the most

common endocrine disorder in women ages reproduction, associated with a lot of cardiometabolic consequences, with obesity, insulin resistance, and hyperandrogenemia. Due to the complications caused by the use of old therapies, today more and more new treatment has been taken into account such as curcumin. Curcumin is one of the principal anti-inflammatory and healthful components of turmeric comprising 2-8% of most turmeric preparation.

Materials and Methods: A literature search was conducted through PubMed and Science direct to identify the effect of curcumin on PCOS

Results: According to studies, curcumin can cause a reduction in lipid profile such as Triglyceride, T-Chol, LDL-C and T-Chol/HDL-C ratio. A number of studies have shown curcumin was associated with a significant reduction in liver fat content and plasma level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and glycated hemoglobin. Curcumin Can reduce in plasma level of glucose and increase the insulin level as a result Can improve insulin resistance in PCOS patient. Curcumin also effects on obesity which is an inflammatory process. As a result, it reduces inflammatory cytokines such as TNF α , adipokines, adiponectin, IL18, and IL6.

Conclusion: Since curcumin effects on inflammatory markers and insulin resistance we can with more studies use it as an adjunct to other drugs in the treatment of PCOS.

Keywords: Polycystic Ovarian Syndrome, Curcumin, Inflammatory Factors, Insulin Resistance

P_{nm}-6: Inflammatory Factors in Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a complex condition that usually affects young women and is known by ovulatory dysfunction and androgen excess. The factors that cause PCOS are multiple, such as; genetic, lifestyle mistakes, and their combinations. PCOS is considered as a mild and chronic inflammatory condition, as a result, inflammatory factors are affected.

Materials and Methods: A literature search was conducted through PubMed.

Results: A low-grade chronic inflammation state is also found in most PCOS women and has been related to metabolic and ovarian abnormalities, including androgen excess secretion. Adipose tissue releases more than 50 cytokines, acute-phase proteins, and other inflammatory mediators, which have an autocrine, paracrine, or systemic function and proinflammatory or anti-inflammatory activities that change in PCOS. According to study increased in circulating level of leptin, Resistin, Visfatin, Chemerin, and RBP4. Furthermore decreased or unchanged level of Adiponectin and Omentin can be seen. On the other side in PCOS patient concentration of inflammatory factors level are increased such as CRP, TNF α , IL17, IL18, IL1B, IL6 but IL10 is an anti-inflammatory factor so the level of it decreased in PCOS.

Conclusion: In PCOS inflammatory factors are affected as a result it can be used to treat and influence this disorder by further studies.

Keywords: PCOS, Inflammatory Factor, Cytokines

P_{nm}-7: The Relationship between Dietary Vitamin A and β -carotene Intake and Parameters of Semen Analysis

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Background: Oxidative stress has been known as a cause of male infertility, and antioxidants have been considered as an effective agent against oxidative stress. Present study was conducted to determine the relationship between dietary intake of vitamin A and β -carotene and sperm analysis parameters.

Materials and Methods: A descriptive-correlational study was performed on 190 healthy men who had inclusion criteria in the study and referred to the Fertility and Infertility Center of Shahid Beheshti in Isfahan, Iran (2016-2017). The dietary intakes of vitamin A and β -carotene were obtained by Food Frequency Questionnaire (FFQ) and Nutritionist IV software. Data analysis was performed using software SPSS version 16.

Results: The average vitamin A and β -carotene intake in men was 2081.81 ± 1451.47 mcg and 1485.026 ± 1316.41 mcg respectively. The results showed that the average amount of vitamin A and β -carotene were not significantly related to sperm analysis ($P > 0.05$).

Conclusion: The dietary intake of vitamin A and β -carotene were not significantly correlated with sperm analysis parameters (volume, count, motility, morphology and number of WBC in semen fluid). Cohort and follow-up studies are needed to track the fertility results of men in different groups of fertility and to examine the integrity of the sperm DNA to achieve more accurate results in male fertility.

Keywords: Male Infertility, Semen Analysis, Antioxidant, vitamin A, β -carotene

P_{nm}-8: Herbal Medicines in Infertility

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Background: Infertility is a condition that pregnancy has not occurred after 12 months of regular and unprotected sexual intercourse. Herbal remedies are medicines made from plants that have been used for thousands of years. This article provides an overview of the use of herbal medicines in infertile women and men.

Materials and Methods: A literature search was conducted through Scopus.

Results: Fertility herbal remedies have an affirmative effect on the hormonal system, reproductive organs, and sex drive. Withania somnifera cures impotence and increase sex appeal and fertility and improves sperm count and motility inhibits

lipid peroxidation and regulates reproductive hormone levels. It is helpful for women by promoting appropriate functioning of the reproductive organs and maintaining hormonal balance, also helps to the tone of the uterus for those who have recurrent miscarriages. *Alpinia officinarum* can be effective in sexual dysfunction and improves sperm morphology and sperm count in idiopathic infertility. *Allium sativum* increases fertility, spermatogenesis and the level of testosterone and improves the testicular structure. *Chasteberry* is effective in hormonal imbalance and improves ovulation due to its high prolactin levels. *Pomegranate* increases blood flow to the uterus and thickens the uterine lining to decrease the chance of miscarriage. *Maca Root* can treat infertility in both women and men. It increases normal hormone production and especially useful for women with hypothyroidism because it supports thyroid function. *Wenshen Yangxue* formula increased the percentage of fertilization and follicles, improved quality of oocytes in mice. Korean herbal medicine (MYOMI-14) improved the sperm concentration, sperm progressive motility, total motile sperm count. *Eurycoma longifolia* Jack improved the male libido, sexual prowess, and fertility. It increased testosterone steroidogenesis in the rat. *Tribulus* provided desirable effects on sperm parameters in idiopathic infertile men.

