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ABSTRACT BOOK

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The *International Journal of Reproductive BioMedicine (IJRM),* formerly published as "Iranian Journal of Reproductive Medicine (ISSN: 1680-6433)", is an international scientific monthly publication of the Research and Clinical Center for Infertility of Shahid Sadoughi University of Medical Sciences and Health Services.

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Abstracts of the 9th Yazd International Congress and Student Award on Reproductive Medicine in association with 4th Congress of Reproductive Genetics

Contents

Key Lectures *(Alphabetic order)*

9th Yazd International Congress and Student Award on Reproductive Medicine

K-1
Ovarian rejuvenation through platelet-rich autologous plasma

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Platelet-rich plasma (PRP) is a novel method that has been successfully employed for a range of medical issues. PRP holds a high concentration of platelets found in plasma many types of protein molecules, cytokines, and growth factors. Therefore, it is considered as a reasonable method with beneficial effects on tissue regeneration, angiogenesis activation, inflammation control, and anabolism. PRP was also proposed as an acceptable and potentially successful approach for improving the fertility outcome in poor ovarian responders (PORs) as well as women with primary ovarian insufficiency (POI). However, there is still insufficient clinical data on the application of PRP in the field of ovarian infertility. Preliminary studies revealed that in women with POI, intraovarian injection of autologous PRP might be an alternative experimental treatment option. Furthermore, it has been shown that PRP injection could be positively applied as an effective and safe approach in PORs before the IVF procedure to increase the clinical pregnancy and live birth rates. Our recently published clinical trial showed a 47% pregnancy among PORs in response to PRP injection; of those, 50% led to healthy live births. In addition, we found menstruation recovery among 22.2% of women with POI after the PRP injection. In the end, ovarian autologous PRP injection could be a chance for conception without donor eggs in poor responder patients, along with improving the life quality of women suffering from early menopause without synthetic hormonal treatment.

K-2
Potential novel treatments in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a common and complex endocrine disorder, which is characterized by the presence of defining reproductive and endocrine defects. PCOS patients also suffer from metabolic features, including obesity, insulin resistance, liver steatosis, and an increased risk of type 2 diabetes. Despite the recent advances in our understanding of the underlying factors involved in the development of PCOS, the exact cause of the syndrome is still obscure. Hence, there is no cure for this condition and the current management strategies are limited to symptomatic treatment options. However, during the past decade, several novel therapeutic agents with a more mechanistic approach have displayed promising results in ameliorating defining PCOS traits. For instance, the administration of resveratrol, a sirtuin 1 activator, in a randomized controlled trial was able to reduce serum androgen levels and decreased insulin resistance in PCOS patients. Precursors of NAD^+, which in turn can induce the activity of sirtuin 1 have also shown promising results in experimental models of PCOS by reversing both metabolic and reproductive features of PCOS. More recently, specific agents have been developed to target kisspeptin, neurokinin B, and dynorphin system, which is suggested to be one of the main role players in the pathophysiology of PCOS. Such experiments have demonstrated the amelioration of PCOS-like traits in animal models of PCOS following the treatment with agents that target the kisspeptin, neurokinin B, and dynorphin system. Additionally, the administration of a number of supplementations has been evaluated in several clinical and experimental studies. Coenzyme Q10, bioflavonoids, N-acetyl-L-cysteine, and berberine with antioxidant properties and melatonin as a key regulator of circadian rhythm have been displayed positive impacts on the clinical and experimental features of PCOS. However, further investigation is still required in order to evaluate and validate the efficacy of such novel therapeutic agents in the management of PCOS.

K-3
ROS and assisted reproduction

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Abstract not received.

K-4
Recurrent implantation failure: Update etiology, diagnosis, treatment, and future direction

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Implantation failure can be used for both patients who have never shown increased level of human chorionic gonadotropin, and those who have increased HCG without later evidence of gestational sac and only applicable in ART.
It is important to consider age, stage of embryo-cleavage, or blastocyst. Therefore, recurrent implantation failure (RIF) is the failure of clinical pregnancy after 4 good-quality embryo transfers at least three fresh or frozen IVF cycles in women under age 40 years. Maternal age, BMI, smoking, and stress considered as the risk factors of RIF. Pathophysiology mechanism of RIF include immunological (maternal killer cells, peripheral, uterine Th1/ th2 ratio, TNF-α level-autoantibodies, and Aps-hereditary thrombophilia), Infection, Leukemia inhibitory factor, anatomical abnormalities, endometrial thickness, and genetic. Therapeutic interventions for RIF are optimal IVF treatment (Embryo factor-transfer methods-ovulation induction protocol-progesterone support) immunotherapy such as Tacrolimus, IVIG, PBMC (peripheral blood mononuclear cell), and granulocyte colony-stimulating factor (G-CSF), treatment of infection, correction of intra uterine pathologies, salpingectomy, endometrial injury, genetic (PGS) – PGD, endometrial receptivity array, male factors, lifestyle modification, supportive treatment. The recommendation for women with RIF perhaps the best is personalized medicine.

K-5
Tissue engineering and regenerative medicine in Iran: Current progress of Iranian Universities

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Tissue engineering and regenerative medicine (TERM) is an emerging field focused on the development of alternative therapies for tissue/organ repair. This highly multidisciplinary field, in which bioengineering and medicine merge, is based on integrative approaches using scaffolds, cell populations from different sources, growth factors, nanomedicine, gene therapy, and other techniques to overcome the limitations that currently exist in the clinics. The field of TERM in Iran, dating back to early 1990s and the advent of stem cell researches. During two decades ago, Iran has exhibited a remarkable increase in scientific publication in different aspects including TERM and today, Iran is one of the privileged countries in stem cell therapy in the Middle East. The main goals in TERM are the application and fabrication of scaffolds for tissue engineering of nerve, heart, liver, bone, and cartilage tissues. Todays some of the product from engineered tissues in laboratory move to the clinic in Iran but there are some problems in the clinical application of constructs that need to be solved them.

K-6
The positive effect of kaempferol on fibroblast cell reprogramming

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The reprogramming of differentiated cells creates a suitable source of pluripotent cells, which could be achieved via using Yamanaka factors, the most common method. The low efficiency of this reprogramming is challenging. In order to increase efficiency, various methods have been tested before.
Using of small molecules, especially natural ones, for elevating of reprogramming efficiency are recently more considered. Of this group, Kaempferol, for the known epigenetic and anti-oxidant effects on stem cells used in our study.
Recombinant fibroblast cells, with inducible Yamanaka factors (FUW-tetO-hOKMS) gene cassette, exposed to different concentrations (1, 5, and 10 µM) of Kaempferol (K group) and 0.5 mM of sodium butyrate (S group) separately for 5 days, before the cassette induction until iPS cell like colonies formation. Cellular morphology changes of these samples were evaluated quantitatively and qualitatively. The molecular assays including qRT-PCR and IF of pluripotent markers will be performed ahead.
The first cell morphological changes were observed on the fifth day after tetO-hOKMS cassette induction in case (K) and control (S) groups. Quantitative and qualitative morphological examinations of cells showed that embryonic-like colonies were only formed among cells treated with 5 µM Kaempferol (two colonies) and cells treated with sodium butyrate (one colony). However, the failure in the expansion of these small numbers of colonies has made it difficult to continue research.
The low number of embryonic-like colonies compare to the results obtained from similar studies indicates the low efficiency of reprogramming likely due to our poor performance and the work requires more repetitions and removal of the reprogramming induction barriers. However, by considering the similar positive efficacy of Kaempferol to the sodium butyrate (as a common reprogramming agent) in cell reprogramming, it makes sense that more studies on Kaempferol effects on cell reprogramming.

K-7
The success of various endometrioma treatments in infertility: A systematic review and meta-analysis of prospective studies

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Endometriosis is seen in 0.5-5% of fertile and 25-40% of infertile women. To investigate this conflict between gynecologists that ovarian endometriomas should be removed or not before making any decision about pregnancy among infertile women, we decided to carry out a systematic review and meta-analysis to compare the effect of various available therapeutic methods and notice the impact of these options on women´s pregnancy rate. This review is based on PRISMA recommendations with an electronic search using the following databases: Pubmed, Scopus, Google scholar, etc from 2000 and 2018, in the English language. The studies compare pregnancy rate based on four different treatment types of OMAs between infertile women: (surgery + ART, surgery + spontaneous pregnancy, aspiration ± sclerotherapy + ART, and ART alone). At least 8 prospective studies were included, in which 553 infertile women were compared in term of treatment methods of OMAs before trying to become pregnant. Treatments are usually based on the patient's clinical condition and must be individual, with the purpose of relieving pain, improving fertility, or both. We haven't any significant difference between our 4 groups of study, however the success of surgical procedure compared to other methods was higher and the success of ART alone was the least.

K-8
Feto-maternal immunological cross talk

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Not only embryo aneuploidies, but also the other factors such as immune-related maternal tolerance to pregnancy might contribute to implantation failure or miscarriage.. Maternal tolerance begins at the uterine level following by successful adaptation to the semiallogeneic fetus is a complicated process. The fetal cells that come into direct contact with the mother's immune cells in the uterus are uterine natural killer (uNK) cells in trophoblast cells, the layer that surrounds the blastocyst. The key function of the materno-fetal tolerance process is the remodeling of the spiral arteries, with the destruction of the media by invading extra villous trophoblasts (EVT) cells. The EVTs that invade the maternal decidua have fetal origin, and express high levels of human leukocyte antigen-C (HLA-C) recognized by uNK killer cell immunoglobulin-like receptors (KIRs). Maternal and paternal HLA-C allotypes are expressed at the same time and at high levels on the EVT cell surface. Placentation is regulated by interactions between maternal KIRs expressed by uNKs, and fetal HLA-C molecules, expressed by EVTs. Insufficient invasion of the uterine lining by trophoblasts and vascular conversion in the decidua are thought to be the primary defect among disorders such as recurrent miscarriage, preeclampsia, and fetal growth restriction. This process is regulated by the interaction between maternal KIRs, expressed by the uNKs, and their ligand HLA-C, expressed by EVTs. Pregnancies are at increased risk of recurrent miscarriage in mothers who are homozygous for KIR haplotype A (KIR AA) when the fetus has more HLA-C2 genes than the mother does and when additional fetal HLA-C2 alleles are of paternal or oocyte donor origin. There is a lower live birth rate and an increased miscarriage rate after double embryo transfer in KIR AA patients. In addition, the live birth rate decreases significantly as the embryo HLA-C2 load increases. The maternal immune system is one of the main actors at the maternal–fetal interface, and its lack of activation but not a rejection seems to influence the placentation and pregnancies outcomes.

K-9
Treatment of poor responder patient

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Decrease of ovarian reserve (DOR) is one important reason that negatively affects female fertility. DOR has been found in the 8-15% of assisted reproductive technology cycle. For women over 40 years DOR is about 50%, and 10% of women may experience an early reduction in ovarian reserve regardless of age. To find a proper treatment, first, we need to define DOR or poor responder patients, which two different criteria, named Bologna or Poseidon, shall be studied. Bologna criteria includeMaternal age ≥ 40 years or any other risk factors for DOR, antral follicle count < 5-7 follicles or anti-mullerian hormone (AMH) < 0.7-1.3 (ng/ml), and a previous DOR < 3 oocytes with a conventional controlled ovarian hyperstimulation (CoH). According to the Poseidon classification patient are subdivided into four subgroups based on first, age, second, antral follicle count or AMH, third, ovarian response, and finally, expected poor responder.
There are different lab tests for diagnosis of DOR including, FSH/E2, inhibin B, AMH (in day 2-3 menstrual cycle), and more recently insulin-like growth factor-1 in day 3 menstrual cycle. Additionally, transvaginal sonography is also recommended as another diagnosis method. To reach the best outcome of treatment in poor responder patient, it is recommended to decrease the required time to cumulative live birth rate per cycle, which is mainly dependent on the number of oocytes and an euploid embryos. For optimization of the oocyte number per ovarian cycle in DOR, there are number of recommendations to follow:
- Long protocol with conventional COH as the first choice for the subgroup of Poseidon 3 or 4.
- Mild stimulation protocol
- Dual stimulation protocol

To achieve better outcome adjuvant therapy in DOR patients is recommended as following:
- Growth hormone (GH)
- DHEA and other androgens
- LH supplementation

As known before, gonadotropin is commonly used in the COH cycle for poor responder patients. Current evidence supports a maximum daily dose of 300 IU of FSH, contrary to the previous recommendation of 600 IU. As mentioned before, the best outcome in poor responder patients is to obtain a euploid embryo. Because the availability of at least one euploid embryo increases the rate of pregnancy in poor responder patients up to approximately 60%, no matter the age. However, new evidence from basic science studies provides a biological explanation for the age-related effect on aneuploidy. This is due to impaired mitochondrial function, increased granulosa cell apoptosis, and increased level of oxidative stress in germline cells.

K-10
Development of synthetic protein-free chemically defined, safe and efficacious media products for human ART: Compliance with safety, regulatory, and cultural norms

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This presentation describes the continuous ongoing research efforts to develop the SYNBIOS MEDIA which are a series of synthetic and chemically defined formulations of protein-free embryo culture, handling and cryopreservation media developed following >20 years of systematic research (first communicated in 1997 at the Fertility Society of Australia Scientific Conference - Ali, 1997; and published in 2000 - Ali et al, 2000, Human Reprod 15:145-156) specifically to address the safety, and compliance with both regulatory issues and cultural norms. Conventional gamete and embryo culture, handling and cryopreservation media utilize donor serum protein supplements, which carry (i) a theoretical risk of disease transmission through protein-bound pathogenic agents; (ii) harmful undeclared protein contaminants; and (iii) donor micro DNA/RNA strands, and (iv) is prone to batch to batch variation in their composition affecting the quality of embryos generated between batches. Proteins cannot be sterilized with absolute certainty. The synthetic SYNBIOS MEDIA are devoid of added serum proteins making it among the safest media for both human and animal application with no (i) no pathogenic agents and thus no disease transmission, (ii) no undeclared harmful proteins contaminants, (iii) no donor micro DNA/RNA contaminants preserving the genetic purity of the embryo, and (iv) with no batch to batch variation; the latter ensures the quality of embryos generated between batches are maintained. It also complies with the cultural norms and lifestyle of many religions and beliefs. The synthetic SYNBIOS MEDIA products do not contain donor DNA/RNA because it is devoid of donor serum proteins. This attribute eliminates the risk of crossover of the embryonic genome with third party genetic material ensuring that embryos generated during ART treatment retain their two-parent genetic constitution thereby preserving the purity of lineage of the progeny, making it Halal-compliant and Halal-certified human embryo media. The efficacy of synthetic SYNBIOS MEDIA is similar to currently available media. The synthetic SYNBIOS MEDIA protein-free embryo culture media can be frozen-stored for up to 24 months without loss of efficacy. Freezing enables longer shelf life making SYNBIOS MEDIA embryo culture and handling media are thus less wasteful and economical for users.

K-11
Current insights in in vitro maturation of oocyte

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Oocyte in vitro maturation (IVM) is an assisted reproductive technology in which oocytes are retrieved from the antral follicles of unstimulated or minimally stimulated ovaries. IVM involves retrieval of immature germinal vesicle stage oocytes and culture of intact cumulus-oocyte complexes in vitro until the metaphase II stage. Maturation of oocytes includes the nuclear and cytoplasmic maturation of oocytes. Only the oocytes whose nucleus and cytoplasm are matured simultaneously can have adequate fertility and the potential for embryo development.

The in vitro maturation of oocytes is mainly affected by culture conditions. Since the metabolic dynamics and required nutrients are not entirely the same in different stages of follicular development, optimization of each step is needed to achieve a higher maturation rate and better oocyte quality, based on the sequential culture system.

The enrichment of culture media, standardization of the stimulation protocols and management of cytoplasmic maturity are strongly recommended for improved IVM cycles. With the development of IVM technology, the combination of natural cycle IVF with the IVM of immature oocytes can be used as an attractive regimen to promote IVM treatment.

K-12
AMH and ovarian surgery

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Ovarian reserve is defined as the number and quality of follicles in the ovary at a set time. The hypothesis that

endometrioma can impact ovarian reserve was established when histology-related research hypothesized that growing cysts, stretching the cortical tissue, might cause structural variations and circulation impairment, possibly leading to a decrease in the primordial follicle cohort in affected ovaries. The effects of ovarian endometrioma (OMA) (without previous surgery for OMA) on the ovarian reserve remain to be elucidated. The most reliable and extensively used ovarian reserve marker has been the level of anti-Mullerian hormone (AMH) due to its consistency throughout the menstrual cycle and following hormonal variations or treatments. AMH levels were noticeably lower in females with endometrioma in contrast to control groups (healthy ovaries and/or benign ovarian cysts). As has been detailed in previous publications, the presence of OMA is correlated with a decrease in AMH levels and adversely affects the ovarian reserve. However, numerous studies call into question the adverse influence of endometrioma on the ovarian reserve. AMH levels were downregulated only in subjects having undergone surgery independently of the presence of current endometriomas. Nieweglowska and co-workers reported that significantly decreased AMH levels were observed only in females with bilateral endometrioma, rather than in those with unilateral endometrioma. Similarly, Esinler reported that endometriomas with ≤ 3 cm in diameter did not impact the ovarian reserve. Of note, data from females with unilateral endometrioma are poorly informative, since the contralateral intact ovary compensates for ovarian function and fertility potential. Since larger cysts may be associated with lower levels of AMH, a relatively small cyst may not cause AMH levels to be significantly altered. women with endometrioma exhibited a progressive decline in serum AMH levels, faster than that in age-matched healthy females. It is emphasized that even experienced surgeons and accurate techniques cannot avoid operative ovarian reserve damage. AMH concentrations decreased noticeably after one year in patients with bilateral endometriomas, in individuals with cyst size >7 cm and in stage IV groups.

AMH hormone is a glycoprotein, a substance produced by granulosa cells in ovarian follicles. It is first made in primary follicles that advance from the primordial follicle stage. At this stage, follicles are microscopic and cannot be seen by ultrasound. An AMH test is often used to check a woman's ability to produce eggs that can be fertilized for pregnancy. A woman's ovaries can make thousands of eggs during her childbearing years. The number declines as a woman gets older. AMH levels help show how many potential egg cells a woman has left.