Conclusion: Based on the evidence of herbal medicines, may present effective treatment for infertility. Only because herbal remedies are obtained from natural herbal sources does not mean that they are not dangerous if taken incorrectly.

Keywords: Infertility, Herbal Medicine, Herbal Remedies

P_{nm}-9: Reproductive Technology Success Rates Nutrition Helps Assistance

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Background: One in six couples around the world experience infertility. Before undertaking expensive and intrusive assisted reproductive treatment such as *in vitro* fertilization, many seek advice from their physicians or dietitians on what foods and supplements might enhance their fertility. But health practitioners are often ill equipped to provide dietary recommendations in a scientifically based manner. Nutrition, Fertility, and Human Reproductive Function provides a comprehensive guide to clinicians on how they can best advise their patients to optimise fertility and reproductive function through optimal nutrition.

Materials and Methods: This is a review article. Literature for the period 2005-2019 was searched in the electronic databases of google scholars, Cochrane, science Direct and Pub Med using the following key words.

Results: Researchers want to include foods that are good for your hormones, for egg development, and for your uterus and other reproductive organs. An easy and effective way to support your female hormones is by making sure you are eating healthy fats and protein with each meal. Blood sugar fluctuations can wreak havoc with female hormones. Insulin is a hormone and when it is fluctuating wildly, it can cause your thyroid and sex hormones (like estrogen) to get out-of-whack. Also, healthy fats and cholesterol are needed for production of both male and female sex hormones. As a bonus, eating more healthy fats and

protein will make you feel fuller and more satisfied. You will want to eat around 1 to 2 Tablespoons of fat with each meal. Antioxidants protect your cells and your eggs from damage. You want to make sure you are eating high in antioxidants like vitamins A, C and E, selenium.

Conclusion: Foods are almost always going to be the best way to nourish your body, but in some instances, it may be wise to supplement. Please talk with a Nutritional Therapy Practitioner about how to get started adding these foods to your diet and if supplementing may be right for you. There have been many research studies done on how nutrition and nutrients affect fertility overall, fertility treatments, egg health, sperm health, and reproductive success. While there is no one large study specifically about nutrition and ART success, there are many studies about various aspects of fertility and ART and nutrition.

Keywords: Assistance Reproductive Technology, Nutrition, Fertility

P_{nm}-10: Placenta Previa after ART

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Background: Placenta previa is a drastic disorders of pregnancy with fatal maternal and neonatal outcomes. The risk of placenta previa can be enhancement in pregnancies received by assisted reproduction technology (ART).

Materials and Methods: A narrative review was performed within articles published a “PubMed”, “Elsevier”, “SID” and original text books to reach the aim.

Results: The treatment of infertility is growing speedily. The placenta has a main role in fetal health and functions as a essential bridge to normal fetal development among pregnancy. By reviewing studies there was a six-fold higher risk of placenta previa in singleton pregnancies conceived by assisted fertilization compared with naturally conceived pregnancies. In IVF treatment, the risk of placenta previa is elevated 4-fold in women with an endometrial thickness of >12 mm against women with an endometrial thickness of <9 mm. The diagnosis is suspected using a combination of gray – scale and color flow Doppler ultrasound. Transvaginal sonography is a useful adjacent, especially with coexistent placenta previa.

Conclusion: It must be considered the couples who start the infertility treatment, spend high cost and time. They need to be awarded of the increased risk of Placenta previa after ART and must be helped on managing the pregnancy.

Keywords: Placenta Previa, ART, Prenatal Care, Infertility,

P_{nm}-11: Epidemiology of Infertility

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Background: The aim of epidemiologic research is to study

the distribution and determinants of disease in human populations with the intention of prevention and treatment of disease. Infertility is a condition of the reproductive system defined as not conceiving after 12 months of regular and unprotected sexual intercourse which have a pervasive adverse impact on the whole family and reproductive health. Female Infertility is classified into primary or secondary. Primary infertility refers to women “who have never been diagnosed with a clinical pregnancy and meets the criteria of being classified as having infertility”. Secondary infertility refers to women who are unable to establish a clinical pregnancy but have previously been diagnosed with a clinical pregnancy. The goal of this article is to provide an overview of epidemiology and infertility.

Materials and Methods: A literature review search was conducted through PubMed.

Results: In addition to the advanced maternal age at conception which is the most important negative predictive factor of fertility, other factors including lifestyle and environmental factors are postulated to play an active role. Nearly, 8-12 % of all couples suffer from infertility, the majority of them live in developing countries. Secondary infertility is the most common type of female infertility in worldwide due to post-abortive and postpartum infections. Most pregnancies (80%) occur within the first six cycles of attempted conception with regular intercourse. After six unsuccessful cycles with fertility, half of the remaining 20% of couples will conceive spontaneously in the next six cycles. After that, 10% of the couples are defined as infertile, however, approximately 55% of them become pregnant in the next 36 months. After 48 months, about 5% of the couples have absolute infertility (no chance of achieving a spontaneous pregnancy). Evidence from review studies documents the alarming global decline of age-related fertility due to the increase of the average age of first-time mothers (between the ages of 30 and 35 or older) and the median age at last birth for females (40–41 years). The data of a study on female age at last birth in a natural fertility population shows that the age-related impaired fertility gradually increases from 4.5% at age 25 years to 20% at age 38 years. Afterward, it rises quickly to about 50% at age 41, 90% at age 45 years and approximately 100% at age 50 years. It reflects the fact that many women are still unaware of postponing childbearing as an important risk factor for fertility. According to the reports of the studies, the true incidence of male infertility is unknown and the prevalence has been weakly estimated in various studies. Ignoring the differences in these research methodologies, there are wide variations in the published data on the prevalence of male infertility ranges from 6% to 50%. Not determining male infertility as a reportable disease, diagnosing and treating male infertility in an outpatient clinical setting, not reporting male infertility on insurance billing (it is paid out of pocket) are some of the factors mentioned in the studies that have impeded the epidemiology study of male infertility.

Conclusion: This report summarizes current knowledge about epidemiology of female and male infertility.

Keywords: Epidemiology, Female Infertility, Male Infertility

P_{nm}-12: Oxidative Stress Markers in Early Pregnancy loss: A Case-Control Study

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Background: Evidence suggests that oxidative stress plays a role in the pathophysiology of pregnancy complications in women. The present study was conducted to determine the levels of oxidative stress markers in early pregnancy loss and to compare the results with those in healthy pregnant women.

Materials and Methods: A total of 32 women with early pregnancy loss and 32 healthy women in the first trimester of pregnancy and with similar demographic characteristics entered this study as the cases and controls. Serum levels of malondialdehyde (MDA), total antioxidant capacity (TAC) and uric acid and bilirubin levels were determined in both groups. The data obtained were then analyzed and compared between the groups using the independent-sample t-test and Mann-Whitney's U-test.

Results: The two groups were matching in terms of personal-demographic characteristics including mother's age, father's age, gravidity and body mass index (BMI). MDA levels increased significantly in the women with spontaneous abortion compared to the healthy pregnant women (4.35 ± 1.47 vs. 3.42 ± 1.68 $\mu\text{M/L}$; $P=0.026$) and TAC decreased significantly in the cases compared to the healthy controls (552.34 ± 212.79 vs. 1003.23 ± 1168.68 U/ml ; $P=0.040$). Uric acid and bilirubin levels did not differ between the groups.

Conclusion: The results of this study provide further evidence on the effect of increased oxidative stress on the incidence of early spontaneous abortion in the first trimester of pregnancy. High serum MDA levels and low TAC during pregnancy were two risk factors for spontaneous abortion. The present findings support the hypothesis that oxidative stress plays a key role in the etiopathogenesis of spontaneous abortion. Further studies are required for assessing the preventive role of antioxidant therapy in this complication.

Keywords: Oxidative Stress, Pregnancy, Spontaneous Abortion

P_{nm}-13: Polycystic Ovary Syndrome and The Role of Weight Loss Surgery in Female Fertility and Adiponectin Level

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Background: Polycystic ovary syndrome (PCO) is a prevalent endocrine disorder. Metabolic syndrome like endometrial hyperplasia (EH), insulin resistance, hypertension and obesity in women in the fertility age is related with PCO. Obesity has an unfavorable effect on pregnancy, fertility techniques and fetal-maternal consequence. We studied the benefit of weight loss surgery as a treatment way in women with PCOS and EH. Bariatric surgery, decrease possibility pre-eclampsia, CRP, diabetes and increase adiponectin level in pregnancy. However, there is a rise in SGA and stillborn or neonatal death.

Materials and Methods: This is a brief review of recent literature on the status research networks such as Google Scholar, PubMed to identify adiponectin level and role of weight loss surgery in PCO women.

Results: Thus, weight loss surgery in corpulent women with infertility can lead to gestation. The effect of weight loss surgery in women fertility is important and increase adiponectin level.

Conclusion: Studies have shown that bariatric surgery is consequential in the managing of female with PCO and enhance

fertility. Bariatric surgery has a beneficial impact on endometrial hyperplasia, endometrial cancer prevention and treatment and surging adiponectin levels in PCOS women. However, this enhancement was slower differentiate to obese non PCOS women. A genetic sensitivity to insulin resistance might describe these conclusions.

Keywords: Polycystic Ovary Syndrome, Obesity, Weight Loss Surgery, Adiponectin

P_{nm}-14: The Impact of Obesity on Male Fertility

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Background: Infertility is defined as a couple's inability to achieve pregnancy following one year of unprotected intercourse. Male factors alone constitute 25-30% of all cases of infertility. It seems that obesity is associated with reduced male fertility. The rate of obesity among young men is constantly increasing and the rate of obesity has more than doubled over the past decade and it is a major health problem throughout the world. This review focuses on negative impact of obesity in men fertility and the benefits of weight loss on reproductive outcomes.