Unlike FSH, which may vary day to day and month to month, AMH is more consistent and, when combined, AMH and FSH provide the best insights compared to FSH alone. AMH is produced by the granulosa cells that line the tiny follicles within the ovaries. AMH serum levels differ widely according to genotype. Very low serum AMH concentration is characteristic of AMH mutations. However low AMH concentration in newborns or in boys undergoing puberty is physiological (Grinspon *et al*., 2011), and should not be interpreted as a sign of AMH mutation. Measurement of serum AMH is even more sensitive and specific than the AFC as it also reflects pre-antral and small antral follicles (< 2 mm), which are hardly seen in the ultrasound. Serum AMH is therefore a deeper "probe" for the growing follicular pool than the AFC (Dewailly *et al.*, 2014a).

The level of AMH in the blood can help doctors estimate the number of follicles inside the ovaries, and therefore, the woman's egg count. A typical AMH level for a fertile woman is 1.0-4.0 ng/ml; under 1.0 ng/ml is considered low and indicative of a diminished ovarian reserve. To encourage your body to naturally raise levels of AMH is to choose a diet that is rich in necessary nutrients, such as vitamin D. Many women with a low AMH get pregnant naturally, though it's less likely as the score falls below "low." AMH levels vary from month to month, and a lower level doesn't say with absolute certainty that you can't get pregnant.

AMH levels below 1.6 ng/mL predict a smaller number of eggs retrieved with IVF. Levels below 0.4 ng/mL are severely low and are not compatible with successful IVF. Women with very low (< 0.5 ng/ml) AMH levels undergoing IVF still have reasonable chances of achieving a pregnancy, but their prognosis is significantly affected by chronological age. Women with PCOS often have elevated AMH levels, likely to be due to the high levels of follicles they have in the early stage of development. Studies showed that stress exposure was related to reproductive failure. In this study, we found that there was a significant correlation between psychological stress and decreased AMH levels for infertile women.

Endometrioma is the cystic lesion of ovaries originating from endometrial glands and stroma; it is identified in 17-44% of patients with endometriosis. Numerous existing studies have reported the association between endometrioma and infertility. Surgical excision has commonly been considered to avoid further ovarian damage. However, the potential adverse effect of this surgery on the ovarian reserve has recently become a focal point. Whether or not surgical excision can facilitate subsequent conception in young women planning to be pregnant is controversial. Whether non endometriotic ovarian cystectomy or unilateral oophorectomy can decrease ovarian reserve will be discussed during the presentation, and few techniques of laparoscopic cystectomy will be demonstrated.

K-13
Regenerative medicine in liver and pancreas

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Many deaths in the world occur every year due to chronic liver and cirrhosis diseases. Liver transplant is the gold standard treatment but organ shortage is a great obstacle. There are several cell sources for liver regeneration such as primary hepatocytes, Mesenchymal (MSCs), embryonic stem cells and induced pluripotent stem cells (iPSCs). Nowadays, tissue-engineering strategies in liver regenerative medicine is an attractive issue; like cell sheet, liver organoids, recellularized liver, and bioartificial liver organs. Regarding diabetes mellitus, a whole pancreas transplant is the treatment of choice. Allogeneic islet transplantation in the liver of carefully selected diabetic recipients via portal vein infusion is an alternative cell-based strategy. Other stem cell sources like chemically induced islet-like cells, embryonic stem cells & iPSCs are alternative sources. The application of ECM scaffold functionalization to present bioactive motifs is an important issue in tissue engineering strategies for the pancreas. Currently, macroencapsulation and microencapsulation systems are used in several clinical studies. However, there are many challenges in both primary and stem cell therapy for liver and pancreas disease such as ethical problem, immune rejection, and best cell number and rout of transplantation.

K-14
Biosensors for cancers of the reproductive system: Novel ideas in detection

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Cancer still is the most challenging disease globally, despite fascinating advances in medicine. Those types that assault reproductive-related organs are among cancers with a higher death rate, and the numbers are overgrowing. Therefore, early detection of cancers is the best way to a most functional treatment and/or inhibition of the progress. Current cancer detection strategies predominantly suffer from low sensitivity and specificity and need expensive instruments with an expert operator. However, biosensors have shown a promising ability to detect/quantify molecular biomarkers associated with Cancer's early stages over the past decades. They can be very sensitive and selective, especially biosensors using nanomaterials and nanostructures, called nanobiosensors. Biosensors are categorized based on their transducer types, and they can be in various working platforms from electrode and lab tubes to papers and chips. Later called lab-on-a-chip devices, a biosensor is integrated into a microfluidics chip and can do sample handling and manipulation before detection. Here, I introduce biosensors, their types and platforms, and their applications to detect reproductive-related cancers.

K-15
Microfluidics in stem cells studies: Applications in reproductive sciences

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Microfluidics is revolutionizing biology and medicine and over the past two decades, their applications are constantly growing. Stem cell studies are one of the most important applications of microfluidics since they can have the variety of advantages over conventional stem cell studies. Microfluidics devices are enabling us to closely monitor the stem cells even in single-cell studies to analyse their metabolism and secretions. In addition, it is possible to stimulate cells with chemical, biological, physical, and mechanical simulates and evaluating the differentiation, reprogramming and performing physiological, immunological, and morphological studies. Moreover, they can be used to co-culture different cells together yet in two separate chambers, study organoids, and other 3D culture applications. Plus, the chance of contamination and cross-contamination is very low because they are sealed in the microfluidics channels and chambers. Cell sorting applications of microfluidics are also a great opportunity for scientists to separate specific stem cells from human sample cells. Last but not least, a very innovative application of microfluidics in medicine named organ-on-a-chip devices that can mimic a human organ can benefit from using stem cells.

K-16
Derivation of pluripotent cells from mouse spermatogonial stem cells (SSCs)

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Although testis-derived embryonic stem cell-like (ES-like) cells have been obtained in several studies, the time window for the shift to pluripotency is not clear yet. Here we describe, that only during a special time window (41 until 125 days) after initiation of germline stem cell (GSCs) cultures from neonate and adult promoter-reporter Oct4-GFP transgenic mouse the spontaneous appearance of germline-derived pluripotent stem (gPS) cells from both neonate and adult GSCs occurred. The isolated and long-term cultured (more than one year) GSCs which were isolated by a

morphology-based selection procedure expressed germ cells markers and exhibited a similar morphology with a high nucleus/cytoplasm ratio in comparison to undifferentiated spermatogonial stem cells (SSCs) in vivo. The generated gPS cells expressed pluripotency marker, in-vitro differentiated into all three germ layer lineages, formed complex teratoma after transplantation in SCID mice and produced chimeric mice. Although the exact mechanism of the development of gPS cells from GSCs is still unclear, this new information could provide an ideal strategy for scheduling natural conversion mechanisms of ES-like cells from mouse testis.

K-17
Mesenchymal stem cell-derived extracellular vesicle transplantation in animal model of premature ovarian failure

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Abstract not received.

K-18
Human endometrial organoids

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Abstract not received.

K-19
Three-dimensional scaffolds and differentiation of embryonic stem cells into oocyte-like cells, mimic cells –matrix interaction model

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Stem cells are undifferentiated cells that present in the embryonic, fetal, and adult stages of life and give rise to differentiated cells that make up the tissue and organs. Due to their unlimited source and high differentiation potential, stem cells are considered as potentially new therapeutic agents for the treatment of infertility. Stem cells could be stimulated in vitro to develop various numbers of specialized cells including male and female gametes suggesting their potential use in reproductive medicine. During the past few years, considerable progress in the derivation of germ cells from pluripotent stem cells has been made. In addition, stem cell-based strategies for ovarian regeneration and oocyte production have been proposed as future clinical therapies for treating infertility in women. Three-dimensional (3D) culture matrices is a new technology in stem cells differentiation mimicking the tissue microenvironment. Based on biomaterials and porous substrates that can support cell proliferation, differentiation and regeneration, using scaffold can make tremendous progress in this field. Some growth factors, such as epidermal growth factor (EGF) also facilitate normal meiosis during oocyte maturation in vivo. A 3D culture system along with scaffolds can apply the induction of oocyte differentiation from embryonic stem cells. Therefore, embryonic stem cells can be induced to differentiate into oocyte-like cells using embryoid body protocol along with the three-dimensional microenvironment in vitro. For the effective differentiation of oocyte-like cells can employ the presence growth factor such as EGF and assessed the markers of germ cell differentiation, meiotic progression and oocyte maturation. In our study, EGF exposed cells in the three-dimensional microenvironment, upregulated the gene expression levels of premeiotic (oct4, Mvh), meiotic (SCP1, SCP3, Stra8, Rec8) and oocyte maturation (GDF9, CX37, ZP2) were significant. The high efficiency of differentiation into oocyte-like cells from embryonic stem cells reflects the culture method employed in the 3D co-culture system. These results showed that this culture system along with EGF improved the rate of in vitro oocyte differentiation.

K-20
Modern and future therapies in endometriosis

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Endometriosis is estimated to affect 7-15% of reproductive-aged women. It has been considered one of the most important and debatable gynecological diseases, however, selecting therapeutic strategies in this field is even more challenging. Even if it's believed that the gold standard for treatment is surgery by some authorities, one should keep in mind that medical treatment is the cornerstone modality in this regard. There is an ongoing need for safe, effective, cheap medical therapies for endometriosis patients, both in conjunction with and independent of surgical interventions. Most conventional therapies for endometriosis work by a similar mechanism, and efficacy is variable. In recent years, there has been increased interest in the development and testing of novel pharmacotherapies for endometriosis.

In the present presentation, we discuss both conventional and emerging treatments for endometriosis, with different presentations across the lifespan and discuss how emerging therapies might fit into future medical management of endometriosis. Conventional therapies include nonsteroidal anti–inflammatory drugs, combined oral contraceptives, progestins, GnRH agonists/antagonists, and aromatase inhibitors. Emerging therapies are focused on disease-specific targets such as endothelial growth factor receptors, etc. It seems that the field of medical treatment of endometriosis is now moving toward modifying the immune and inflammatory responses in endometrial implants. If these drugs show efficacy in clinical trials, combining them with current medical treatment is expected to result in a profound impact on symptom and disease burden for patients who suffer from endometriosis worldwide.

K-21
The reproductive microbiome

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The microbiota is defined as all of the micro-organisms that live in the human organs. Five distinct microbiota are determined in different anatomical locations of human body. Lactobacillus species are dominant healthy microbiota in the lower reproductive tract. These micro-organisms provide asuitable environment with low PH in the vagina that inhibits the growth of most pathogenic bacteria. Recently we know that the upper reproductive tract is not sterile and the endometrium has some own microbiota. However, the role of these micro-organisms in reproductive outcomes is less investigated. Researches confirmed lactobacillus-dominant vagina can lead to better outcomes with ART. Some studies showed endometrial dysbiosis can disrupt endometrial receptivity and lead to infertility. It seems non-lactobacillus-dominant microbiota in the reproductive tract can cause the lower chance of successful implantation and the high miscarriage rate. In recent years some oral and vaginal supplements are introduced as probiotic products and claimed that may recover vaginal environments with better reproductive outcomes. Some studies recommended that pre-conception counselling and lifestyle modification can impact reproductive outcomes but more investigations are needed. It is important to know what factors can disrupt normal reproductive tract microbiota and how we can determine them.

K-22
Sperm selection using Zeta potential method

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In routine ICSI, the selection is based on morphology and viability, which does not necessarily preclude the chance injection of DNA-damaged or apoptotic sperm into the oocyte. Sperm with a high negative surface electrical charge, named "Zeta potential", are mature and more likely to have intact chromatin. In this procedure, sperm is selected based on the presence of a negative charge on sperm membrane. Thus, the aim of our work is the comparison between pure gradient and Zeta method, to select spermatozoa with normal chromatin. Our aim is to develop a simple Zeta potential method for sperm isolation; and to analyze the sperm maturity, morphology, and DNA parameters.

K-23
The use of time-lapse technology for embryo culture, is it necessary?

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Setting up efficient criteria and reproducible approach to identify the best embryo is an important challenge in in vitro fertilization laboratory. Also, embryo culture in optimal conditions is a crucial factor for assisted reproductive techniques success. Conventional incubators and morphological microscopy assessment are routinely used to culture and select embryos with the highest developmental potential to transfer. Conventional microscopy analysis requires embryo removal from the stable condition of the incubator. So, it exposes the embryos to temperature, pH and oxygen level changes. However, the morphological analysis may include discrete data of blastomere size, number and symmetry, fragmentation, the appearance of inner-cell mass, and trophectoderm of the blastocyst.
In recent years, new incubators and culture medium have been improved which provide better development of embryos. Time-lapse technology provides continuous culture and observation of embryos. It eliminates the need for embryo removal from the stable condition of the incubator. Also, time-lapse technology allows embryologists to assess the exact developmental events of embryos. The embryologists are allowed to access and register the embryo development events from extrusion of the second polar body to blastocyst formation. Time-lapse technology has spread rapidly and a large number of in-vitro fertilization labs produced considerable data. Although, there is no consensus on which morphokinetics parameters, or combination of them, should have a main role in the selection of an embryo. Several confounding factors including patient characteristics and clinical procedures have been seen to influence the development of embryos.

However, there is not sufficient research of difference in clinical pregnancy, live birth, miscarriage rates between Time-lapse technology and conventional incubation. The application of this technology is quickly growing, becoming increasingly more accurate. Studies contain deep-learning models, artificial intelligence, and embryo morphokinetics are currently increasing. It enhances hopes for time-lapse technology for clinical use in the near future.

K-24
The language of life

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Language is a means of communication between individuals. Languages can have different forms. The majority of languages are spoken with words but other forms of language using signs and chemical codes as means of communication do exist too. These means of communication may vary between individuals of different species and even of the same species. One can even extend the concept of language and communication, to cells signalling each other and communicating between each other. Extracellular Vesicles (EVs) are nanoparticles that vary in size from around 50 nanometer (nm) to 1000 nm. These vesicles are found in all biofluids such as blood, saliva and semen. EVs are made of bio-membranes and carry a cargo consisting of mRNAs, microRNA, long non-coding RNAs, proteins and even DNA. Based on their size and route of their biogenesis, EVs are regarded as exosomes, microvesicles and apoptotic bodies. EVs are exceedingly regarded as a universal means of communication between different cells and cell types within one or between different species. In recent years, scientific literature has pointed to the role that EVs play in communication between the embryo and the maternal tract. This communication prepares the mother for the process of implantation and may have important implications for the success of the implantation process. Due to this capacity, EVs provide an excellent opportunity for biomarker discovery and understanding the events involved during the implantation process. During my presentation, I will discuss the recent research conducted in my laboratory to establish sensitivity and specificity of EV's as biomarkers for predicting the success of the implantation process in IVF clinics.

K-25
Realtime and multilevel digital monitoring in ART: Are we ready to move IVF to the next level?

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Although tremendous improvements have been made in both clinical and laboratory approaches in recent years, current success rates in assisted reproductive technology are far below the expected levels. Among many, the most pronounced reasons include suboptimal diagnosis as well as treatment techniques or models, limits of morphology-based gamete and embryo selection, operator- and infrastructure-dependent variations in culture environment and manipulations as well as patient-specific characteristics that require personalized approaches. Recent studies indicate that electronic or AI-based novel approaches in coping with each limitation/obstacle can potentially show improvements in the outcome per se. However, promising reports usually suffer from either being enforced to fit in a certain isolated patient population or having problems associated with reproducibility in different clinical and laboratory settings. There is no doubt that such approaches will be used in routine applications in the future of assisted reproductive technology. However, overestimating the possible benefits and/or premature use of such approaches/technologies can also carry a risk of creating loss of interest among peers. To minimize such obstacles and upgrade the system for the complete digital transformation, we have developed in our group a customized, multilevel digital security, data management, and process monitoring system that can effectively be integrated and used real-time in different departments (clinical, laboratory, and genetics), simultaneously. Its gradual implementation has resulted in numerous positive and beneficial outcomes while we have also found out several key areas that are needed further improvements, as well as areas that created new risks associated with digitalization. In general, the transition from paper-based to digital data management and tracking platform usually come with certain infrastructural costs and risks that should be individually assessed for each clinic. Besides investing and planning for a dedicated and skillful IT team, management and training of the critical personnel are found to be one of the most important parameters for a successful implementation of such transformation. Furthermore, digital transformation should also be considered as a continuous process. Our customized digital monitoring system has now been effectively communicating and interacting with not only the medical and laboratory departments but also with other departments such as patient relations and accounting. With this opportunity, we are now in the process of developing AI-based applications that can help the key stakeholders (doctors, embryologists, and managers) to

decide which is the best for the patient at any given time during his/her treatment. In this talk, together with the authors' personal experiences, current approaches, and status of digital transformation in reproductive medicine will be summarized and discussed.

K-26
Microfluidics in embryo culture system

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Microfluidics is a multidisciplinary field of study that is rapidly growing in reproductive biology and assisted reproductive technology, based on its scientific and technical aspects. Using sub-microliter amounts of fluid in the microfluidic device instead of conventional micro-droplets might help to emerge a new horizon in assisted reproductive technology to investigate fluid behaviour, embryo culture, and embryo selection. Mechanical and biochemical features of microfluidics can improve all steps conducting in IVF laboratories from sperm selection and oocyte preparation to embryo culture and embryo assessment. Main studies have been focused on the application of microfluidics in rodents, nonetheless, recently some of them have turned to clinical usage.
In order to achieve success in in vitro embryo culture and selection, mimicking in vivo environment is logically necessary. Therefore, in microfluidic devices, embryo surrounding microenvironment, dynamic fluid environment, and chemical components should be considered. There are some challenges in investigating the microfluidic novel systems for embryo culture which include; disturbance of fluid flows, media evaporation, osmolality alteration, design and structure of devices, and possible toxicity of polymers used in 3D printed devices. Due to eliminating some of them, setting up computer-controlled Braille platform, and, using PDMS-parylene-PDMS hybrid membrane or polystyrene (PS) had been suggested. In this regards, dynamic fluid condition (microfunnel-pulsatile) could show a greater number of cells per blastocyst compared with static culture. In conclusion, despite some pros and cons, microfluidics has been rapidly noticed in the embryo culture system. Owing to complexities and multi-parametric issue of embryo culture, comprehensive and comparative investigations are suggested.