Materials and Methods: This review article has been extracted from 28 articles that has indexed in most valid scientific cites that has published from year 2012 to 2019.

Results: Some etiology for male infertility include cryptorchidism, testicular torsion or trauma, varicocele, seminal tract infections, antisperm antibodies, gonadal dysgenesis, and obstruction of the reproductive channels. Obesity was recently proposed for addition to this list. the relationship between obesity and semen characteristics, reproductive endocrine function, sexual function, and male infertility is found. Obesity increase estrogen and it is an inverse correlation between obesity and testosterone and a direct correlation with estrogen. Obesity is probably due to the interaction between multiple genes and several environmental factors, including diet and activity level. Obesity is often associated with a lifestyle characterized by decreased physical activity with prolonged periods of sitting, which has been shown to affect sperm production by increasing local testicular temperature. Obese infertile men can also have a characteristic scrotal fat deposition that can increase the local testicular temperature and affect sperm production. Another potential cause for infertility in obese men is the reduced coital frequency associated with obesity. In men reporting symptoms of erectile dysfunction, overweight or obesity are found in 79% of subjects.

Conclusion: In obese males, evidence suggests that increased estrogen as a result of aromatization in the fatty tissue may be an important mechanism for the hypoandrogenemia and altered sperm parameters. There is evidence that weight reduction can correct this hormonal imbalance. Weight loss is the cornerstone of the treatment of obesity-associated infertility. Most studies have focused on the effects of weight loss on the hormonal profile of obese men. In addition, physical activity and leanness are associated with a reduced risk for erectile dysfunction. Lifestyle changes that encourage weight loss, including diet and exercise Then can improve in erectile dysfunction.

Keywords: Infertility, Obesity, Men, Exercise

P_{nm}-15: The Effects of Surgical Treatment for Endometriosis on Female Sexual Functioning: A Systematic Review

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Background: Some diseases such as endometriosis can affect sexual satisfaction and quality of life. The aim of this study were to systematically review the literature on the effects of surgical treatment of endometriosis on female sexual functioning

Materials and Methods: The present study is a systematic review that is performed by electronic database search of studies on the association between Endometriosis, Endometriosis Surgery and Sexual Dysfunction Which published between 2000 and 2019.

Results: 16 study (14 quantitative and 2 qualitative) were included. Data were analyzed by using narrative synthesis. According to the results of the studies, positive changes after laparoscopic surgery have been seen and dyspareunia, sexual performance index and sexual quality of life had the most positive changes.

Conclusion: Laparoscopic surgery has a positive effect on the improvement of sexual dysfunction.

Keywords: Endometriosis, Laparoscopic Surgery, Sexual Dysfunction

P_{nm}-16: Serum Adiponectin Levels and Phenotypes of Polycystic Ovary Syndrome: A Literature Review

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Background: Adiponectin is a circulating protein produced by adipocytes. Circulating levels of adiponectin are inversely related to adipocyte mass. Low levels occur with insulin resistance, type 2 diabetes, metabolic syndrome, and obesity-related cardiovascular disease. The purpose of this article is to review the literature to determine what is known about the connection of adiponectin And phenotypes of PCOS.

Materials and Methods: Comprehensive searches in the electronic databases MEDLINE, EMBASE and Science Citation Index Expanded were conducted to identify published studies evaluating the association between Adiponectin and phenotypes of PCOS.

Results: Studies have indicated that a significant relationship was found between adiponectin and phenotypes of Polycystic Ovary Syndrome.

Conclusion: adiponectin serum concentrations vary according to the phenotypic expression of PCOS. The relationships between adiponectin and insulin resistance and sensitivity, metabolic syndrome, and BMI in women with PCOS suggest that adiponectin could be used as a biochemical marker to identify phenotypes of PCOS.

Keywords: Adiponectin, Polycystic Ovary Syndrome, Phenotypes, PCOS,

P_{nm}-17: Inflammatory Factors in Endometriosis

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Background: Endometriosis affects up to 15 % of reproductive age women. The growth of endometrial epithelial and stromal cells outside the uterine cavity characterizes the endometriosis. It is associated with chronic pelvic pain, infertility and reduced quality of life. The histogenesis of endometriosis remains unclear, accumulating evidence indicates that combinations of hormonal, immunologic, genetic and environmental factors are involved in the origin and development of endometriosis. In this article, an overview to identify an association between altered serum inflammatory factors and endometriosis.

Materials and Methods: A literature search was conducted through ScienceDirect.

Results: Endometriosis is a chronic local inflammatory disease and alterations in the immune system might play an important role in its pathogenesis. The growth of endometriotic lesions relies on estrogen. These lesions increase pro-inflammatory cytokine, chemokine and growth factor concentrations in the local environment. It has been evidenced that the expression of some inflammatory factors including interleukin (IL) IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-15, IL-17, IL-18, IL-27, and IL-37, in the serum, peritoneal, and follicular fluid of women with endometriosis differs from that of women without this condition. Previous studies have demonstrated a role for elevated chemokines and MMPs in endometriosis lesions, peritoneal fluid, and peritoneal macrophage gene expression. there are at least six (CXCL1, CXCL8, CXCL13, CXCL14, CCL2, and CCL5) different chemokines that were evaluated in association with infertility. the pelvic fluid concentration of the B-chemokine CCL5, also called RANTES (Regulated upon Activation, Normal T Cell Expressed and Secreted), was significantly elevated in women with endometriosis. and that these levels correlated positively with the stage of the disease MCP-1 (monocyte chemotactic protein-1) or B-chemokine CCL2 and Eotaxin – or CCL11 – is another B-chemokine (CC chemokine) in women with endometriosis increase in the peritoneal fluid that correlates with the severity of the disease.