K-27
The effect of supporting the one carbon cycle on in vitro and in vivo fertilization outcomes

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One-carbon cycle (1-CC) is comprised of a series of metabolic pathways that can be categorized into folate and methionine cycles, transsulfuration pathway and recently formaldehyde cycle. These metabolic pathways are central to various important cellular functions that provide one-carbon units for essential biosynthetic reactions and for writing epigenetics marks. Folate is one of the most important promoters of 1-CC. It is well recognized that serious deficiency of folate during pregnancy is associated with adverse outcomes such as neural tube defects in the child. However, there is a growing concern about the potential adverse effects of high dose folate supplementation before and during pregnancy. Regard to this, there are few studies which report that excess folate consumption can cause developmental abnormalities and cognitive abnormalities in offsprings in mice. Moreover, two common inherited human mutations are the 677C > T and 1298A > C mutations in the gene encoding methylenetetrahydrofolate reductase, which converts 5, 10-me-THF to 5-methyl-THF (m-THF, the active form of folate). These mutations may increase the un-metabolized form of folate and decrease m-THF which may explain to some extent why these mutations might be more prone to infertility and certain chronic illnesses. Therefore, monitoring of pregnant women for adequate dietary folate intake regarding the presence or absence of these mutations is required.
In addition, despite extensive improvements in assisted reproductive techniques (ARTs), the pre-implantation and post-implantation outcomes of ARTs have remained low. One of the main causes of these insufficiencies may be related to the in vitro manipulations and composition of the culture medium. Therefore, any changes that can bring these conditions closer to the in vivo situations can probably have a significant impact on ARTs. One of the important metabolic pathways during the process of folliculogenesis, oocyte maturation, and embryonic development is 1-CC. So, adding the substrates and cofactors of 1-CC may improve ARTs outcomes. In this presentation, first, we discuss the effect of folate deficiency and also the excessive dose of folate on fertility outcomes and then discuss the importance of various micronutrients involved in 1-CC during in vitro conditions.

K-28
PGT-A in ART anno 2021: An update

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Preimplantation genetic testing for aneuploidy (PGT-A) is increasingly employed in assisted reproductive

technology (ART). By sifting out embryos with abnormal chromosome numbers (aneuploid), PGT-A should theoretically improve pregnancy success. However, earlier versions of PGT-A were ineffective, and in some cases, detrimental, due to biopsy-induced trauma and because the technology at the time could analyse only a fraction of all chromosomes. More recently, the emergence of technologies enabling all chromosomes to be analysed and a switch to less traumatic blastocyst-stage biopsy have seen widespread uptake of PGT-A. Assessing the full impact of blastocyst biopsy PGT-A requires consideration of multiple factors, including embryonic mosaicism, the sensitivity of the technological platform used, embryo loss during long-term in vitro culture, embryo cryopreservation, and inter-clinic variability in expertise. PGT-A has been shown to increase the success of in vitro fertilization (IVF), by reducing the risk of miscarriage, shortening the time to pregnancy, and allowing more confident single embryo transfers without compromising outcomes. While it is widely accepted that chromosome aneuploidy, a common feature of human embryos, is a major cause of IVF failure, miscarriage and congenital defects, controversy remains around the routine implementation of PGT-A in clinical practice. The possibility of bypassing biopsy with non-invasive PGT-A (niPGT-A) is becoming highly attractive. niPGT-A via the analysis of cell-free DNA (cfDNA) present in the spent culture media is currently an area of active development. Furthermore, next-generation sequencing increased the resolution and sensitivity of PGT-A allowing the identification of chromosomal mosaicism and the detection of segmental copy number variations (CNVs), both areas of disagreement due to their uncertain clinical significance. The concordance rates between PGT-A results from the original trophectoderms samples and re-biopsies have been shown to be reduced for mosaic or segmental CNVs compared to euploid and whole chromosomal aneuploidy results. Nonetheless, segmental errors are believed to be responsible for 6% of clinical miscarriages and can result in genetic conditions, such as Cri-du-chat, accounting for approximately 0.05% of births. Variable mosaicism rates based on single TE biopsies (2-13%) are influenced by the next-generation sequencing assay adopted, the stimulation protocols and the IVF laboratory culture conditions. As biopsies are not necessarily representative of the whole embryos, the true prevalence and clinical implications of blastocyst mosaicism are still under question. CNVs are increasingly recognised as natural events in the preimplantation embryo. Furthermore, observations of developmental competence in a subset of mosaic blastocysts reaching healthy live births lead to the suggestion of corrective mechanisms. However, there is no direct evidence to support this theory at present. The effects of low-moderate level mosaicism on ongoing pregnancy rates remain unclear, with studies examining the transfer of such embryos reporting conflicting data. Elucidating the uncertainties around mosaicism and segmental CNVs within well designed studies would lead towards improved PGT-A and patient management in ART.

K-29
Endometrial scratching: What is the end of role?

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Embryo implantation is one of the most important factors influencing pregnancy in assisted reproductive technology cycles and is usually attributed to a lack of uterine receptivity. Although some studies suggest that endometrial scratching may double the chances of getting pregnant, conclusive scientific evidence on its benefits and knowledge of the underlying mechanisms is limited. It is not clear exactly how endometrial scratching works, but it is thought that scratching the uterine lining may induce an inflammatory response. The subsequent repair process may improve the chances of implantation by:

- The release of growth factors, hormones, and proinflammatory cytokines, which make the newly-formed lining more receptive to an implanting embryo
- Activating genes that are important for the preparation of the endometrium, which may not otherwise be turned on, at the time of attempted implantation.

Mechanical disruption to the endometrium has been shown to modulate the genetic expression of factors important for implantation, including laminin alpha 4, integrin alpha 6, matrix metalloproteinase 1, and glycodelin A. The optimum time for endometrial scratching is controversial and should be discussed in the presentation. A Cochrane review of nine randomized trials including 1512 women with unexplained subfertility suggested an overall benefit from endometrial scratching. However, the review also stated significant limitations to the included studies, and cautioned against drawing confident conclusions from these findings. The authors also emphasized the importance of balancing the possible benefits against the potential risks. Although Olesen in 2019 was shown that the pregnancy rate is higher after endometrial scratching but other studies showed differences in live birth and clinical pregnancy rates among the endometrial scratch and control groups were not significant. But, recently in 2020 results of the SCRaTCH trial that was a non-blinded randomised controlled trial in women with one unsuccessful IVF/ICSI cycle would lead to a higher live birth rate after the subsequent IVF/ICSI treatment compared to no scratch. According to available data, more research is needed in order to determine whether endometrial scratching significantly improves the chances of pregnancy with IVF. Finally, this procedure should not be offered in daily practice, and its use in patients undergoing IVF for the first time is not supported.

K-30
ART and male infertility

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I had the opportunity that my clinical practice has begun when the assisted reproductive technology (ART) has begun in Iran. In fact, I was a member of the early team of Yazd Infertility Center.We have witnessed dramatic advances and success of this high technology how to treat male infertility. Before the ART era, there were many limitations for the treatment of infertile men, especially those who were azoospermic or even oligospermic. But in spite of this progress, some side effects have grown. Most harm is to ignore men's health, while male fertility and health are closely interrelated. Another problem is paying less attention to the best and near most natural therapy process and the trend to bypass it with ART.
Unfortunately, as some recent data, almost one third of men in infertile couples were never evaluated. Therefore, due to both major reasons as men's health and the best treatment of infertility, the andrology department is essential for any ART centers.

K-31
In vitro spermatogenesis in artificial testis for male infertility

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Increasing male infertility rates have led to a greater need for new artificial testicular systems in order to preserve fertility. Laboratory studies have shown that preservation, proliferation, differentiation, and transplantation of spermatogonial stem cells or testicular tissue could be ways to maintain the fertility of childhood cancer patients and azoospermic men in the future. In this regard, tissue and cell culture, supplements and 3D scaffolds have created a new perspective on the differentiation of stem cells in vitro, which could improve the outcomes of male infertility. The 3D matrix appears to allow for the formation of colonies and the proper arrangement of testicular cells, although differentiation has not yet been fully obtained. Therefore, in the future, new systems will be needed so that they can cause proliferation and maturation of germ cells in laboratory conditions and ultimately produce functional sperm by emphasizing regeneration of the germ cell microenvironment.

K-32
New horizons in onco-fertility

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Improvements in the management of many common cancers in young people have led to significant growth in the number of long-term survivors of cancer in people of reproductive age. However, survival comes at a price, with many young people suffering significant side effects from chemotherapy and radiotherapy. These include significant damage to reproduction due to the gonadotoxicity of cancer treatments. Recognition of the importance of future fertility to cancer survivors has led to increasing interest in the subspecialty of "oncofertility" the preservation of reproductive options for young men and women before cancer treatment. Fertility preservation is relatively straightforward for most post-pubertal males and cryopreservation of semen has been performed for over half a century for this group of patients. More recently, technological developments in cryopreservation of oocytes and embryos have allowed realistic chances of future fertility of female patients who preserve gametes or embryos before treatment. In younger patients or those who do not have time to undertake a stimulated IVF cycle to obtain oocytes, ovarian tissue cryopreservation is an increasingly used option. Testicular tissue is also frozen for pre-pubertal boys, but cannot currently be used to reinitiate spermatogenesis. Future prospects include novel means of ovarian protection providing improvement on the largely ineffective use of gonadotropin-releasing hormone agonists, and increasingly, in vitro maturation of immature oocytes to reduce the time taken to perform fertility preservation before cancer treatment can be started.

K-33
Sperm selection using motile sperm organelle morphology examination (MSOME) in ART

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The ultimate aim of any sperm selection method is to provide the best-quality sperm possible so as to maximize the outcome of whatever assisted reproductive technology (ART) procedures are to be undertaken. Gamete micromanipulation, such as intracytoplasmic sperm. injection (ICSI), is very useful for treating couples with compromised sperm parameters. An alternative method of sperm selection has been described; the spermatozoa are selected under high magnification (over 6000x) with sperm organelle morphology examination (MSOME) criteria and used for ICSI. This technique, named intracytoplasmic morphologically selected sperm injection (IMSI), has a theoretical potential to improve reproductive outcomes among couples undergoing assisted reproduction technology (ART). According to the majority of studies,

it is not recommended to use MSOME/IMSI routinely in the ART program. The couples with repeated implantation failures, patients with severe male factor infertility, sperm DNA damage, advanced male, and maternal ages are the populations who will have higher chances to conceive from this technique. It is also recommended that diagnostic morphological evaluation of semen samples with MSOME is done before ICSI/IMSI procedure. The effectiveness of IMSI is still controversial mainly due to differences in inclusion criteria, stimulation protocols, seminal and oocyte qualities, and many other confounding variables within the ART program. However, there is no doubt that the use of MSOME/IMSI techniques can be helpful for some infertile couples to have a baby.

K-34
The indication of surgery in endometriosis

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Endometriosis is highly prevalent, yet compared to equally prevalent conditions it is poorly understood and a challenge to manage. It has been estimated that more than 176 million women worldwide suffer from endometriosis and its associated symptoms including infertility, cyclical and non-cyclical abdominal pain, dysmenorrhea, dyspareunia, dysuria, and dyschezia. Endometriosis may be categorized into three entities: peritoneal endometriosis, ovarian endometriotic cysts (endometrioma), and deep endometriosis (DE) (previously known as deep infiltrating endometriosis or DIE). Indications for endoscopic diagnosis and treatment in endometriosis are as follows: Pain, Organ destruction and/or Infertility. Surgical removal of the lesions is considered the "gold standard" for symptom control. Surgery is an important treatment option for women with DE.

However, like medical intervention, surgery is not always successful and is also associated with clinically relevant risks (Chapron et al., 1998; Becker et al., 2017). Surgical treatment failure can be partially attributed to the heterogeneity of endometriosis but it is also correlated with factors such as surgical experience, the complexity of each case, and anatomical locations of the disease. Surgeons must have significant knowledge of pelvic anatomy in order to have an approach to a grossly distorted surgical field. Thus, pelvic anatomical landmarks represent essential points of reference to start procedures such as mobilization of the pelvic viscera, wide peritoneal resections, or the identification of further anatomical structures to be preserved, such as bowel, ureter, vessels, and parasympathetic and orthosympathetic pelvic neural fibres in nerve-sparing procedures (Ceccaroni et al., 2018). The principles for identifying and treating deep endometriotic lesions and the good practice recommendations in the text aim to support clinicians and surgeons in counselling and treating (or referring) women presenting with DE.

K-35
Sharing beliefs-triangle of sexuality, religion, and infertility: Argumentative review

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The influences of culture are present in different areas of sexual and reproductive behaviours as well as help-seeking behaviours in the case of infertility. The conflict between religious teachings, sexuality, and infertility related interventions is of increasing importance in traditional societies today. In other words, the clash between religious tradition, sexuality, and infertility can be a controversial scientific issue in clinical settings. This article explores these issues and draws insights from Iranian's religiosity, where this challenging triangle is considerable in the context of competing sexuality and infertility related help-seeking behaviours. It raises far-reaching issues concerning the distinction between belief and practice, as well as the role of religious teaching in the sexual and reproductive sphere.

In this article, we have attempted to find a way forward which not only recognizes cultural limitations in terms of the religiosity and sexuality dichotomy but also its strengths in terms of the need for acknowledgement and civility in the assisted reproduction technology (ART) sphere. The authors also advocate that Muslim countries with advanced ART should adopt a fact-specific approach that is sensitive to the sexuality issues in the contexts with specific religious interpretation (Tafsir), and which focuses upon the values of religiosity, tolerance and mutual respect to one's sexuality.

In sum, we argue that ART policies in Iran may lend themselves to a model of accommodation and compromise which avoids stubbornness and instead seeks out common ground in "religious teachings, sexuality educations, and infertility management. Although the task may be challenging, the consequences of failure, to our mind, justify the effort.

K-36
Potential regenerative effects of amniotic-fluid derived exosomes on the rat model of azoospermia

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Any defect during the spermatogenesis process may cause temporary or permanent male infertility. Cell-free therapies and by-products such as exosomes have been used as alternative modalities for the treatment of tissue injuries. There is no data on the use of extracellular vesicles to restore male fertility. This study aimed to explore the therapeutic effects of amniotic fluid-derived extracellular vesicles including exosomes (AF-Exos) on the recovery of sperm production capacity in a rat model of azoospermia. Exosomes were isolated from amniotic fluid samples via ultracentrifugation and characterized by scanning and transmission electron microscopy (SEM and TEM), dynamic light scattering (DLS), and western blotting techniques. The induction of non-obstructive azoospermia (NOA) in rats was performed by intratesticular administration of 5 mg/kg/testes Busulfan. Azoospermia was confirmed with histological and spermiogram analysis. AF-Exos samples (10 and 40 µg exosomal protein) were injected into the testes of NOA rats. Two months after intervention, the spermatogenesis rejuvenation was evaluated via histopathology (H & E staining), spermiogram, and hormonal analysis. The expression level of a regeneration marker (OCT-3/4) was also studied via immunohistochemistry staining and the number of spermatogonial progenitors was as well evaluated. AF-derived Exos showed sphere-shaped morphology with 50 ± 7.521 nm mean diameter and -7.16 mV zeta potential, and are positive in specific surface markers (CD63, CD9, and CD81). Histopathological and spermiogram data revealed that the spermatogenesis index and sperm parameters were significantly improved after AF-Exos injection compared to azoospermic groups. Also, after AF-Exos injection the OCT-3/4+ cells were increased in NOA rats exhibited spermatogenesis restoration. Both doses of exosome (10 and 40 µg) restored the testicular function in NOA rats. Except in a high dose of AF-Exos (40 µg) for testosterone and FSH, no statistically significant differences were found regarding hormonal levels post-exosome injection. Our study demonstrated that AF-Exos have the potential capacity to facilitate regeneration in the spermatogenesis process and improve sperm quality through paracrine effects via releasing potential restoratives factors into the site of injury. This study provides a novel therapeutic insight on the NOA treatment.

K-37
Cycle regimens for frozen-thawed embryo transfer

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Frozen–thawed embryo transfer (FET) enables the excess embryos generated by in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) to be stored and utilized at a later date. This reduces wastage after IVF and increases the chance of conceiving after one cycle of ovarian stimulation and oocyte retrieval. In recent years, the number of FET cycles performed has increased dramatically due to the trend towards transferring fewer embryos after a fresh IVF cycle, and as a result of improved laboratory techniques. In contrast to the complex stimulation protocols employed to stimulate multiple follicular growth for IVF, frozen embryo transfer (FET) protocols are simpler, with the primary aim limited to adequate preparation of the endometrium to receive the thawed, transferred embryo(s). However, despite the growing importance of FET in the treatment of subfertility, there is little consensus on the best method for endometrium preparation in ovulatory women. In order to optimize pregnancy rates, the development of embryos and endometrium should be synchronized. This can be achieved in various ways. The simplest method of endometrium preparation is represented by natural cycle FET (NC-FET), in which the endocrine preparation of the endometrium is achieved by endogenous sex steroid production from a developing follicle. Timing of embryo transfer is determined by detecting the spontaneous luteinizing hormone surge or by administering human chorionic gonadotropin to initiate luteinization. A frequently employed alternative approach is represented by 'artificial cycle protocols' in which exogenous estrogens and progesterones are administered, with or without co-treatment with gonadotropin-releasing hormone agonists. In artificial cycle FET (AC-FET), estrogen and progesterone are administered in a sequential regimen that aims to mimick the endocrine exposure of the endometrium in the normal cycle. Initially, estradiol is given in order to cause proliferation of the endometrium, while suppressing the development of the dominant follicle. This is continued until the endometrium is observed to be 7-9 mm thick on ultrasound, at which time progesterone is added to initiate secretory changes. The physiological mid-cycle shift from estrogen to progesterone is thus emulated. The timing of embryo thawing and transfer is planned according to the moment of progesterone supplementation.
In conclusion, natural cycle treatment has a higher chance of live birth and lower risks of PIH, PPH and VPTB than AC for endometrial preparation in women receiving FET cycles. Ovarian stimulation with Gn/FSH or AI may be promising, but the evidence is scare and needs to be evaluated in future studiespregnancies after NCFET have a more favourable outcome compared

with AC-FET, with lower rates of HDP, preeclampsia, LGA and macrosomia. The development of gestational hypertension in FET cycles seems not to be influenced by the mode of endometrial preparation. This is valuable information, as the number of FET cycles has increased, including the 'freeze-all' strategy. Future studies are required to clarify the underlying biologic mechanisms of our findings, and further randomized controlled trials are needed to improve the quality of evidence.