Conclusion: It is imperative that we now understand that endometriosis is a complex and possibly a systemic disease, with multiple factors involved in its pathophysiology. A panel of biomarkers will most be necessary to diagnose and treat a complex disease such as endometriosis.

Keywords: Endometriosis, Inflammatory Factor, Etiology of Endometriosis

P_{nm}-18: Antioxidant in Endometriosis Treatment

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Background: The etiology and pathogenesis of endometriosis is controversial. there are many studies investigating the relationship between endometriosis and oxidative stress. Apoptotic endometrial tissues in endometrial implants produces

reactive oxygen metabolites as metabolic intermediate product. The increase of the production of reactive oxygen and nitrogen species (RONS) known as free radicals or the reduction of antioxidants bioavailability trigger the oxidative stress leaving organisms more susceptible to oxidative damage. An overview to identify an association between antioxidative treatment and improvement of endometriosis symptoms.

Materials and Methods: A literature search was conducted through Pubmed and ScienceDirect.

Results: Antioxidants are a defense mechanism created by the body to neutralize ROS. Antioxidants can protect against the cell damage that free radicals cause, known as oxidative stress. Some of them that may be effective in endometriosis treatment are: Vitamin C is an effective antioxidant that acts directly with superoxide, OH radicals, and singlet oxygen. Vitamin E acts as a peroxyl radical scavenger, inhibiting the effect of free radicals by forming a tocopheryl radical. Resveratrol is a natural polyphenolic flavonoid synthesized by plants subsequent to ultraviolet radiation that inhibits ROS production in monocytes, macrophages and lymphocytes also were found to affect cell proliferation and apoptosis by inhibiting the NF-KB. Melatonin is a main secretory product of the pineal gland synthesized from tryptophan that is both a powerful free-radicals scavenger and an antioxidant enzymes stimulator with potent anti-inflammatory attributes. Xanthohumol is a polyphenol chalcone from hops that has antioxidative and anti-inflammatory effects are achieved by the inhibition of NF- kB signaling pathway. Epigallocatechin-3-gallate (EGCG) is the most abundant polyphenol found in green tea acts by reducing OS via inhibition of angiogenesis through VEGF reduction. EGCG suppresses estrogen-stimulated activation, proliferation and VEGF expression of endometrial cells. N-acetyl-L-cysteine (NAC) is a thiol antioxidant and the precursor of glutathione that is apart from its ability to protect against damage from both hydroperoxides and other alkylating agents. Activation of the immune system by increasing glutathione levels, it suppresses the activation of NF-Kb an important transcriptional factor. Omega-3 fatty acids inhibit the release of inflammatory mediators like IL-8 and prostaglandins in human endometrial stromal cells (HESC).

Conclusion: Antioxidative stress agents showed a significant inhibitory effect on different studied aspects of the development and progression of endometriosis

Keywords: Endometriosis, Antioxidant, Stressoxidative

P_{nm}-19: Nutrition and Infertility

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Background: There is a growing body of evidence that nutritional habits may have an important role in fertility. The objective of this study is to provide a comprehensive assessment of existing studies on the relationship between nutrition and infertility.

Materials and Methods: Using an integrative review, articles for infertility and nutrition published from Jan1990 to Feb2019 in databases including MEDLINE, ISI Web of Knowledge, Scopus, Google Scholar, IranDoc, IranMedex, MagIran and SID in English and Persian languages were extracted. After assessment of inclusion and exclusion criteria, 41 articles were selected and reviewed.

Results: Adequate intake of monounsaturated fatty acids, derived mainly from vegetable fats, as well as avoidance of unsaturated fatty acids may be effective in the prevention of infertility in females. Choice of plant rather than animal sources of proteins, decrease in glycemic load of the diet, use of dietary supplements containing iron and folic acid, could also be beneficial. Also a positive correlation is found between the consumption of too much carbohydrate and abnormal sperm motility. Excessive consumption of sweet junk food and drinks and saturated fat are reported to cause the sperm count to decrease. There is a positive relationship between the intake of vitamin C and β -carotene and sperm count, concentration and the total progressive motile sperm count; between the intake of vitamin E, oral zinc supplement and progressive motile sperm count.

Conclusion: According to the role of nutrition in infertility, it is suggested to integrate nutrition counseling into clinical guidelines of infertility for individuals of reproductive age.

Keywords: Diet and Fertility, Nutrition and Fertility, Infertility, Infertility Treatment,

P_{nm}-20: Prevalence and Causes of Infertility in Iran Comparison with other Parts of The World

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Background: Infertility is a common problem in couples and is now viewed as a global reproductive health issue. The causes of infertility vary widely, between countries and within the country. Environmental and geographical factors can affect women and men's infertility. The purpose of this study is to determine the prevalence and causes of infertility in Iran in comparison with other parts of the world.