K-38
Ex vivo spermatogenesis: Static or dynamic culture system

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Infertility is one of the most important problems in human societies, today. This issue can change the social life of infertile couples and has nothing to do with the cause of infertility. However, it should be noted that about 50% of infertility cases are related to men. In vitro germ cell maturation and enrichment transfer techniques could potentially help to preserve fertility, especially in pubertal males without mature germ cells. In addition, this technique could also be potentially used for the treatment and the maintenance of biological paternity of oligozoospermic or azoospermic patients. Today, with advances in reproductive biotechnology, it is possible to produce in vitro male haploid cells. This matter can help a large group of infertile patients. To achieve this goal, many researchers have studied different culture systems and other factors involved in stimulating ex vivo spermatogenesis. Various methods have been proposed, including organ culture system, two/three-dimensional culture and isolated cell culture method or adding the required supplements of tissue or cell in the culture medium. In order to bring the culture system closer to the in vivo conditions with the aim of spermatogenesis, major changes are necessary. One of these changes is the use of dynamite culture instead of static culture. Recently, bioreactor, in which biological or biochemical processes are developed under a closely monitored and tightly controlled environment, is one of the latest approaches that often used to culture cell and tissue in-vitro. It is suggested that the organotypic culture of testicular tissue or fragments is capable of maintaining the architecture and viability of germ cells, and induction of in-vitro spermatogenesis. Moreover, the addition of a mini-bioreactor or microfluidic device has shown the potential to improve organotypic culture systems, as it can lead to long-term ex vivo maintenance of testis tissues which is required for producing sperm. Although these techniques have only been applied in lab animals, there is reproductive technology advancement hope for the near future that these methods will also give surprising results in humans.

K-39
Sperm selection using magnetic activated cell sorting (MACS) in assisted reproduction

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Magnetic separation has been successfully applied to many aspects of both biomedical and biological research and also in clinical areas like cellular therapies for human autoimmune disease, like rheumatoid arthritis, diabetes, multiple sclerosis, and SLE. Infertile men with poor sperm motility and morphology were found to have increased sperm DNA fragmentation compared with individuals with normal semen analysis may also have a high degree of sperm DNA fragmentation, which can be a major cause of unexplained infertility, and sperm DNA fragmentation. Aberrant chromatin packaging during spermatogenesis, defective apoptosis before ejaculation, or excessive production of reactive oxygen species (ROS) in the ejaculate. Exposures to environmental or industrial toxins, genetics, and lifestyle are also known factors that may cause sperm DNA fragmentation and infertility. Although the factors present in the paternal genome that may have an impact on poor reproductive outcome are still not wall defined, there is accumulating evidence linking sperm nuclear DNA abnormalities to poor reproductive we come one of the most suspected organelles in the sperm nucleus. Several studies using the magnetic activated cell sorting (MACS) technique with human spermatozoa have been published over the years. Interests in these studies were mainly the molecular efficiency of the technique and improving the post preparation sperm quality. Researchers have evaluated the percentage of sperm recovery following the use of MACS as a sperm preparation technique, and they concluded that the integration of MACs with density gradient centrifugation (DGC) is an effective sperm preparation technique that does not lead to significant cell loss and separating a distinctive population of non-apoptotic spermatozoa with intact Membranes might optimize the outcome of assisted reproduction. Reduction of apoptotic spermatozoa within the ejaculate using the MACs system results in a distinc reduction of spermatozoa with DNA fragmentation, enrichment of spermatozoa free of apoptosis, improvement of sperm viability, motility, and mitochondrial membrane integrity.

K-40
Sperm selection using PICSI method

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It has estimated that up to 50% of the infertility cases are predominantly or partly caused by male factors. About 10-20% of couples suffer from unexplained infertility, the male partner has sperm DNA fragmentation index (DFI) above 20%. It has been shown that increased sperm DFI may lead to decrease chance of natural pregnancy and assisted reproductive techniques success rate. In this patients, the potential underlying causes should be treated first. However, in non-responders, other strategies such as antioxidant medical treatment, sperm selection techniques and testicular sperm extraction may be useful. In this regard we want to talk about physiologic intracytoplasmic sperm injection as a probable useful sperm selection technique.

K-41
Regenerative medicine for female reproductive system

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Tissue engineering and regenerative medicine make a bright future for the regeneration, repair, and replacement of various tissues and organs. In the reproductive system, most of the major acquired or congenital organ failures lead to a great functional problem, infertility. Everybody with infertility will have great social and family obstacles, often with psychological consequences for the couples.

Regenerating the absent organ or repairing and replacing the diseased tissue are the novel choices for the treatment of reproductive system diseases due to organ or tissue failure. By tissue engineering for female patients, that is using the triad of potent cells, scaffolds and growth factors could make an artificial uterus, tubal organs, ovary, and follicles. Selection of the best cells, scaffolds, and stimulation factors to make a functional tissue is the aim of many research programs around the world. Various types of stem cells, organic and inorganic biocompatible scaffolds, and different types of proteins, enzymes, and small molecules as stimulators have been used. Engineered tissues could apply as the in vitro research models and for clinical use to restore reproductive function.

Taken together, this medical technology prepares the introductory facilities for germ cell support and in vitro fetal growth and complete artificial uterus for ex vivo embryo growth and maturation (biobag). For all the possible instances, religious beliefs, law and reproductive health ethics should be considered.

K-42
Using embryo culture medium as a diagnostic factor

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The success rate of assisted reproduction is remained low despite performing several studies around the world. Different strategies have been used for the improvement of assisted reproduction technology (ART) outcomes. More attention has been paid for omics technology in recent decades. Metabolomics is a non-invasive technique to evaluate oocyte quality, and competence, embryo viability, and endometrial receptivity. In fact, metabolomics provides sufficient data about the oocyte, embryo, and endometrium for the treatment of patients with subfertility. Also, this method, by selecting the best embryo for transfer, can reduce the number of transferred embryos, and the risk of multiple pregnancies as well. Evaluation of oocytes based on metabolomics can replace other methods of selection with the high variability like morphology or invasive method like polar body biopsy. Amino acids turnover can predict embryo viability with high rate of implantation resulting to a live birth. Metabolomics evaluation of endometrium is also associated with the receptivity of endometrium and also for diagnosis of endometriosis. High-quality researches are needed for drawing the final conclusion about the efficacy of metabolomics on ART outcomes including live birth and miscarriage rates.

K-43
Sperm selection using Microfluidic method

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Abstract not received.

K-44
Uterine myoma and infertility

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Uterine myoma or fibroid is the most common benign gynecological tumors in women of reproductive age. Fibroids are hormone-dependent smooth-muscle tumors with a wide heterogeneity in composition, size, and number. Most women with fibroids are fertile; however, fibroids may affect fertility by distorting the pelvic anatomy and the intrauterine environment. The way by which myoma result in infertility remains to clearly understood. Besides anatomical distortion, the possible mechanism impairing fertility are; endometrial function

alteration (increased uterine contractility and impairment of the endometrial and myometrial vascularization and blood supply, alters the local hormone balance that could affect gamete transport and/or reduce embryo implantation. Submucosal and intramural myomas with pressure effect on uterine cavity are associated with decreased pregnancy and implantation rates after ART cycles. The management method highly depends on the size, number, and location.

K-45
Organoids: A paradigm-shifting stem cell-based technology for drug discovery and development, companion diagnostics, and preclinical patient stratification

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Hubrecht organoid technology (HUB) has developed the 3D culture system to establish and expand human and animal epithelial tissue from a variety of organs, both healthy and diseased, such as cancer. The organoid technology is based on the work of Hans Clevers that identified adult stem cells in many human tissues, including intestine, liver, pancreas, breast, and lung. Organoid cultures have the virtually unlimited expansion, genetically and phenotypically stable and retain biological and functional properties of the original tissue (Barker et al., Nature 2007; Sato et al., Nature 2009, 2011; Gastroenterology 2011; Huch et al., Nature 2013; Karthaus et al., Cell 2014; Cell 2015; Boj et al., Cell 2015). Organoids recapitulate the original tissue response to external stimuli and provide a unique and robust in vitro model for drug development, diagnostics, and patient stratification.
The HUB is collaborating with and licensing the technology to the Pharmaceutical and Biotech industry. In addition, HUB has built a comprehensive Living Biobank of well-characterized Organoids from different healthy, disease, and cancerous tissues of multiple organs. In combination with the Living Biobank and Organoid technology, HUB offers a unique platform to develop assays and provide preclinical drug discovery, toxicity, personalized medicine services.

K-46
Treatment of male infertility

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Some drugs and medical illnesses suppress the fertility capacity of men, so the first step of medical treatment of male infertility is identifying these drugs and withdraw them and diagnosing the diseases like hypogonadotropic hypogonadism, hyperprolactinemia, and genital tract infections and manage them.
One of the male infertility causes is excessive reactive oxygen species (ROS) production. Physiologic amount of ROS is necessary for the reproductive system, but the excessive amount of it causes cellular damage. Different sources of ROS production are smoking, varicocele, heat, etc. and eliminating these sources is necessary.
The other option for decreasing ROS in the seminal fluid is antioxidant drugs. Each of these drugs has the different effects on semen parameters and it is proven that multiple antioxidant therapy is superior to solo antioxidant therapy.
For antioxidant therapy, we should know the minimum and maximum dosage of each drug because over and under the administration of antioxidants may have the deleterious effects on semen parameters.
Not all infertile men are a good candidate for antioxidant therapy and also we cannot administered all antioxidant drugs for every patient so for precise selection of patients and antioxidant drugs, it is highly recommended to evaluate oxidative stress of semen and start antioxidant therapy based on it.

K-47
Anti-müllerian hormone and polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive women. The prevalence of PCOS varied based on the recruitment process of the study population, the criteria used for its definition and the method used to define each criterion. Rotterdam criteria introduced in 2004 and included polycystic ovarian morphology (PCOM) as a criterion for PCOS. This definition made a lot of concern in terms of validity and reliability of its assessment and the necessity for revising its definition given using advanced high-resolution ultrasounds devices. Several efforts were made to introduce a more reliable substitute for PCOM, among all candidate options anti-Müllerian hormone (AMH) was the best marker reflecting antral follicular count (AFC) given its exclusive production by granulosa cells of the ovary. Attempt was also made to identify the optimal diagnostic threshold for AMH for substitution of PCOM in PCOS criteria, however, a universally approved threshold has not yet been introduced. Moreover, there are studies that suggested AMH as a surrogate marker for diagnosis of PCOS due to overproduction of AMH by granulosa cells in anovulatory status, cross-communication of AMH with FSH and LH which leads to hyperandrogenism. It seems that the AMH cut-off value of ~5.7 and ~3.7 ng/ml in the early and late reproductive period has adequate sensitivity and specificity for making PCOS diagnosis.

K-48
Somatic and germ line gene therapy

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Genetic and molecular approaches are applied to diagnose or predict a disorder then the offers are dealing with the disease or termination of pregnancy. This is called the diagnostic therapeutic gap. Gene therapy, as an old dream, aims to fill this gap. It involves the introduction of genetic material into the cells to cure or prevent the inherited and non-inherited diseases and expected to be curative and durable. By developing efficient gene delivery and gene editing systems, now gene therapy is applicable. To meet the expectations, there are two alternative strategies: somatic and germ line gene therapy with their advantages and challenges.

K-49
Can in vitro culture condition influence pre-implantation embryo aneuploidy?

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Natural fecundity has a low rate in humans compared to other mammals, so that the probability of pregnancy for a healthy fertile couple in each menstrual cycle is less than 25%. On the other hand, according to the available evidence, about 10-40% of pre-implantation embryos are lost after fertilization in normal healthy women. Aneuploidy is the most important cause of embryo implantation failure. Aneuploidy increases as women are aging. Some studies have reported the rate of 20 to 40 % of chromosomal abnormalities in embryos among healthy fertile women following natural conception. However, the rate of aneuploidy of in vitro fertilized embryos is much more than embryos from natural conception, and this rate increases with maternal age from 73% in women under 35 to 87% in women aged 41 or older.

The causes of aneuploidy in human embryos remain largely unknown. In addition, the level of aneuploidy in the gametes which produce these embryos is lower than the level of aneuploidy in the resulting embryos, so that approximately 20% of the retrieved oocytes in IVF cycles and about 9% of the sperm in each ejaculate have aneuploidy on their haploid chromosomes. Interestingly, about 90% of aneuploidy of human embryos originates from oocytes. In particular, the type of chromosomal abnormalities in embryos and oocytes is quite similar, mainly aneuploidy, while most chromosomal abnormalities in sperm are structural.

Furthermore, the rate of aneuploidy in embryos that underwent intracytoplasmic sperm injection (ICSI) procedure is higher compared to embryos obtained from IVF. Therefore, it seems that in vitro oocyte manipulation for ICSI has adverse effects on oocytes and leads to aneuploidy in embryos. Two hypotheses can be considered for the relatively high rate of embryo aneuploidy in IVF/ICSI cycles; controlled ovarian hyperstimulation (COH) is the first exogenous factor and the second is IVF laboratory conditions. For COH, various factors such as the type and dose of drugs, duration of ovarian stimulation, and even the aspiration from ovarian follicles can affect the integrity of the oocyte chromosomes.

However, multiple environmental variables in embryology laboratories can affect the chromosomal integrity of oocytes and embryos. These variables include the type of culture medium, culture conditions, pH, temperature, osmolality and oxygen tension, contaminants and volatile compounds, gamete manipulation, and gamete aging in terms of immaturity or post-maturity of the oocytes. Serious variations in the IVF laboratory environment may lead to mitotic spindle alterations, centrosome amplification, cell-cycle checkpoint defects, non-separation of chromatids, and telomere stability, causing defects in chromosome segregation and aneuploidy.

Studies conducted in IVF centers show as much as 40% variations in aneuploidy rates between different IVF centers; donated oocytes of young women and even the oocytes of the same donors were used in these centers by implementing a similar screening method for chromosomal abnormalities.

This represents the importance of controlling the environment of the embryology laboratory using quality assurance and quality control policy to minimize the rate of embryo aneuploidy and subsequently increase the success rate of IVF. Therefore, the aneuploidy rate of preimplantation embryos can be a key performance indicator to measure the effectiveness of an IVF laboratory and IVF center.

K-50
Sperm DNA fragmentation, contributing factors, and clinical setting

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Sperm DNA fragmentation index (DFI) along with semen parameters evaluation, is considered an important diagnostic method in assessing male fertility potential. Researches Evidence support the association between DFI with male infertility, natural conception, and assisted reproductive technique outcomes. Various factors contributed to DNA fragmentation generation in men, such as testicular dysfunction, diseases correlated with testicular such as varicocele, exposure to high-risk chemicals components, hyperthermia, and poor lifestyle

and nutrition. There are various laboratory methods for assessing DFI clinically. Patients with varicocele, unexplained infertility, recurrent pregnancy loss, recurrent failure of assisted reproductive techniques, and those at risk of lifestyle/environmental exposures are recognized candidates for DFI evaluation. Although no comprehensive treatment has yet been developed to overcome sperm DNA fragmentation, physicians offer different treatments to reduce the DNA fragmentation and improve sperm DNA and chromatin integrity. It seems in the clinical setting, oral antioxidants therapy, varicocele repair, use of recurrent ejaculations alone or combined with micromanipulation-based sperm selection techniques, and the use of testicular sperm for intracytoplasmic sperm injection are suitable options for improving the quality of DNA.

K-51
Long life endometriosis

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Endometriosis is defined as the presence of endometrial like tissue outside the uterus which induces a chronic, inflammatory reaction. The condition is predominantly found in reproductive age, from all ethnic and social groups. The associated symptoms can impact on general physical, mental, and social well-being in all over the life of the patient. Therefore it is crucial to give a careful note to women's complaint and history and make a precise decision. Treatment must be individualized taking the impact of the disease and the effects of treatment into account. For a pain management before any decision for surgery, a multidisciplinary clinic including pain manager, psychologist, physical medicine, nutritionist, and gynecologist must be involved. And then the first line of treatment of pain should be medical. For infertility, the first line of treatment should be IUI or IVF. Although there is some evidence that the success of infertility treatment and ART is lower in the presence of endometriosis, but the side effects of early surgery and decrease ovarian reserve and recurrence rate of 20-50% after surgery should be included into account.

K-52
Fetal lung cells for cell therapy of lung injury in an animal model

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The regeneration-inducing capacity of the cells derived from human fetal lung has not been systematically studied for cell therapy of lung injuries. We hypothesize that due to the commitment of these cells to the the respiratory system, they have a high potential to promote regeneration in respiratory system. In this study, lower respiratory tissues were isolated from 12-19 weeks human fetuses. The cells were characterized by their morphological and gene expression profiles and their ability to form organoids. The cells were then intra-tracheally delivered to rats with pulmonary injury induced by bleomycin at day 0 and 14 after induction of injury. Rats were sacrificed on day 28 after injury and their lungs were evaluated histologically. We have shown that cell therapy reduced fibrosis and collagen deposition and promoted the regeneration of alveoli. Also, cell therapy increased the expression of surfactant protein C and IL-10 and decreased the expression of aquaporin 5 and transforming growth factor beta. Here, we show that fetal human lower respiratory tract cells can significantly increase the process of lung regeneration. This finding not only introduces a potential cell source in this area but also suggests a potential phenotypic target for the derivation of regenerative cells from multipotent or pluripotent stem cells.

K-53
AZF_C delitions Y chromosome and male infertility

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Male infertility is a common condition with heterogeneous causes. Genetic or epigenetic variations or both contribute up to 15%–30% of cases of male infertility. Genes control a variety of physiologic processes, such as spermatogenesis which occurs in a sequential manner with mitotic, meiotic, and postmeiotic differentiation phases and secretion of hormones. The genetic abnormalities involved in male infertility may be chromosomal (numerical and structural aberrations) or monogenic disorders, mitochondrial DNA (mtDNA) mutations, microdeletion of the Y chromosome, multifactorial disorders, imprinting disorders, or endocrine disorders of genetic origin. The Y chromosome is one of the smallest human chromosomes and microdeletions on the long arm of this chromosome (Yq) is one of the most significant causes of male infertility. Y chromosome microdeletions were present in about 5.2% -12.1% of Iranian infertile men with azoospermia and severe oligozoospermia. The azoospermia factor (AZF) region is further subdivided into 3 non-overlapping regions termed as AZFa, AZFb, and AZFc. Deletion of AZFa and A2Fb which are less common are associated with non-treatable azoospermia. Deletions of the AZFc region are most common in men with idiopathic oligozoospermia or azoospermia. Cases with AZFc deletions show a progressive deterioration in spermatogenesis and cases develop azoospermia over a period of time. The clinical outcomes of intracytoplasmic sperm injection (ICSI) for oligozoospermic patients with Y chromosome AZF

microdeletion are comparable to those of infertile patients with normal Y chromosomes. For azoospermic men with AZFc deletion microdissection, TESE is recommended with a success rate of sperm retrieval about of 36.3%. In conclusion, the pregnancy and delivery in oligozoospermic patients with AZFc deletion are comparable with other studies, but despite of sperm retrieval in azoospermic patients with AZFc deletion, the chance of pregnancy or delivery in these patients is very low. More attention to surgical points for sperm retrieval and more extensive search for sperm finding and refinement of sperm freezing and ICSI procedure is needed for better results.

K-54
An introduction to tissue engineering: How to select the best scaffold

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Tissue engineering and regenerative medicine are fast developing approaches in the production of new organs and body tissues. On the other hand, it is a field that seeks to replace/repair or enhance the biological function of a tissue or an organ by manipulating cells via their extra-cellular environment. The concept of directly engineering tissue was articulated in detail in 1985 by Fung co-workers and the term "tissue engineering" was first used during a meeting sponsored by the National Science Foundation in 1987. Even though everyone believes that the field of tissue engineering may be relatively new, the idea of replacing tissue with another goes as far back as the 16th century. Over the past few decades, there has been a wide range of researches that have been conducted on the provision of tissue-engineered and regenerative medicine which lead to a significant improvement in the production of scaffolds with similar characteristics to a natural tissue/organ. These scaffolds needed due to either trauma/injury, genetic disorders and diseases where can lead to damage and degeneration of tissues in the human body, which necessitates treatments to facilitate their repair, replacement or regeneration. The aim of this talk is to talk about basic principles of tissue engineering and regenerative medicine and in particular show the path for selecting the best biomaterials known as scaffolds to complete the treatment of damaged/diseased tissue or organs.

K-55
Practical use of tissue engineering in treatment of ovarian cancer and vaginal infections

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The female vaginal tract has its own innate defence system that involves the natural microbiota, the vaginal epithelium and proteins that help manage a healthy microbiome. In this project, we are concerned with what happens when things go wrong with this balance. Bacterial vaginosis (BV) is a common vaginal infection with 1 in 3 pre-menopausal women in the UK suffering from BV at some point in their life. Intravaginal treatment can be impeded during menstruation and BV can often recur within a few weeks if not treated effectively. Management of the vaginal biofilm is an emerging sector in women's health. Globally, 20-30% of women of reproductive age suffer from BV, as yet only partially understood but associated with an increased chance of preterm abortion, pelvic inflammatory disease and sexually transmitted diseases. The symptoms of discharge and offensive smell can cause considerable distress. The aim of this project is to offer women a temporary scaffold encapsulated with a drug that can manage the biofilm on the epithelial layer of the vagina.

K-56
Genetic aspects of ovarian reserve

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Ovarian aging exhibits no obvious signs therefore women who delay pregnancy later in life may be faced with unexpected fertility issues. The ability to accurately predict a woman's reproductive lifespan is becoming of increasing importance. Ovarian reserve tests are typically a measure of antral follicles and roughly correlate with the number of primordial follicles remaining in the ovary. The rate of decline of the ovarian reserve is variable between women, and current ovarian reserve tests have limitations. Current techniques, such as the anti-mullerian hormone (AMH) test, lack long-term accuracy and predictability. The identification of genetic variants associated with an increased risk of accelerated ovarian aging may allow for a more accurate and predictive screening tool. To present the current literature on genetic prediction of ovarian reserve and determine whether genetics markers of the ovarian reserve may complement the best current predictors of ovarian reserve. The advantage of using genetic markers of ovarian reserve is that they are present throughout life, and analysis only needs to be done once. Our data suggest that common variants influencing age at menopause may also modify the risk of accelerated ovarian ageing. Our results extend findings from recent genome-wide association studies and may guide future research efforts in identifying further genetic biomarkers influencing ovarian reserve.

K-57
Exosome therapy in treatment of infertility

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Abstract not received.

K-58
Etiologies of male oxidative stress

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Reactive oxygen species (ROS) are formed during normal cellular metabolism. In the male reproductive system, they are involved in many physiological processes, including capacitation, hyper-activation, acrosome reaction and sperm-oocyte fusion. The generation of the ROS occurs via three methods in spermatozoa: (1) in the cell membranes, using nicotinamide adenine dinucleotide phosphateoxidases, and (2) in the mitochondria, using nicotinamide adenine dinucleotide phosphateoxido-reductase and finally, (3) immature spermatozoa with residual cytoplasm contains high levels of glucose-6-phosphate dehydrogenase, a cytosolic enzyme that utilizes the hexose monophosphate shunt to produce abnormally high levels of NADPH. Excessive NADPH results in a greater production of superoxide anions by NADPH oxidases. Activated leukocytes (peroxidase positive) also produce large quantities of ROS and it has been shown that leukocytes are the predominant source of ROS in raw human semen. When ROS increases beyond the physiological levels, overwhelming opposing antioxidant forces, oxidative stress (OS) results. When this occurs, ROS can lead to lipid peroxidation, DNA damage, and OS-induced apoptosis and autophagy, which can be harmful to the highly susceptible sperm cells.

In addition to several diseases like diabetes and varicocele, there are many extrinsic factors affecting human spermatozoa by elevating ROS level. Smoking is one of the most clinically relevant and preventable causes of OS. Alcohol consumption is considered as another etiological factor for ROS production. Malnutrition and poor diet can also induce ROS production. On the other hand, obesity and over-nutrition also play a significant role in inducing OS in the reproductive system. Stress is linked to increasing in ROS in the seminal plasma and impaired sperm quality. There are many proposed mechanistic effects of medicines on ROS and its related OS. Bactericidal antibiotics can overproduce ROS and lead to mitochondrial dysfunction in mammalian cells including spermatozoa. Environmental pollutants such as nitric oxide, sulfur dioxide, carbon tetrachloride, ozone, wood dust, particulate matter, volatile organic compounds, bisphenol A, xenoestrogens, and phthalates can potentially induce OS and decrease sperm quality. Additionally, studies have shown that pesticides such as lindane, methoxychlorate and dioxin-TCDD have been related to testicular OS in animals and humans. Finally, the radiation and electromagnetic fields such as cellular phones also can cause an elevation in ROS production and decrease sperm fertility potential.

K-59
Reproductive tissue engineering by marine scaffolds: Decellularization and 3D bio-printing

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The body structure of marine invertebrates is made up of a variety of scaffolds of proteins, glycoproteins, carbohydrates, and bio-glass, all of which can be used to engineer human tissues, either intact or manipulated. The combination of existing scientific evidence shows that marine invertebrate scaffolds can create new capabilities in the pharmaceutical and medical industries. Production and recombination of human cell-compatible scaffolds from marine invertebrates or the removal of cells in invertebrates and the use of the natural structure of marine scaffolds are applications of marine tissue engineering to produce soft reproductive tissues. As a result, the biodiversity of marine invertebrates of the Persian Gulf with simple body scaffolds for the extraction of bio-ink compounds for 3D bio-printers and the decellularization of their tissues has provided vast research potential. In this direction, the Marine Comparative and Experimental Medical Center in the Persian Gulf Marine Biotechnology Research Center has completed various projects in the construction of marine scaffolds. Three invertebrate species of the Persian Gulf have been studied for this purpose, which are jellyfish, brown algae, and sponge. In the 3D bio-printer made by the researchers of this center, alginate compounds extracted from brown algae were used for flexible scaffold printing. Furthermore, jellyfish tissue and sponge after decellularization were used to culture human fibroblasts.

K-60
Challenge of cell therapy in endometriosis

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Endometriosis is a common medical problem, occurring in 10–15% of reproductive-age women and 20 to 50% of infertile women. Endometriosis is an inflammatory, mostly estrogen-dependent condition that happened due to the development of endometrial tissue outside of the uterus. Manifestations include pelvic pain, dysmenorrhea, infertility, and in some cases ovarian cancer. Despite the high prevalence of endometriosis, the pathogenesis of this disease remains poorly understood. However, the most accepted theories are angiogenesis, cellular invasion, adhesion formation, fibrosis, neuronal infiltration, and abnormal cell growth. Also, some recent studies suggest that endometrial stem/progenitor cells function in the development of endometriosis. In other words, potential stem cells can form endometriotic implants. However, regarding the fact that endometrium becoming fibrosis during the progress of endometriosis, stem cell therapy offers the potential treatment of tissue injury and fibrosis. It is accepted that stem cell has the capacity of self-renewal and differentiation into other cells simultaneously confirming its important role in maintaining the homeostasis, repair, and renewal of endometrial fibrosis. Regarding the controversial role of stem cells, more efforts are needed to explore the specific stem cell-based therapy and make its clinical use.

K-61
Mimicking nature in wise strategy

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Controlled ovarian stimulation (COS) remains a challenging clinical step in assisted reproductive technique, especially in some specific patients group in which no evidence-based guidelines are available. Up to now, the clinical approach to the infertile women needs several decisions that reside in the clinician's hands. For this reason, COS is one of the cornerstones of in vitro fertilization (IVF). Today the measure of success in IVF must be the cumulative live birth rate per started cycle. Its purpose is to obtain an adequate response in terms of oocytes' number and quality to improve treatments' efficacy and efficiency by obtaining several competent embryos. The ability to predict the ovary response is the priority to obtain the right number of oocytes and to define the right individual treatment for the right patients. Many factors can be used as predictors of ovarian response such as: age, biochemical parameters, follicle-stimulating hormone (FSH), anti-müllerian hormone, and morphological parameters (antral follicular count) but also some clinical conditions like polycystic ovary syndrome and low body mass index.

Although some data suggested that recombinant-FSH and human menopausal gonadotropins (hMG) for COS in long agonist protocols perform similarly, the evidence is limited in antagonist protocols, i.e. the most commonly used at present. Therefore, the decision on which gonadotrophins should be used for COS is still uncertain, especially in patients at their first COS (naïve) and/or in freeze-all strategies. However according to the evidence already published r-FSH and hMG have a different endocrine profile, the serum levels of FSH, androgens, and estradiol were significantly higher with hMG than r-FSH in conventional COS. Moreover r-FSH significantly increases the number of oocytes retrieved and embryos obtained compared with hMG after COS. The duration of COS was significantly longer and the total amount/dose of gonadotropin was significantly higher with hMG than with r-FSH.
Finally, no difference has been reported in term of ovarian hyperstimulation syndrome risk and ovarian hyperstimulation syndrome profile between hMG and r-FSH Regarding luteinizing hormone (LH) activity during COS, LH supplementation in COS continues to be actively debated and controversial, causing some confusion between practitioners. Current evidence suggests that r-LH supplementation appears to be beneficial in i) hypo-hypo, ii) patients with hyporesponse to FSH monotherapy, iii) advanced maternal age, iv) patients with very low endogenous LH during COS.
Finally, r-FSH + r-LH combination may be effectively used to obtain COS in IVF patients without increasing the overall costs for the patients or the National Health Service in a specific setting.

K-62
An introduction to tissue engineering: How to select the best cells

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Stem cells are special cells that are able to develop into many different cell types and or tissues, ranging from bone cells to brain cells. Stem cells are divided into embryonic stem cells and adult stem cells Subtypes. Embryonic stem cells (ESC's) and induced pluripotent stem cells (iPSC's) can turn into more than one type of cell from different origins. Adult stem cells can differentiate toward one tissue (such as the liver) or to multiple tissues (such as the bone, brain, and cartilage) from the same origin in the case of mesenchymal stem cells. Given their unique regenerative abilities, stem cells offer new potentials to repair or treat diseases such as bone and cartilage defects, diabetes, and heart disease.
The general principles of tissue-engineering involve combining stem cells, a natural/synthetic scaffold, and

required chemical/mechanical factors to build a functionally, structurally and mechanically compatible living tissue.

An ideal tissue engineered construct needs an excellent microenvironment with the optimal cell adhesion, growth, and differentiation capacity in controlled pH, temperature, oxygen tension and be properly adapted to the mechanical force of the microenvironment. Therefore, besides the choice of stem cells, the development of such a construct requires a careful selection of three key components: 1) scaffold, 2) growth factors, 3) extracellular matrix.

For the permanent repair of damaged tissues, the following criteria are essential to consider:

- An adequate number of cells and their ability to differentiate into desired phenotypes,
- Cells must be able to adapt to the three-dimensional structure and produce their own extracellular matrix
- Tissue-engineered cells also must be structurally and mechanically compliant with the native cells and be able to integrate with native cells without the risk of immunological rejection and pose no biological risks.

The source of cells utilized in tissue engineering can be autologous (from the patient), allogenic (from a human donor but not immunologically identical), or xenogeneic (from a different species donor). Each stem cells source has its (dis) advantages in usage. For example, the autologous cells represent an excellent source for use in tissue engineering because of the low association with immune complications but their use is in general not cost-effective for batch controlled clinical use. In contrast, allogenic cells and xenogeneic stem cells offer advantages over autologous cells in terms of uniformity, standardization of procedure, quality control, and cost-effectiveness. Their disadvantage may lie in immunogenic related adverse effects.

In conclusion, the use of an appropriate multipotent or pluripotent stem cell in tissue engineering is an emerging concept. Many technical questions need to be answered and require close collaborations between scientists, clinicians, engineers, and legal and ethical regulating bodies (for example when embryonic stem cells or genome-edited iPSC's are used) to obtain the goal of functional tissue restoration.

4th Congress of Reproductive Genetics

K-63
Evaluating ovarian impairments in implantation failure

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Recurrent implantation failure (RIF) can be defined as a failure to achieve a clinical pregnancy after transfer of at least four embryo of good quality in a minimum of three fresh or frozen cycles in women under the age of 40.RIF is often a complex problem with a wide variety of etiologies and mechanisms as well as treatment option.

Recurrent miscarriage (RM) is defined as two or more consecutive pregnancy before the 20st week of pregnancy or a fetal weight of less than 500 grams that affect approximately 5% of conceived women worldwide.

RIF and RM are multifactorial disorders with three main causes including gamete and embryo factors, factors affecting endometrial receptivity and ovarian factors affect gamete and embryo factors and include premature ovarian failure (POF) and polycystic ovarian syndrome. Primary ovarian insufficiency (POI) is a heterogeneous disease caused by a variety of mechanisms that affects ~1-2% of under-forty years old women. The etiology of POI has been found to be genetic mutations, chromosomal, and autoimmune.

About 10% of cases of POI are related to genetic diseases and over 50 genes are known to be causally related to POI. The most frequent conditions associated with POI are Turner syndrome and fragile X pre-mutation; mutation of BRCA 1-2 genes and several other mutations and genetic syndromes that have recently been highlighted, although they rarely occur.

Fragile X mental retardation type 1 gene pre mutation has been frequently found in POF or POI. Tet (Ten-eleven translocation) Tet1 deficiency leads to POF by influencing the quality of oocytes and reduces expression of X-chromosome-linked genes, such as Fragile X mental retardation type 1. ATG7 (autophagy-related genes) and ATG9A variants have also a functional link with POI. If a diagnosis of genetic-based POI is determined before the onset of POI, counseling on currently available fertility preservation techniques is advisable.

Advanced maternal age, high follicle-stimulating hormone, low antral follicle count, and low Anti-Mullerian Hormone result in fewer number of oocytes retrieved, high number of immature oocytes, reduced fertilization and embryo utilization rate. Advanced age also cause aneuploidy, increase mitochondrial damage and decrease in mitochondrial membrane potential. Its impact on zona hardening and subsequent defective hatching as a cause for RIF has been suggested by a few studies. PCOS is the most common cause of anovulatory infertility in the developed countries and also the most commonly identified abnormality among women with recurrent miscarriage. Spontaneous loss of fetus occurs in 40% of women with PCOS and the possible causes may include obesity, hyperinsulinemia, insulin resistance, hyperandrogenemia, hyperhomocysteinemia, high levels of plasminogen activator inhibitor-1 factor, poor endometrial receptivity, and elevated levels of luteinizing hormone. Subacute Cd (cadmium) exposure disrupt the Hypothalamic–pituitary–gonadal axis function, leading to PCOS and POF features and other abnormalities in female rats.Chromosomal abnormalities of the male or female partner (deletions and duplications-dicentric and ring chromosomes-balanced and unbalanced translocation-single gene defect) and Maternal cytoplasmic factors or mutations in cell cycle control gene also can cause RIF and RM.

K-64
Genetics and pharmacogenetics aspects of PCOS

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The aim of treatment of polycystic ovarian syndrome (PCOS) is restoring the ovulation; due to PCOS is the most common cause of anovulatory infertility and the most prevalent endocrine disorder in reproductive age women (5-20%) with the main symptoms including oligomenorrhea/anovulation, clinical and/or biochemical hyperandrogenism and polycystic ovaries. PCOS is a multifactorial disease including hormonal, genetic, environmental and immunological factors. Alteration in genetic factors can be related to etiology of disease are most related to its symptoms. These genes are estradiol biosynthesis genes (CYP19 or Aromatase); gonadotropin-releasing hormone, follicle-stimulating hormone receptor, luteinizing hormone, anti-müllerian hormone; and transforming growth factor beta gene family.
Commonly applied treatments in PCOS consist of clomiphene citrate, aromatase inhibitors and recombinant follicle-stimulating hormone to make ovulation induction, however outcomes of this treatment are often unpredictable. In some cases, patients are resistance to treatment. Therefore, an era of predicting drug response on the basis of one's genome is drawing close to reality, entitled pharmacogenomics needs to be studied. We aimed to summarize the way in which genetic variability might modify effects of drug-metabolizing enzymes, transporters and receptors, thereby altering response to drugs used in ovulation induction in PCOS patients. For example CYP2D6 is responsible for CC metabolism and is mostly expressed in liver.

K-65
Sperm DNA damage and male infertility

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Abstract not received.

K-66
Pharmacogenomics in infertility

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With 80 million couples worldwide having difficulty to conceive a child naturally, infertility sounds to be a real serious medical issue that requires a great attention. Despite the carried out studies and the great efforts to understand this problem, there is still a gap between our current knowledge/capabilities and the available options for the treatment. One possible reason for such a gap may be related to factors other than advanced maternal age, ovarian reservation, cigarette smoking, and hormonal changes. Pharmacogenetics is a relatively new approach that has been considered to fill in this gap, and utilized to develop personalized therapies. The main aim of pharmacogenetics is to study the genetic variations in order to optimize the success and minimize adverse effects of drugs. Therefore, the approach has been used to study the molecular changes in various biochemical markers involved in reproduction. It is being put forward as an alternative to the "one-size-fits-all" approach that has been ineffective in many conditions.

K-67
New genetic finding in PGD

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Abstract not received.

K-68
What is important of male infertility environment?