Materials and Methods: This review study was conducted by searching articles from 2015 to 2019 in Persian and English in Scientific Direct, Google Scholar, PubMed and SID databases. The keywords used are "infertility", "prevalence", "causes" and similar words. Review articles and non-epidemiological studies were excluded from this study.

Results: Infertility prevalence was about 5 to 17.5% in different areas of Iran. Primary infertility incidence was 60-70% and the secondary infertility incidence was 30-35%. The most prevalent cause of infertility in women was found to be in relationship with ovarian factors (33.5%) and menstruation disorders. Abnormal semen parameters was Infertility was significantly correlated with age, educational level, body mass index, waist circumference, history of abortion, smoking and family history of infertility. Infertility prevalence was about 14% in other countries such as China and Nigeria. Primary infertility incidence was 13-24% and the secondary infertility incidence was 34-38%. Tubal factor was the predominant cause of infertility cases among the women. Women with a history of vaginal discharge, puerperal sepsis, induced abortion and pelvic surgery were more likely to have tubal factor infertility.

Conclusion: Infertility is a common presentation in the clinics and the secondary type is predominant in this setting. The cause of infertility most commonly identified in the studied Iranian population was ovarian factors and menstruation disorders.

Keywords: Infertility, Prevalence, Causes

P_{nm}-21: Intellectual Disability and Assisted Reproductive Technology

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Background: Assisted reproductive technology (ART) is an important method for the infertile couples to have child. Children born of these methods may be exposed to some complications in the long term. This study was performed with aim to assess intellectual disability (ID) in ART.

Materials and Methods: In this literature review article, the related articles in English were collected from databases of PubMed, Scopus, Google Scholar using the keywords "intellectual disability", "intellectual disorders", "intellectual impairment" and "assisted reproductive technology" from 2016 to 2019. Proper articles were included in final analysis.

Results: Generally, there is a risk of an increase in the incidence of childhood ID in children was born by ART Compared with spontaneous conception. But the increase is low. Some statistically significant studies have been reported. For specific procedures in most of these studies, the rate of increase in ICSI is more than IVF and the increase in absolute risk associated with IVF was small. Other articles have not reported a significant difference.

Conclusion: There is a controversy in studies in relation to the this subject due to differences in research environment, follow-up period and other variables related to the study. Given that there is no decisive results in increasing the incidence of ID in children were born by ART, More extensive cohort studies are needed in this regard. Considering the possibility of an increase in this complication, it is imperative that this issue be taken into account in the infertile couples counseling program.

Keywords: Intellectual Disability, Intellectual Disorders, Intellectual Impairment, Assisted Reproductive Technology

P_{nm}-22: Effects Obesity on *In Vitro* Fertilization Treatment Outcomes: A Systematic Review

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Background: A worldwide increase in the prevalence of obesity has been observed in the past three decades, A worldwide increase in the prevalence of obesity has been observed in the past three decades, particularly in women of reproductive age. The number of young obese women assisted reproductive treatment (ART) is on the rise. Obesity also contributes to adverse clinical outcomes following *In vitro* fertilization (IVF) procedures. The aim of this study was to evaluate the influence of obesity (BMI > 30 kg/m²) on the IVF treatment outcomes.

Materials and Methods: A systematic review was performed using the following key words: Obesity, ART, IVF in Medline, Embase, Cochrane Library, Pubmed, Science Direct databases from 1986 to 2018. Study selection was based on title and ab-

stract. Based on the Jdad scale, studies with scoring 3 or more were enrolled in the study. Finally, out of a total of 30 articles were related, the full text of 23 studies with inclusion criteria were evaluated.

Results: Obesity induces changes in the hypothalamus-pituitary-gonadal (HPG) axis, hormone levels, gametogenesis, and adverse ART outcomes. During the IVF cycle, serum CRP levels are significantly higher in women with obesity at all time points. High serum CRP level on day of ovum pick-up (OPU) day negatively affects embryo quality. Obesity is associated with decrease FSH receptor (FSHR) expression and E2 production, causes by dysfunctional insulin pathway and insulin reduces reaction of follicular granulosa cell to FSH Stimulation in Obesity-Related Infertility Women during IVF. Also in this group of women, Obesity negatively influence the number of oocytes retrieves and is associated with an increased failure to achieve a clinical intrauterine gestation and live birth.

Conclusion: Obesity have detrimental effects on IVF treatment outcomes, therefore results support the clinical recommendation of advising obese women to lose weight prior to ART.

Keywords: Obesity, *In vitro* fertilization, Assisted Reproductive Treatment, Obesity, IVF, ART

P_{nm}-23: The Psychological Complications of Surrogate Mothers

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Background: Advanced infertility technology is rapidly expanding in the world and one of the most controversial of reproductive innovations is surrogacy. The effects of this approach on Surrogates and intended parents vary from one country to another and in different cultures. Surrogate's Mothers Motivations are different including financial reasons, empathy for childless couples, gain a sense of achievement or enhance their self-esteem. Regardless of these Specific factors may cause psychological harm to surrogates. This study conducted to determine the psychological complications of surrogate mothers.