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K-69
Covid 19, Vaccines and infertility

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ACE2-based changes in the testicles, hormonal changes in patients, and the possible formation of antisperm antibodies (ASA) can cause male infertility as a complication of coronavirus infection. Although some studies have shown abnormalities in spermatogenesis and sperm motility, there is still no evidence of long-term persistence of these disorders as chronic complications of the disease. Also, after the availability of the vaccine against this virus, the possibility of the vaccine affecting the fertility of men and women has been considered. In the case of female infertility, one cause for concern is the presence of a protein called syncytine 1, which is similar to part of the new corona virus spike. It is claimed that corona vaccines can produce antibodies against syncytine 1 and cause infertility in women. Further studies will be needed to conclude, which will be discussed in this article.

K-70
Chromosomal microarray methods for detection of aneuploidy and structural chromosomal abnormality in Paroxysmal nocturnal dyspnea

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Since the identification of the exact number of human chromosomes in 1956, different techniques have been developed to identify the number of chromosomes and structural chromosomal abnormalities. Some of them such as karyotyping and fluorescence in situ hybridization (FISH) are valuable tools in both research and diagnosis. Resolution limitation is one of the important limitations of these techniques. The inability to study the entire genome simultaneously was the next limiting factor. Fortunately, the advent of new technologies to help identify postnatal and prenatal chromosomal abnormalities has had a positive effect on reducing abnormal birth rates and increasing success in assisted reproductive techniques. In 1997, microarray-based comparative genomic hybridization (array CGH) was introduced. Array CGH has the high resolution of FISH and the ability to study all chromosomes simultaneously. This technique has led to great advances in medical genetics.

K-71
Prenatal screening and prenatal diagnosis, new challenges in Iran

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Approximately 3% to 5% of pregnancies are complicated by birth defects or genetic disorders. The rapid evolution of cytogenetic methods and the advancement of molecular genetics have greatly contributed to the reduction of births with genetic defects. Prenatal tests include prenatal screening tests and prenatal diagnostic tests, which aim to detect metabolic, chromosomal, and anatomical abnormalities of the fetus as soon as possible during pregnancy. In 2007 American Congress of Obstetricians and Gynecologists (ACOG) released "ACOG Practice Bulletin No. 77," which recommended making aneuploidy screening or invasive testing available for all women, ideally at their first prenatal visit. This idea was revolutionary at the time, as previously only women who were considered to be at high risk had been offered these tests. In Iran, prenatal screening tests are considered the health care system and every pregnant woman with a positive screening is referred for prenatal diagnostic tests. But Iran is currently facing a serious challenge for PGS and PGD. The Iran parliament has decided to eliminate prenatal screening tests from PHS to prevent population decline.

K-72
Non-invasive prenatal testing (NIPT) challenges in future

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Non-invasive prenatal testing (NIPT) for select fetal trisomies became clinically available in 2011 and transformed the landscape of prenatal screening in many countries. With very high sensitivity and very low false-positive rates, this advance represented a tremendous step forward in Down syndrome detection. However in the case of the following conditions the interpretation of NIPT results might be challenging and even error-prone: mosaicism, co-twin demise/vanishing twin, maternal genetic abnormalities, maternal medical conditions (e.g. organ transplant, malignancy), copy number variation of the portion of a fetal chromosome too small to be detected by the standard cytogenetic testing.
Since it is noninvasive, safe and allows the early detection of abnormalities, NIPT expanded rapidly and the test is currently commercially available in most of the world. As NIPT is being introduced globally, its clinical implementation should consider various challenges, including financial-economic, social, and organizational/ educational challenges. Subsequently, with a higher depth of sequencing and improved bioinformatics analyses, NIPT expanded to include detection of a number of microdeletions. Genome-wide screening for copy number variants has also been reported and is now offered clinically.
Noninvasive identification of fetal single-gene disorders, and ultimately analysis of the fetal genome, has become the "next frontier" in prenatal diagnosis. By increasing the portfolio of what can be offered by NIPT it will be necessary to think ahead the challenges of interpreting the incidental findings that will come up and how to share these findings with the families in future.

K-73
Preimplantation genetic testing (PGT) using next generation sequencing (NGS)

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Abstract not received.

K-74
Genetic impairments of implantation failure

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Embryo implantation is a critical stage of pregnancy, and failure in implantation is a main factor in early pregnancy loss or assisted reproductive failure. In humans, natural pregnancy per cycle is poor (~30%), and 75% of pregnancy are terminated because of implantation failure.
A variety of cellular actions and molecular pathways implicated in embryo-uterine interaction during implantation have been recognized, which through gene expression and genetically engineered mouse models studies was found.
However, multiple molecules are engaged in the control of implantation, but their particular actions stay uncertain. Successful implantation of a good quality human embryo in a receptive endometrium needs a significant and complicated cooperation of factors. Studies on gene- and protein expression have guided to recognition of many endometrial biomarkers and genes of both successful and unsuccessful implantations.
The functions of many candidate genes stayed important because their knocked out commanded to embryonic lethality or systemic faults. Increasing numbers of studies represented that genetic factors influence invasion and angiogenesis developments and they are crucial in embryo implantation. Molecular and genetic studies specify that ovarian hormones produced signaling molecules, containing cytokines, growth factors, homeobox transcription factors, lipid mediators and morphogen genes, work by way of autocrine, paracrine and juxtacrine relations to indicate the complex process of implantation. However, the categorized environment of the molecular signaling pathways that administrate embryo-uterine interactions in early pregnancy remains to be discovered in depth. This could be so, genetic defects and even genetic polymorphisms of genes involved in the developments of implantation could control, or at least intensify effects to implantation failure.

K-75
NIPT application and detection of genetic diseases in PND

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Different genetic conditions associated with congenital anomalies/intellectual disability can contribute to long-term disablement, with significant impacts on individuals, families, health-care systems, and societies. Although congenital anomalies may be the result of one or more genetic, infectious, nutritional or environmental insults, it is often difficult to identify the exact causes. Conventional karyotype, quantitative fluorescence-polymerase chain reaction (QF-PCR) and chromosomal microarray are able to detect about 40-50% of the causes of fetal anomalies, with therefore about 50-60% of cases in which it is not possible to define the etiology. In fact, 10-20% of isolated or syndromic congenital anomalies can be associated with monogenic diseases, whose diagnosis is often established based on a family anamnesis, clinical examination, and pedigree pattern and confirmed through genetic examination. Next-generation sequencing enables to sequence of the fetal exomes, furnishing a broader diagnostic capability compared to traditional and molecular cytogenetics prenatal tests. This approach adds at least an extra 10% of clinically relevant information in cases of fetuses with structural anomalies. Moreover, the same noninvasive prenatal test can now be performed for definitive diagnosis of some monogenic recessive and X-linked conditions, or also in paternally inherited dominant and de novo conditions.
We recently demonstrated that trio-whole exome sequencing (trio-WES) using fetal cell free-DNA (cff-DNA) can be analyzed with sufficient sensitivity and that an adequate strategy can identify the cause of pregnancies at risk for malformative disorders. Our work suggested that for fetuses with proven congenital malformation an extended US examination together with trio-WES on cff-DNA may be helpful in detecting an underlying congenital disease.
Furthermore, subsequent analyzes also in couples with low risk or negative anamnesis for genetic pathologies, allowed us to further demonstrate that genome-wide sequencing is an effective method that will likely be more used in the coming years as a clinical tool for diagnosis. Moreover, we are able to demonstrate that the WES analysis on cell-free DNA has a better diagnostic yield than the same test performed on DNA extracted from the amniotic fluid, proving that WES on cff-DNA is especially suitable, with an appropriate genetic counseling, in fetuses with genetic diseases.

K-76
Chromosome X-inactivation and infertility

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Abstract not received.

K-77
Genetic aspects of male infertility

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About 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility. One in eight couples encounters problems when attempting to conceive a first child and one in six when attempting to conceive a subsequent child. Three percent of women remain involuntarily childless, while 6% of parous women are not able to have as many children as they would wish. Infertility affects both men and women. In

50% of involuntarily childless couples, a male-infertility-associated factor is found together with abnormal semen parameters. Male infertility is a common and severe health problem. Infertility not only affects one's ability to have children, but also has emotional, psychological, family, and societal effects. The incidence may be increasing during the time of those affected, roughly 40% have idiopathic infertility. It is likely that the majority of those patients have genetic abnormalities that are the cause of their infertility. The understanding of the genes involved in spermatogenesis, sperm maturation, and normal sperm function is key, but we must also focus on better methods of accelerating advances into meaningful clinical diagnostic tests and therapies. During the past 30 years, significant improvements in technology have advanced the treatment of male infertility and Genetic evaluation as well. The primary advance has been intracytoplasmic sperm injection (ICSI) in conjunction with in vitro fertilization through ART cycles. Although this technological leap has allowed thousands of men to father a child who otherwise would have been unable to do so, the scientific study of the causes of male infertility has not kept pace. All urologists working in the field of Andrology must have an understanding of genetic abnormalities associated with infertility so that they can provide correct advice to couples seeking fertility treatment. Men with very low sperm counts can be offered a reasonable chance of paternity, using IVF, ICSI, and sperm harvesting from the testes in case of azoospermia

In fact, the clinical application of ICSI proceeded without sufficient scientific study of its safety to the offspring, or the future genetic ramifications several researchers and clinicians, and an international audience of experts in the field, reviewed the study of the genetics of male infertility, the tools available in the laboratory and clinic, the current state of knowledge, and the future of research and translation into clinical diagnostics and in this webinar the colleagues discussing the following aspects as:

- The genetics of male infertility in the era of genomics
- Important environmental factors on fertility potential
- DNA damage how can affect the fertility potential of male
- Methods and tools for the study of the genetics of male infertility
- Clinical approach for evaluation of azoospermia men
- Management of azoospermia due to microdeletions in y chromosome
- Regeneration approach as new tools for now and near future to generate spermatogenesis.

K-78
Personalized medicine for the embryo and the fetus

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Since the conception of precision medicine has been put forward in reproductive medicine, this idea has been popularized and applied in many specialties. Significant progress has been made toward personalizing the entire process, including diagnosis, treatment planning, and embryo identification, and combining large-scale genetic information data and knowledge discovery can offer better prospects in reproductive medicine. The causes of infertility are various, and many factors influence the success rates of ART which are complicated; hence, different genetic diagnostic methods of reproductive medicine for the diagnosis of infertility causes and transfer of healthy embryos, needs to be precise. During the last decay, next generation sequencing influenced reproductive medicine to personalize the diagnostic methods. Prenatal genetic diagnostics and preimplantation genetic diagnosis can and should be expanded to incorporate genetic, genomic and transcriptomic data to develop new approaches to diagnosis and fetal treatment. I would like to review recent advances in prenatal genetic diagnostics and preimplantation genetic diagnosis, the challenges associated with these new technologies such as next generation sequencing and how the information derived from them can be used to personalize and advance fetal care.

K-79
New strategies for the diagnosis of male infertility: Current developments and prospects

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Infertility is one of the common health issues around the world. Although Female factors are the main cause of infertility, male factor infertility involves approximately 30%- 35% of infertile couples. Genetic factors account for 15% of infertility male factors. By emerging the next generation sequencing technology, the role of male genetic factors is becoming increasingly prominent. Various whole-exome sequencing approaches such as family-based whole-exome sequencing are powerful tools for accurately identifying the novel variants responsible for infertility. In this talk, we try to outline different strategies for studying the causes of male infertility and the latest findings in this area. Eventually, it seems that male monogenic factors play a much greater role in infertility problems than previously thought. Despite the remarkable advances in male infertility, due to the high prevalence of this disease, we expect to see many more discoveries in the near coming years.

K-80
Azoospermia

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Infertility affects 15% of couples, and male factors are implicated as a cause in 50% of patients. Azoospermia is considered the most severe form of male factor causes and is defined as the absence of sperm in the semen sample. It can be further categorized into obstructive and non-obstructive azoospermia. Non-obstructive azoospermia may be due to testicular (primary testicular failure) or pre-testicular (secondary testicular failure) causes. Obstructive azoospermia could be due to congenital and/or acquired etiologies. Genetic factors may play an important role in both types of azoospermia. Before the advent of intra-cytoplasmic sperm injection (ICSI) azoospermic men considered sterile and were in need of sperm or fetus donation. In the era of new assisted reproductive technique, these male patients could have their own child. Since ICSI could surpass be natural selection, the genetic abnormalities could easily transmitted to the offspring. Therefore genetic counseling should be considered before ART.

K-81
Personalized ovarian stimulation for assisted reproductive technology

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When one talks about personalized medicine in the field of assisted reproduction technology, the main focus is mainly on achieving the optimal number of oocytes in successful ovarian stimulation at the same time considering the safety, success, and potential of individuals to respond. Considering that more oocytes mean higher clinical live birth rate but one single protocol for stimulation does not fit all. Considering every individual has different egg reserve which can be assessed based on anti mullerian hormone or antral follicular count, therefore, amount of Follicle-stimulating hormone given for induction of stimulation should be related to one of these two parameters at the same time considering safety which here means avoiding ovarian hyperstimulation syndrome. Literature shows that around 15 oocytes are the optimal number of oocytes for achieving a live birth and avoiding ovarian hyperstimulation syndrome. Achieving this number of oocytes may not be possible in one single cycle and multiple cycles are required and a standard dose of FSH is not justified and a tailoring Follicle-stimulating hormone dose should be defined based on the individuals age, antral follicular count and previous cycle performance.

K-82
Treatment of infertile male causing by AZF deletion

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Male infertility is a common condition with heterogeneous causes. Genetic or epigenetic variations or both contribute up to 15%-30% of cases of male infertility. Genes control a variety of physiologic processes, such as spermatogenesis which occurs in a sequential manner with mitotic, meiotic, and postmeiotic differentiation phases and secretion of hormones. The genetic abnormalities involved in male infertility may be chromosomal (numerical and structural aberrations) or monogenic disorders, mitochondrial DNA (mtDNA) mutations, microdeletion of the Y chromosome, multifactorial disorders, imprinting disorders, or endocrine disorders of genetic origin. The Y chromosome is one of the smallest human chromosomes and microdeletions on the long arm of this chromosome (Yq) is one of the most significant causes of male infertility. Y chromosome microdeletions were present in about 5.2% - 12.1% of Iranian infertile men with azoospermia and severe oligozoospermia. The AZF region is further subdivided into 3 non-overlapping regions termed as AZFa, AZFb, and AZFc. Deletion of AZFa and A2Fb which are less common are associated with non-treatable azoospermia. Deletions of the AZFc region are most common in men with idiopathic oligozoospermia or azoospermia. Cases with AZFc deletions show a progressive deterioration in spermatogenesis and cases develop azoospermia over a period of time. The clinical outcomes of ICSI for oligozoospermic patients with Y chromosome AZF microdeletion are comparable to those of infertile patients with normal Y chromosomes. For azoospermic men with AZFc deletion microdissection, testicular sperm extractions is recommended with a success rate of sperm retrieval about 36.3%. In conclusion, the pregnancy and delivery in oligozoospermic patients with AZFc deletion are comparable with other studies, but despite of sperm retrieval in azoospermic patients with AZFc deletion, the chance of pregnancy or delivery in these patients is very low. More attention to surgical points for sperm retrieval and more extensive search for sperm finding and refinement of sperm freezing and ICSI procedure is needed for better results.

K-83
Personalized medicine in infertile men

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As in all fields of medicine, each patient has different details in male infertility. Treatments made without taking these details into account are more likely to fail. Today, widely used approaches in the world are generally in the form of applying some close-to-standard procedures that the centers have created for them to the patients. Such approaches are effective on a certain number of patients, but other groups cannot be successful despite repetitive procedures.

Today, thanks to the developing genomic technologies, it is possible to examine thousands of genes and copy number changes in humans at the same time. Considering that hundreds of factors in infertility have entered the literature so far, conducting extensive research is an indispensable need for most patients. The genetic change detected in some of these patients changes the treatment algorithm of the patient significantly, and in some patients, diagnoses that are not possible to treat can be made. In particular, patients in this last group are exposed to repeated in vitro fertilization or other medical practices, even though they have no hope of treatment, and lose time and money.

Although genetic tests were often put at the end of the list of studies to be carried out in infertility practices in recent years, due to the high cost of genetic tests, it is possible to move forward in the examination order due to the fact that these applications have gradually lower costs. One of the most important stages of personalized medicine applications is to evaluate the patient with large genetic panels and to evaluate the effects of other diseases related or unrelated to infertility with very wide eyeglasses.

K-84
Genetic and epigenetic aspects of endometriosis

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Endometriosis is a major gynecological disease that affects over 10% of women worldwide. It is characterized by the implantation of functional endometrial tissue at ectopic positions generally within the peritoneum. Endometriosis is recognized as a steroid-dependent disorder; however, the cause of endometriosis is unknown and there is no definite cure for it. This is mainly because of our limited knowledge about the pathophysiology of this disease at the cellular and molecular level. A PubMed search summarizes the key mediators of pain, abnormal uterine, bleeding, and infertility in endometriosis, including sex steroid hormone receptors, inflammatory molecules, extracellular matrix enzymes, growth factors, and neuroangiogenic factors. Therefore, clarifying the molecular mechanisms underlying endometriosis is essential in order to develop advanced therapies for the disease. In this regard, access to a precise genetic/epigenetic profile of endometriosis would be helpful for the diagnosis and treatment of the disease.

K-85
Can personalized medicine help infertility treatment?

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Different surveys showed that the rate of infertility is above 10% in many parts of the world. This means that becoming pregnant can be a stressful process for many couples, and they have to seek assisted reproductive technology (ART) and use different drugs to help them for having a baby. It is shown that there are differences in response to different drugs in these individuals. It is proved that these differences are genetically determined. Individualized or personalized medicine is defined as using genetic information to select the most appropriate choice of pharmacological therapy. Personalized medicine is based on polymorphic DNA sequence variations. If a polymorphism occurs in the coding or regulatory regions of a gene, it can alter the function, activity, or level of expression of that gene. Using the new advanced methods of genetics testing such as automated analysis of genome-wide single nucleotide polymorphisms allows identifying polymorphisms in genes involved in drug metabolism, transport and receptors. In ART, the efficiency of the protocol is a problem that needs to be solved. If we can utilize genome sequencing as a routine clinical approach to create an individual's own pharmacogenomics profile, therefore we can provide valuable information to help infertility specialists to use the optimal drug dosage for ART. So far, significant progress has been made toward personalizing the entire ART process, including diagnosis, treatment planning, and embryo identification. In fact, reproductive medicine is among one of the first subjects that used the concept of personalized medicine even before its popularization. The application of personalized medicine in ART starts in fact when the studies showed that the causes of infertility are various, and factors influencing the success rates of ART are complicated. In fact, there are variations in different individuals regarding their oocyte and embryo grading, endometrial condition, and semen analysis. Therefore, different steps of reproductive medicine need to be personalized, such as the diagnosis of infertility causes and transfer of the healthy embryo. One of the most important areas of using personalized medicine in reproductive medicine is discovering and validating genomic, protein, and metabolite biomarkers. To overcome the concept of "one size does not fit all" we should consider patients' specific molecular profiles. Finally, the main difference in personalized medicine between ART and the other subjects is that in reproductive medicine, the matter is "personalized" to not only one individual but in three different individuals; the mother, father, and embryos. Another complication of ART is that we have to apply the personalization in different biological systems including; the egg, sperm, embryo, and uterus, as different systems may be involved in etiology of infertility in different subjects.