Materials and Methods: This review is based on new Studies were identified by searching multiple literature databases, including PubMed, SCOPUS, ISI and Google Scholar related to psychological complications of surrogate mothers which published up to May 2019.

Results: In the reviewed articles, countries in which surrogacy agencies using a mental health professional in order to minimize potential psychological problems and other negative effects of the surrogacy process, the amount of psychological disorders after delivery was low also women from lower-income populations in developing nations suffer from high levels of emotional problems, primarily prenatal and postnatal depression, compared to women from higher-income nations. Because women form a deep bond with the unborn baby. Usually gestational surrogates that were hosting pregnancies for international intended parents, had higher levels of depression compared to another surrogates. Social stigma is another factor that likely to generate feelings of depression and anxiety.

Conclusion: The following factors were considered as effective factors in depression, anger and a sense of fault of surrogate's mothers: demographic factors (socio-economic status, educational status and marital status), pregnancy (support dur-

ing pregnancy), bonding with the fetus (Emotional Prenatal Bonding and Instrumental Prenatal Bonding), surrogacy arrangement (satisfaction with payment and feelings towards surrogate house) and stigmatization (hiding surrogacy and facing criticism so psychological preparation, giving enough information about issues when making a decision and pay attention to cultural factors are effective in reducing surrogates psychological problems.

Keywords: Surrogate, Surrogacy, Psychological Complications, Depression

P_{nm}-24: Assisted Reproductive Technology and Risk of Autism

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Background: In the last decades, prevalence of Autism Spectrum Disorders (ASD) has dramatically increased. Though genetic factors are known to be involved in the etiology of ASD, research demonstrates that environmental factors such as congenital rubella, maternal use of thalidomide, or valproic acid during pregnancy have been associated with autistic behaviors. Among the environmental factors, some authors reported association between ASD and assisted reproductive technology. Their hypothesis was the association between hormonal factors and ASD. This study conducted to determine assisted reproductive technologies and its relation to autism.

Materials and Methods: This review is based on a number of new Studies were identified by searching multiple literature databases, including PubMed, SCOPUS, ISI and Google Scholar related to *In vitro* fertilization and risk of autism which published up to May 2019.

Results: In the reviewed articles, opposite results were reported. Some reports have suggested an increased risk of ASD with use of infertility treatments but most of these studies have been case-control and retrospective without rigorous confirmation of exposures or have had very small case numbers and did not adjust for potential confounders. For example increased parental age which has been associated with both use of infertility treatments and having a child with ASD, in some studies have not been considered. Also in some studies a higher risk of ASD in children born after assisted conception might be related to the higher rates of multiplicity, preterm birth and low birth weight deliveries. Against, few studies reported that assisted reproductive technology is not a strong independent risk factor for ASD and finally one study has reported favoring a protective role of ART.

Conclusion: According to contradictory results, prospective, large and high-quality studies are still needed.

Keywords: Assisted Reproductive Technology, Autism, Autism Spectrum Disorders, Intra Cytoplasmic Sperm Injection, *In Vitro* Fertilization

P_{nm}-25: Psychological Changes in Infertile Couples and Their Management Title

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Background: The World Health Organization has identified infertility as an important problem in reproductive health. Infertility is one of the individual and social problems that can lead to significant emotional, psychological and social disorders, and has multiple consequences and consequences.

Materials and Methods: This is an overview article and has been compiled using library resources through a search of valid databases such as Google Scholar, SID, IranMedex, PubMed and Scopus from 2008 to 2018.

Results: Infertility has been reported as a stressful, frustrating and frustrating incident in infertile couples (especially women), which can lead to sexual dysfunction, reduced self-esteem, increased sense of humiliation, decreased self-esteem, loneliness and rejection, feelings of guilty, anxiety, depression, disability, frustration, anger, feelings of defect and disadvantage, defect in social identity and emotional imbalance. Attention to these psychological changes and management is essential, and the following strategies for coping and managing them are recommended: Detecting the differences between men and women, communicating with their feelings, questioning each other's needs, participating in treatment, meeting new people, developing feelings, sharing psychological burden with others, increasing self-esteem with the best in everything, searching for hidden concepts of infertility, and etc.

Conclusion: Because attention to psychological changes in the infertile couple is an essential part in their treatment, lack of knowledge about the psychological aspects of infertility can have adverse consequences. Therefore, actions such as raising the level of people's awareness about emotional dimensions Psychological infertility, helping the infertile couples to better balance stress, helping the decision-making process, and so on.

Keywords: Infertility, Psychological Emotional Changes, Coping Strategies

P_{nm}-26: The Prevalence and Clinical Pattern of Infertility in Sabzevar, Northeastern Iran

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Background: According to different geographical conditions, human health in different sub-regions of the world and cultural differences, infertility has heterogeneous causes in the world. Objectives: This study was performed to prevalence and clinical pattern of infertility in Sabzevar, northeastern Iran.

Materials and Methods: This was a analytical descriptive study of infertile couple who were referred at the infertility clinic in Sabzevar between December 2016 to May 2018. A structured form was used to collect relevant clinical information on each participant's clinical presentation and outcome of management. Data were analysed by descriptive and inferential statistics using SPSS version 18.

Results: There were a total of 101 infertile cases. 58 (57.4%) had primary infertility while 43 (42.6%) had secondary infertility. The male factors 30(29.8%) such as sperm disturbance was most common causes of infertility. In 29 (28.7 %) of couples the cause of infertility was PCOD and in 23(22.8 %) of them the problem were related to male and female factor both. Irregular menstruation, hirsutism, Oligomenorrhea were most common symptom in women.

Conclusion: The prevalence of primary infertility in Iran seems to be higher than the world average. Therefore, it is crucially

important to support the large number of couples who face this problem.

Keywords: Infertility, Prevalence, Women, Iran,

P_{nm}-27: Psychological Disorders in Infertile Women Treated with IVF

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Background: Infertility and treatment is a source of psychological disorders in infertile women. Although *in vitro* fertilization (IVF) has been able to open up many hopefuls for infertile couples, it also has its own problems and has led them to tolerate more stress and longer treatment cycles. Many evidence suggests psychological problems caused by infertility can be a contributing factor in intensifying infertility and failure of its therapies as long as anxiety and depression have been identified as the possible cause of failure of the first period of IVF. The present study aimed at investigate the review of psychological disorders in infertile women undergoing IVF treatment.

Materials and Methods: This review study was conducted by searching the Proquest, Science direct, PubMed, Iran doc, Sid (2000-2018) databases with the keywords " Infertility, Psychological disorders, IVF ", from domestic and foreign studies.

Results: IVF is a stress reliever for patients that requires a lot of invasive procedures. Evidence suggests that in addition to biomedical factors, psychological factors and psychological status of people such as anxiety and depression are effective on the outcome of treatment. Women who are treated with IVF are usually due to infertility and fear of anxiety and depression. Some of the common psychological responses to IVF treatment are: depression, anger, feelings of guilt and sadness, anxiety and depression. Some scholars believe that psychological counseling is necessary before and during treatment for the success of treatment.

Conclusion: The results of the studies indicate that psychological disorders can be a threat to the outcome of IVF treatment. Anxiety also leads to increased intestinal cortisol and prolactin levels. Therefore, it is necessary to attend infertility treatment centers, along with the medical group, psychologists and family counselors, and provide their services in different stages, which will reduce the psychological symptoms, increase mental health and the rate of pregnancy in infertile women

Keywords: Infertility, Psychological Disorders, *In Vitro* Fertilization

P_{nm}-28: Sexual Function in Polycystic Ovary Syndrome: A literature Review

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Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. In addition to mood disturbances such as depression and limitations in emotional well-being, and quality of life, the diagnosis of PCOS also has a negative impact on sexual function. The

aim of this paper is to review the evidence from studies that determine the characteristics of sexual dysfunction in women with PCOS.

Materials and Methods: Searching performed in some databases like PubMed, google scholar, Proquest, Scopus, Springer and Science Direct. 28 full text articles in English from 2000 until 2019 were found which their topic was similar to our topic. Main outcome measures were Female Sexual Function Index (FSFI), socio-demographic details and clinical information of PCOS. Major clinical PCOS features including BMI, excessive body hair (hirsutism score), acne, menstrual cycle disturbances, infertility and endocrine profile.

Results: Sexual problems (especially desire and arousal) are more common in women with PCOS. BMI had a significant effect on sexual desire and arousal while the effect of hirsutism was significant on all domains except for dyspareunia. Moreover, a positive association was found between sexual dysfunction and menstrual disturbance.

Conclusion: PCOS patients markedly suffer from sexual dysfunction as comorbidity. Awareness and knowledge of health-care professionals regarding the sexual dysfunction in women should be increased.

Keywords: Sexual Dysfunction, Female Sexual Function Index, Polycystic Ovary Syndrome

P_{nm}-29: Female and Male Obesity and IVF Outcome: Knowns and unknowns

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Background: Obesity reduces fecundity in both males and females. Although most reports show generally poorer outcomes of assisted reproduction for overweight or obese women, there are important discrepancies in the literature that have been reviewed in this study.

Materials and Methods: This study was conducted as a review of the texts. In the Study key words are considered by using databases Cochrane, PubMed / Medline, Embrace/Scopus, ProQuest from 2000 to 2019.

Results: Obese women especially with abdominal fat distribution, have a lower chance of pregnancy following IVF, require higher dosage of gonadotropins. Although the possible effects of obesity on male reproductive potential have been less studied, it is clear that, obesity are associated with reduced ejaculate volume, sperm concentration and total sperm count. However, these findings are not always consistent. BMI is not the best measure to reflect body fat and does not account for racial and ethnic differences in body build nor higher BMI due to increased muscularity. A recent randomized trial for weight loss among obese infertile women did not show improved birth rates compared with prompt infertility treatment within 24 months of randomization. As suggested by studies using oocyte donation as a research model, it seems that the negative impact of obesity affects both ovarian and uterine functions.

Conclusion: Although most reports show generally poorer outcomes of assisted reproduction for overweight or obese women, there are important discrepancies in the literature. This may be due to observational studies using heterogeneous inclusion criteria and outcome measures. However, the precise mechanisms are still in need of further clarification.

Keywords: Obesity, Female, Male, IVF,

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