K-86
The genetics of premature ovarian failure: Current perspectives

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Premature ovarian failure (POF) is a common cause of infertility in women, characterised by amenorrhea, hypoestrogenism, and elevated gonadotropin levels in women under the age of 40. Many genes have been identified over the past few years that contribute to the development of POF. However, few genes have been identified that can explain a substantial proportion of cases of POF. The unbiased approaches of genome wide association studies and next generation sequencing technologies have identified several novel genes implicated in POF. As only a small proportion of genes influencing idiopathic POF have been identified thus far, it remains to be determined how many genes and molecular pathways may influence idiopathic POF development. However, due to POF's diverse etiology and genetic heterogeneity, we expect to see the contribution of several new and novel molecular pathways that will greatly enhance our understanding of the regulation of ovarian function. Future genetic studies in large cohorts of well defined, unrelated, idiopathic POF patients will provide a great opportunity to identify the missing heritability of idiopathic POF. The identification of several causative genes may allow for early detection and would provide a better opportunity for early intervention, and furthermore, the identification of specific gene defects will help direct potential targets for future treatment.

K-87
Current state of art use of stem cells for regenerating tissues and possibility of using iPSCs to generate mature spermatozoa in the future

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Integrated research of stem cells and tissue engineering is essential to improve health issues in the field of regenerative medicine. Tissue engineering combines various fields such as biochemistry, cell biology, materials science, transplantation and hardware engineering in an effort to repair or replace damaged tissues. Stem cells are defined by their ability to self-renew and differentiate into a variety of cell types. Stem cells are divided into 3 groups depending on multilineage differentiation capacity (Totipotent, pluripotent and multipotent). To date, in contrast with tissue-engineered bone, cartilage, muscle, nerve, and skin, tissue engineering of other tissues and organs is much less advanced. This is caused by the fact that there is a limited choice of appropriate cells, biomaterials, chemical stimuli such as hormones and physical stimuli such as mechanical loading. Infertility affects 15% of men of reproductive age worldwide. Spermatogenesis is the proliferation and differentiation of spermatogonial stem cells. For spermatogonial stem cell therapy to be a success, and cultured sperm stem cells to become mature sperm, they need the proper stimuli such as the right microenvironment. However, until now these microenvironment conditions remain the object of speculation. Besides spermatogonial stem cells, the use of adult-derived induced pluripotent stem cells (iPSCs) might be a promising cell source to generate mature sperms. These cells, like embryonic stem cells, have the potential to form eggs and sperm. Although controversy surrounds their use, iPSCs have a huge potential for biological and therapeutic applications for male infertility, provided that in vitro spermatogenesis models for iPSCs can be established, thereby providing insights into the mechanism of human spermatogenesis and its regulation. The challenge remains that the molecular mechanisms underlying human male germ cell development remain poorly understood. But the iPSCs will have extra therapeutic implications for male infertility when combined with genome editing technology in near future.

Award Winners

A-1
Designing and implementation of mental health intervention for oocyte donor women

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Background: Oocyte donors face medical risks, socio-cultural challenges and the psychological consequences toward participating in assisted reproductive technology (ART). Developing a documentation-based intervention reduces the psychological burden from participation in this process.

Objective: The present study was conducted with the aim of designing and implementing a mental health intervention for oocyte donor women.

Materials and Methods: The present study was conducted by using mixed methods study in four stages. In the first stage, a qualitative study was conducted to explain the experiences of women who donate oocytes and providers of ART. In the second stage, the draft of mental health intervention plan for oocyte donors was designed based on qualitative study and review of literature, and a list of needs and strategies of the plan were provided to faculty members using a Delphi technique. Then the intervention was designed based on the strategies with the highest score. In the third stage, in order to review the content of the intervention, an expert panel was held and then approved by the panel members. The fourth stage of the study was a quantitative stage and was conducted as a two-group field trial with the aim of determining the effect of the intervention plan on the mental health of oocyte donors. The study sample size was 72 participants (36 participants in each group). Data collection tool was depression, anxiety and stress scale (DASS-21) questionnaire which was completed in two stages before superovulation induction and after oocyte retrieval. Also, the researched made questionnaire for measuring worry and satisfaction with participation in ART which was completed after oocyte retrieval and data analyzed using SPSS software version 19.

Results: The findings of the qualitative study led to the formation of 7 main categories including "decision challenge","donation complications","challenges of the oocyte donation process", "emotional experiences",“donor perspective versus recipient perspective","Needs and requests" and"structural defects"resulted. In designing the draft of the plan, the intervention was designed in the form of educational pamphlets, consultations and Instagram posts according to the strategies with the highest score. The results of the intervention showed before ovulation induction, the scores of DASS-21 in the intervention group were significantly lower than the control group. After oocyte retrieval, the scores of the DASS-21 and also, worry in the intervention group were significantly lower than the control group and the score of satisfaction with the donation process was significantly higher than the control group. The mean score of the depression and stress in the control group before ovulation induction and after oocyte retrieval were not statistically significant, but in the intervention group the mean score of the depression and stress after oocyte retrieval were less before ovulation induction ($p < 0.001$). After oocyte retrieval, the mean score of anxiety in the control group, was increased significantly ($p = 0.02$); while in the intervention group, there was decreased significantly($p < 0.001$).

Conclusion: The results of this study showed the designed intervention plan was effective on mental health in oocyte donors.Therefore,the implementation of the designed intervention in fertility centers could be useful in promoting the mental health of women who donate oocytes.

Key words: *Mental health, Oocyte donation, Mixed methods study.*

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A-2
Whole-exome sequencing in patients with primary ovarian insufficiency

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Background: Primary ovarian insufficiency (POI) is a complex and relatively poorly understood disorder. It affects 1% of women below the age of 40 accompanied by raised gonadotropins and estradiol deprivation. Women with POI have no antral follicles and size of ovaries are below normal.
Objective: Our objective was to identify the genetic cause of POI in affected members of a consanguineous Iranian family.
Materials and Methods: In this study, we recruited an Iranian family with 2 affected members to be studied by whole exome sequencing (WES). Validation of WES results and cosegregation analysis was performed by Sanger Sequencing. In silico analysis was used to predict the effect and pathogenicity level of the discovered variants.
Results: The proband and her affected sister tested normal for karyotype and *FMR1* CGG repeats. A final list of pathogenic variants was prepared according to WES results, and one specific variant in a conserved domain of transcription factor protein was confirmed to be mutual among the affected. The discovered variant is very rare in the gnomAD database.
Conclusion: This study supports the clinical applicability of WES for cost-effective molecular diagnosis and improves the understanding of the genetic basis of female infertility and ovarian function. Therefore, our finding provides yet another piece of evidence that Loss of function variations in transcription factors with limited expression in ovaries may be the cause of POI.
Key words: *Primary ovarian insufficiency, Familial exome sequencing, Causative mutation.*

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A-3
Does sperm DNA fragmentation have negative impact on embryo morphology and morphokinetics in IVF program?

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Background: Evaluation of sperm DNA integrity may predict the in vitro fertilization (IVF) outcomes.
Objective: The aim was to evaluate the relationship between the sperm DNA fragmentation (sDNAf) with embryo morphology and morphokinetic using time-laps monitoring and to select the best time points for normalization in IVF setting.
Materials and Methods: After evaluating the fertilization and pronuclei scoring, 328 normally fertilized oocytes were assessed to time of pronuclei fading (tPNf), time of 2 to 8 discrete cells (t2-t8) and abnormal cleavage patterns, such as multinucleation, direct cleavage, reverse cleavage and fragmentation. Sperm chromatin dispersion (SCD) assay was used for assessment of prepared sperm chromatin status. SCD was categorized into 4 groups of < 6.5, 6.5-10.7, 10.7-20.1 and > 20.1.
Results: Significant differences were found in t6 ($p = 0.012$), t7 ($p = 0.045$(, t8 ($p = 0.013$) and s1 ($p = 0.001$) between 4 SCD groups. When, morphokinetic variables were normalized to tPNf, this difference was observed in t2 ($p = 0.003$) and t6 ($p = 0.017$). Subsequently, the percentage of top quality embryos and Z1 scoring were dependent to the sDNAf rate.
Conclusion: In conclusion, tPNf was the best reference time point in IVF cycles. Also, we found high sDNAf rate had no negative impact on embryo morphology and morphokinetics in conventional IVF.
Key words: *In vitro fertilization, Embryonic development, Time-laps monitoring.*

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A-4
Gangliogenesis with folliculogenesis of ovary: Three-dimensional and two-dimensional analyses of Golgi-Cox-staining in mouse ovary

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Background: The ovarian follicular development of rodents begins at neonatal period, the stage at which primordial follicles are formed. During estrous cycle,

most of the follicles undergoes atresia and some of them continue their development process. The mammalian's ovary is regulated by some factors including hormonal factors and direct neuron effects. Previous studies have shown that the fate of follicles in this cycle are affected by hormones such as follicle-stimulating hormone and luteinizing hormone. In addition, there are two different populations of neurons in ovary, the internal and external neurons. External nervous system of mouse ovary has many roles. Several studies have shown its role in developmental process, cyclic stages, pregnancy, and aging process. These nerves and also ganglia are responsible for ovarian estradiol secretion. Some studies implied that the ganglia in ovary takes part in some functions such as hormone secretion but to best of our knowledge, their relationship with follicular and ovarian development have not fully understood.

Objective: The present study was set out to investigate two-dimensional (2D) and three-dimensional (3D) evaluations of ovarian nervous network development and the structural relationship between folliculogenesis and gangliogenesis in mouse ovary.

Materials and Methods: Adult mice ovarian tissue samples were collected from diestrus and estrus stages. In details, firstly, the cardiac perfusion was performed. The collected ovarian samples were stained by a Golgi-Cox protocol. Following staining, tissues were serially sectioned with thickness of 30 μm for each section for imaging and further analysis. Ovarian tissue serial images were evaluated with Image J software for 2D analysis and with Imaris software for 3D analysis. The images of estrus and diestrus ovaries were separately compared. In addition, the 2D and 3D data of estrus ovary were comparably analyzed. IBM SPSS Statistics 26 software was used for statistical analysis. The mean differences between follicular groups were analyzed by one-way ANOVA and post hoc Tukey test.

Results: Neural filaments and ganglia were detected in the ovaries by Golgi-Cox staining. In both 2D and 3D studies, an increase in the number and area of ganglia was seen during the follicular growth ($p < 0.05$). The same pattern was also seen in corpora lutea development. However, in some cases such as ratio of ganglia number to follicle area, the ratio of ganglia area to follicular area, 2D findings were different compared with the 3D results. 3D analysis of ovarian gangliogenesis showed the possible direct effect of them on folliculogenesis. Golgi-Cox staining was used in this study for 3D evaluation in non-brain tissue. The results of 3D analysis of the present study showed that, in some cases, the information provided by 2D analysis does not match the reality of ovarian neuronal function. This confirmed the importance of 3D analysis for evaluation of ovarian function.

Conclusion: It was demonstrated that there was positive relationship between gangliogenesis and folliculogenesis in mouse ovary. Ovarian ganglia, as an independent part of ovarian nervous system, is likely to have an important role in folliculogenesis and luteogenesis. Additionally, Golgi-Cox staining and 3D tissue imaging, instead of 2D imaging, are promising protocols for study of ganglia in ovarian tissue.

Key words: *3D Imaging, Ganglia, Ovarian follicle, Golgi-Cox staining, Mice.*

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A-5

Sperm telomere length in infertile men with previous failed fertilization post- ICSI

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Background: Intracytoplasmic sperm injection (ICSI) technique is used mostly for the treatment of male infertility. However, failed fertilization was observed in a litter percentage of infertile couples post- ICSI. Several factors such as abnormal sperm quality, DNA fragmentation, low or absence of sperm factors involved in oocyte a3ctivation are associated with failed fertilization post- ICSI. Sperm telomere length is one of the sperm factor at the end of the eukaryotic chromosomes that protects chromosomes from damage and positive significant correlation was observed between this parameter with sperm DNA fragmentation.

Objective: In the light of these considerations, we aimed to assess sperm telomere length and DNA fragmentation in infertile men with previous failed fertilization post-ICSI.

Materials and Methods: In this study, semen and blood sample were obtained from 10 infertile men with a failure in ICSI fertilization and 10 fertile as a control group. Telomere length was evaluated both in sperm and blood samples by Realtime-PCR. Finally, Independent *t* test, and the correlation coefficient were used for analysis of data.

Results: Mean of sperm and blood telomere length were significantly shorter in infertile with previous failed fertilization compared to fertile men ($p < 0.05$). Moreover, in infertile men, percentage of sperm DNA fragmentation was significantly higher than fertile men ($p = 0.01$). In addition, we observed a significant correlation between sperm telomere length with fertilization rate ($p < 0.05$).

Conclusion: In this study, for the first time, we showed in infertile men with previous failed fertilization, sperm telomere length was low. Therefore, the reduction of sperm telomere length as one of sperm factors could associated with low fertilization potential.

Key words: *Sperm telomere, DNA fragmentation, ICSI, Failed fertilization.*

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- *Darmishonnejad Z, Tavalaee M, Izadi T, Tanhaei S, Nasr-Esfahani MH. Evaluation of sperm telomere length in infertile men with failed/low fertilization after intracytoplasmic sperm injection. Reproductive Biomedicine Online 2019; 38(4): 579-587.*
- *Darmishonnejad Z, Zarei-Kheirabadi F, Tavalaee M, Zarei-Kheirabadi M, Zohrabi D, Nasr-Esfahani MH. Relationship between sperm telomere length and sperm quality in infertile men. Andrologia 2020; 52(5): e13546.*

A-6

Follicular reconstruction in artificial ovary made by human isolated ovarian cells from chemotherapy-induced POF patient seeded into human ovarian decellularized ECM after xenotransplantation

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Background: Depletion of ovarian reserve due to dismiss of follicular growth in chemotherapy induced premature ovarian failure (Chemo- POF) is the main concern for oncofertility researchers who try to find a practical way to restore the ovarian function.

Objective: Artificial ovary is preparing a new niche for ovarian cells and reconstruction of follicular activity may be developed to aid infertility treatment by transplantation of engineered ovary.

Materials and Methods: Ovarian tissues were taken from 8 Chemo- POF women and 15 transsexuals. The medulla was carefully removed and the cortical tissue was cut into 5×5×5 mm3 strips and then cryopreserved. Ovarian cells were removed with NaOH as main detergent from human ovarian pieces of transsexual patients and then decellularized cortical tissue (DCT) was evaluated by DNA content analysis, hematoxylin & eosin and DAPI staining techniques. Human ovarian cortical cells (HOCCS) were finely minced and enzymatically isolated and characterized by real time PCR for IFITM3, vimentin, *FSH-R* and *KI67* genes and immunostaining of vimentin, Inhibin-α and IFITM3 markers. Then the isolated HOCCS (2×10^6 cells) from both Chemo- POF and transsexual ovaries were seeded into DCT by injection and spinner flask culturing for one wk (AO; artificial ovary). Also, MTT assay was performed to measure the in vitro cytocompatibility of ovarian scaffold. Then AO was xenotransplanted to NMRI mice beneath the abdominal sub-serosal fascia for two months. Finally, H&E, hormonal tests (FSH, AMH and E2), real time PCR (GDF-9, ZP3, VEGF, CD34 and KI67) and immunohistochemistry (IHC) (GDF-9) assessments were applied for the calculation of transplantation outcomes.

Results: H&E, DAPI and DNA content confirmed over 95% decellularization. Immunofluorescence showed that isolated HOCCS from transsexual and Chemo- POF ovarian tissues included 80-85% stromal, 5-10% granulosa and < 5% oogonial stem cells by expressing the vimentin, Inhibin-α and IFITM3 markers in passage one. Expression patterns of the mentioned proteins in passage two were appraised 70-75%, 5-10% and > 10%, respectively. Also, HOCCS well expressed Vimentin, *FSH-R*, *FRAGILIS*, *DDX4*, *STELLA* and *KI67* genes in real time PCR technique. One wk culture of AO in spinner flask indicated the HOCCs could penetrate not only into the exterior surfaces also to the depth of the ovarian scaffold (H&E). Histological study and quantitative evaluation of Estradiol, FSH AND AMH production after two months of AO xenotransplantation confirmed the presence of morphologically health and secretory active reconstructed human ovarian primordial and primary follicles. IHC for GDF9 confirmed the paracrine activity of oocytes within the follicles. To approve existing active follicles within AO, the real time PCR demonstrated a good expression of the follicle-related genes like GDF9 and ZP3 in both groups. Furthermore, the angiogenesis genes VEGF and CD34, in both transplanted groups showed high expression. At the end, the expression of kI67, cell proliferation and survival factor, approved the cellular multiplication and health in AO of the both groups.

Conclusion: Our results approved that ovarian follicular reconstruction and function is possible in the case of ovarian insufficiency through xenotransplantation of AO made by DCT seeded by Chemo-POF ovarian isolated cells.

Key words: *Human artificial ovary, Ovarian follicular reconstruction, Oogonial stem cells, Spinner flask.*

A-7

Four hours or more preincubated oocytes in the simple medium provide low transcript levels of maternal effect genes for the embryos

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Background: Preincubation is the temporary cultivation of oocytes at 37°C and 5-6% of CO2 before ART procedures. There is not any explanation regarding

a standard preincubation time in ART laboratory guidelines and it is dependent completely on the laboratory workload. Myo-inositol as the most important form of inositol, is involved in several systemic processes and its antioxidant action has been suggested recently. The study aimed to evaluate the effect of oocyte preincubation time and also myo-inositol as a medium supplement on the oocyte Zar1, Nlrp5, Npm2 transcript levels as well as the fertilization and first cleavage rates.

Objective: The study aimed to evaluate the effect of oocyte preincubation time and also myo-inositol as a medium supplement on the oocyte Zar1, Nlrp5, Npm2 transcript levels as well as the fertilization and first cleavage rates.

Materials and Methods: Cumulus Oocyte Complexes which were retrieved from superovulated NMRI mices were 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 were denuded and transcript levels of Zar1, Nlrp5 and Npm2, selected by bioinformatics, were assessed by real time qPCR method. 2PN and 2-cell rates were analyzed following oocytes and sperms co-incubation. One-way ANOVA and Kruskal-Wallis were respectively used for parametric and nonparametric variables. Statistical significance was defined as P-value ≤ 0.05.

Results: Zar1 (1-fold vs 0.4-fold) and Npm2 (1-fold vs 0.2-fold) transcript levels, as well as 2PN (84.64 ± 4.02 vs 78.90 ± 1.11) and 2-cell rates (79.58 ± 1.45 vs 59.85 ± 9.44) were reduced after 4 h of preincubation time in the simple medium compared to the control group. While Nlrp5 transcript level (1-fold vs 0.07-fold) was significantly decreased following 8 h of preincubation time in the simple medium ($p < 0.001$). Addition of myo-inositol to the culture medium could ameliorate maternal effect genes levels and fertilization and first cleavage rates in the oocytes preincubated for 4 and 8 hours ($p < 0.001$).

Conclusion: However it has not found a clear boundary between optimal and non-optimal oocyte preincubation time, our findings addressed that 4 h or more preincubation time can influence the oocyte mRNA storage and ultimately leads to reduce oocyte fertilization and first cleavage rates. Besides, medium supplementation with myo-inositol could preserve the mRNAs inherited to the embryos and consequently improve fertilization and first cleavage developmental rates.

Key words: *Oocyte preincubation time, Maternal effect genes, Fertilization potential, First cleavage rate, Myo-inositol supplement.*

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A-8

Melatonin prevents wistar rats testes from bleomycin, etoposide, and cisplatin (BEP) chemotherapy-induced reproductive toxicity: A biochemical, immunohistochemical and apoptosis-related genes based evidence

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Background: Recently, the prevalence of testicular cancer, accounting for the most common cancer among young people of reproductive age (15-40 yr), has risen internationally. Bleomycin, Etoposide, and Cisplatin (BEP) chemotherapy has increased the 5-year survival rate of patients with testicular cancer at all stages of testicular germ cell tumors to 90-95%. However, nowadays there is growing concern that some cytotoxic regimens for cancer like BEP create a high incidence of male infertility and even long-term genotoxic effects, which emerge as a critical health issue. Melatonin is a well-known potent antioxidant with widespread clinical applications that recently has been giving increasing attention to its role in male sub/infertility.

Objective: In order to investigate the protective and alleviative effects of melatonin following BEPchemotherapy exposure on sperm characteristics and parameters, nitro-oxidative status, as well as histopathological, inflammatory, and apoptotic alternations in testes. Moreover, to elucidate whether exogenous melatonin attenuates BEP-induced damage in testicular cells and spermatogenesis in a dose-dependent manner?

Materials and Methods: 60 adult male Wistar rats (n = 10/group) were treated with one cycle of 21 days of 0.33 therapeutically relevant dose levels of BEP (0.5 mg/kg Bleomycin, 5 mg/kg Etoposide and 1 mg/kg Cisplatin) with or without melatonin. At the end of the study (day 35), body weight, testes weight, sperm parameters, testosterone hormone level, testicular histopathology, stereological parameters, testicular level of malondialdehyde, nitric oxide and total antioxidant capacity, the expression of apoptosis-associated genes

such as Bcl2, Bax, Caspase3, p53 (Real-time PCR and immunohistochemistry), and the expression of pro-inflammatory cytokine TNF-α (immunohistochemistry) were evaluated.

Results: Our findings showed that melatonin restores the BEP-induced reduction in the body and testes weight ($p < 0.05$). The evaluation of quantitative analysis of the testes stereological procedures, QRT-PCR examination, and immunohistochemical staining revealed that melatonin reversed the BEP-induced impaired spermatogenesis ($p < 0.05$). Furthermore, melatonin rectified BEP-induced disturbance on sperm count, motility, viability, and morphology. Additionally, co-administration of 10 and 20 mg/kg of melatonin could restore BEP-induced alteration in serum testosterone level. Moreover, melatonin enhanced the antioxidant status of the testis by elevating total antioxidant capacity and ameliorating malondialdehyde and nitric oxide levels. More notably, QRT-PCR examination indicated that melatonin therapy suppressed BEP-induced apoptosis by modulating apoptosis-associated genes such as Bcl-2, Bax, Caspase-3, p53 in the testis ($p < 0.01$). In this continuum, the co-administration of 10 and 20 mg/kg of melatonin with the BEP regimen decreased significantly the population of p53 and TNF-α positive cells by comparison to the BEP group. Also, melatonin with low and high doses could enhance the expression of Bcl-2 protein in spermatogenic cells line compared to the BEP-treated group.

Conclusion: This study demonstrated that melatonin protected testes against BEP-induced damage by preventing and ameliorating histopathological and stereological alterations, spermatotoxicity, nitro-oxidative stress, inflammation, and apoptosis. These findings can draw attention to the clinical application of melatonin and also suggest that melatonin may be an attractive agent for attenuating chemotherapy-associated male sub/infertility. This indolamine may also shorten the fertility recovery period in patients undergoing chemotherapy with the BEP regimen.

Key words: *BEP chemotherapy, Melatonin, Sperm, Male infertility, Apoptosis.*

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A-9

Suppression of transforming growth factor-beta signaling enhances spermatogonial proliferation and spermatogenesis recovery following chemotherapy

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Background: Spermatogonial stem cells hold great promise for fertility preservation in prepubertal boys diagnosed with cancer. However, the low number of Spermatogonial stem cells limits their clinical applications. Could small molecules (SM) are chemically synthesized molecules that diffuse across the cell membrane to specifically target proteins involved in signaling pathways, and studies have reported their ability to increase the proliferation or differentiation of germ cells.

Objective: SM which target (or modify) signaling pathways lead to increased proliferation of undifferentiated spermatogonia following chemotherapy?

Materials and Methods: In our experimental study, spermatogonia were collected from four brain-dead individuals and used for SM screening in vitro. For in vivo assessments, busulfan-treated mice were treated with the selected SM (or vehicle, the control) and assayed after 2 (three mice per group) and 5 weeks (two mice per group). We investigated the effect of six SM on the proliferation of human undifferentiated spermatogonia in vitro using a top-bottom approach for screening. We used histological, hormonal and gene-expression analyses to assess the effect of selected SM on mouse spermatogenesis. All experiments were performed at least in triplicate and were statistically evaluated by Student's t-test and/or one-way ANOVA followed by Scheffe's or Tukey's post-hoc.

Results: We found that administration of SB431542, as a specific inhibitor of the TGFb1 receptor (TGFbR1), leads to a two-fold increase in mouse and human undifferentiated spermatogonia proliferation. Furthermore, injection of SB to busulfan-treated mice accelerated spermatogenesis recovery as revealed by increased testicular size, weight and serum level of inhibin B. Moreover, SB administration accelerated both the onset and completion of spermatogenesis. We demonstrated that SB promotes proliferation in testicular tissue by regulating the cyclin-dependent kinase inhibitors 4Ebp1 and P57 (proliferation inhibitor genes) and up-regulating Cdc25a and Cdk4 (cell cycle promoting genes).

Conclusion: This is the first study to report acceleration of spermatogenesis recovery following chemotherapy by administration of a single SM. Our findings suggest that SB is a promising SM and should be assessed in

future clinical trials for preservation of fertility in men diagnosed with cancer or in certain infertility cases (e.g. oligospermia).

Key words: *Spermatogonial stem cells, Small molecules, Fertility preservation.*

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A-10
Mimicking the ovarian extracellular matrix, the role of natural polymrrs

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Background: Female infertility treatment has been entered into a new world, constructed by Regenerative Medicine, which tries to get mature female gamete by help of the Tissue Engineering. So, ovarian follicles growth, ovarian in vitro activation, and ovarian follicle & tissue culture are the processes that can develop under the progress of tissue engineering. Development of ovarian follicles depends on endocrine and paracrine signals, the follicles micro-environment and 3-dimensional architecture. So, mimicking the ovarian extra cellular matrix by ovarian tissue engineering is a possible approach in fertility treatment for patients with premature ovarian failure and onco-fertility patients who cryopreserved the ovarian cortical tissue.

Objective: The current study aimed to assemble the electrospinning scaffolds by natural polymers, for comparison with collagen, as the natural ovarian tissue polymer.

Materials and Methods: After Ethical Committee permission, a full thickness section of human ovary from surface to hilum, was prepared. After chopping and enzymatic digestion by collagenase, the isolated cells were cultured. Besides, the electrospinning scaffolds were fabricated, using the natural polymers including agarose, human serum albumin, chitosan, collagen, silk fibroin, gelatin and a synthetic polymer (poly lactic acid (PLA)). Electrospun blended scaffolds of natural polymers with PLA in ratio of 30/70; 50/50 and 70/30 were prepared. Chemical properties of manufactured scaffolds were characterized by Fourier-Transform Infrared Spectroscopy analysis. Also, water contact angle was measured to quantify the surface wettability of the prepared scaffolds. Scanning electron microscope images analyses were performed before and after cell culture and the porosity and the average of fiber diameter distribution was calculated by ImageJ software. Cytotoxicity was evaluated by MTT assay after 14 days cell culture. Also, cell morphology and growth pattern was followed by hematoxylin and eosin staining.

Results: The blend of all natural polymers with PLA led to the fiber formation in electrospinning process, except for chitosan 70% + PLA 30%. Also, electrospinning for all the polymers separately led to fiber formation except chitosan and albumin. Therefore, 21 variables of electrospun scaffolds were assembled and Fourier-Transform Infrared Spectroscopy results confirmed the polymer accuracy. The results of fiber diameter diversity showed that the thickest fibers were related to the blended electrospun scaffold (50% agarose + 50% PLA) followed by (70% collagen + 30% PLA) ($\geq$ 200 nm). The other scaffolds fibers were below $\leq$ 150 nm. Compared to dish culture plate, MTT assay test after 4, 8 and 14 days cell culture on the scaffolds showed that the blends of (70% gelatin + 30% PLA) followed by (70% collagen + 30% PLA) led to the highest cell proliferation and the lowest cells toxicity, respectively.

Conclusion: Gelatin can be replaced by collagen, as the native extra cellular matrix of the ovary. Our results showed that gelatin, as an accessible natural polymer provided higher cell proliferation and lower fiber diameter than collagen. It is more accessible and cost effective with lower cell toxicity which makes it an optimized polymer for ovarian tissue engineering instead of collagen.

Key words: *Artificial ovary, Extracellular matrix, Natural polymers, Scaffolds, Poly lactic acid.*

A-11
Transcriptomic alternation of chemokines secreted from fallopian tube epithelial cells in response to spermatozoa

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Background: Immunological response of female reproductive system to the allogenic spermatozoa is very essential for a successful fertilization and pregnancy. Fallopian tubes are the focus of this investigation since they are places in which spermatozoa are stored for a period until oocytes are ready for fertilization. Chemokines have been shown to play important roles in reproductive immunology by their chemo-attraction potential and inflammatory function. Previous studies also showed that specific chemokines can increase the survival chance of spermatozoa by modulating female immune system.
Objective: For a better understanding of how chemokines contribute with the maternal immune system in the presence of spermatozoa, we evaluated transcriptional changes of different chemokines from fallopian tube cell line in the presence of spermatozoa by PCR-array.
Materials and Methods: Semen samples were collected from 10 healthy donors who had at least one child. Samples were analyzed according to the WHO standard. To investigate the impact of spermatozoa on chemokines' expression from epithelial cells, the fallopian tube cell line (OE-E6/E7) was co-cultured with the spermatozoa for 24 hr. The cell line without any spermatozoon was analyzed as the control group. After the co-incubation period, RNA extraction was done from washed cells. cDNA was synthetized and chemokines' expression were evaluated by PCR-array. Finally, Independent sample *t* test was applied to compare differences between the groups. Furthermore, IL-8 which had the most expression compared to other chemokines is evaluated in the culture medium by ELISA.
Results: Data analysis indicated that the spermatozoa resulted in down regulation of chemokines. It has been shown that CX3CL1 which involves in T cell migration, and inflammatory chemokines such as IL-8, CXCL9 and CXCL13 were significantly decreased in the presence of the spermatozoa. IL-8 concentration in the case group was also lower than the control. Furthermore, CCL chemokines with the role in migration of inflammatory cells to the target tissue were significantly down regulated. These chemokines alternation can cause higher survival chance of the spermatozoa by preparing an anti-inflammatory condition in the fallopian tubes.
Conclusion: Compatible with previous studies our results have shown that spermatozoa can adapt to the immune system of female reproductive tract by regulating specific chemokines expression. Chemokines are appeared to be essential for preparing a safe microenvironment in the fallopian tubes for the spermatozoa.

Key words: *Chemokine, Fallopian tube, Spermatozoa, Fertilization, PCR array.*

A-12

The study of embryonic causes of recurrent pregnancy loss in couples with consanguineous marriage and normal karyotype with a specific approach to determine the importance of single genes

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Background: Pregnancy loss is a significant health concern, especially in developing countries. Nearly 3-5% of couples trying to have children experience recurrent pregnancy loss (RPL). Unfortunately, advancing maternal age is highly associated with miscarriage while the available reproductive years are shortened, therefore determining why miscarriage occurs and how to prevent further miscarriages has become a major clinical and research focus. It has been estimated that the cause remains unexplained in more than 50% of cases, which strongly suggests that genetic factors may contribute towards the phenotype. This might be considered the fact that hundreds of genes and several critical pathways are involved in each physiological step necessary for guaranteeing reproductive success.
Objective: Mendelian forms of embryonic lethality offer a window into the essential genetic components of early embryonic development in humans. The study hypothesis was that exome sequencing can identify genetic causes of idiopathic recurrent pregnancy loss (RPL), which will further our understanding of human development at a molecular level.
Materials and Methods: This study involved 10 consanguineous couples having suffered at least 3 consecutive embryonic losses. Patients having risk factors such as abnormal karyotype, infectious disease during pregnancy, metabolic, autoimmune, endocrine disease, and uterine anomalies were excluded from the study. DNA was extracted from the tissue sample of ten aborted fetuses (probands) from ten different families with a history of idiopathic recurrent pregnancy loss. Parental peripheral blood samples were collected for confirmatory analysis and follow-up testing. Whole exome sequencing (WES) was performed using illumine HiSeq 2000 platform. Cytoscape 3.7.2 and BINGO were

used for pathway and biological enrichment analysis of putatively pathogenic variants in miscarriages. All variants identified by exome sequencing were verified by Sanger sequencing in all parents.

Results: We were able to identify 32 variants (7 pathogenic, 9 likely pathogenic, and 16 VOUS) of which three genes are already known to be involved in lethal recessive disorders. These genes were compatible with the clinical phenotypes. In cases 1, 2,4 pathogenic variants were identified in know genes (CHRNG, RYR1) responsible for disorders with a clinical description that correlated with the phenotypic description. Of fetuses, in case 3,8,6 novel variants were identified in MYH3, ERBB3, FRAS1 responsible for muscular disorders and Fraser syndrome. Bioinformatics analysis of genes with mutation showed enrichment in biological processes of importance for embryonic development e.g. Fibrin clot formation complement, coagulation cascades, and Striated muscle contraction/muscle contraction, actin-myosin filament sliding. Variants with potential diagnostic value were reported to the patients referring physicians for genetic counseling and further diagnostic and reproductive action.

Conclusion: Next-generation sequencing (NGS) has been reported as being a useful tool for identifying variants in genes related to rare disorders leading to RPL. It has also helped to identify variants related to fetal molecular pathways in pregnancies having unexplained embryonic lethality or unexplained fetal malformations. This approach thus helps, genetic counseling regarding lethal fetal disorders of high-risk families and preimplantation genetic diagnosis. Future efforts should be directed towards increasing the number of sequencing families with RPL.

Key words: *Multiple pterygium syndromes, Whole-exome sequencing, Recurrent pregnancy loss, Thrombophilia.*

A-13
Design and fabrication of a microfluidics device for sperm sorting application

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Background: In vitro sperm selection has been of great interest in order to optimize the selection process based on biological parameters of sperm. On the other hand, microfluidics has recently cast light on how deep human being can sort and manipulate cells and fluids, respectively. Hence, using microfluidic technology to design and fabricate a bio-chip for sperm selection seems to be a promising bet. The insane biocompatibility of the microfluidic polymers as well as a wide customizability enriches us with a variety of design and mechanism selections.

Objective: Hereby, we discuss the design, optimization, fabrication of a microfluidic biochip based on rheotaxical capability of sperms, as well as their attraction to electrostatic charge. Our chip will enable us to have a controlled flow of motile sperms attracted to electrostatic charge and guiding the cells towards groups of selection.

Materials and Methods: The mold for the chip was in Silicon and fabricated using e-beam layer deposition, photolithography and reactive ion etching. Then, polydimethylsiloxane fabricated chips were made and patched by plasma to glass to build up the chip. The system is then connected to a syringe pump and monitored by an inverted microscope.

Results: Our chip, showed high biocompatibility and was able to selects a sub population of vital sperms with a greatly higher motility profile. We also provided evidence, that electrostatic charge has the capability to stimulate the sperm cells towards target. The suggested design provides great quality sperm selection in a quicker time manner with high throughput, besides being feasible in costs.

Conclusion: Based on the results, the fabricated chip showed promise to be used in future medical applications, after taking necessary tests.

Key words: *Sperm, Sorting, Microfluidics.*

A-14
Cumulus cells conditioned medium facilitates germ cell development from human embryonic stem cells

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Background: Human embryonic stem cells (hESCs) can differentiate to germ cells as confirmed using gene expression assessments. Cumulus cells are physically closest cells to the developing oocyte and they have positive effects on oocytes maturation by secreting some factors that have a crucial role in the process of oogenesis. Thus, in vitro differentiation of embryonic stem cells may also be affected of cumulus-secreted factors.

Objective: The aim of this study is assessment the effect of the cumulus cells conditioned medium (CCCM) on differentiation of hESCs to female germ cells.

Materials and Methods: Embryoid bodies (EBs) from Yazd4-hESCs were formed and cultured for 14 days into 4 different conditions: 1) spontaneously

differentiation in EB medium (SD-EB), 2) treated with 40% CCCM (CCCM-EB), 3) spontaneously differentiation in 40% DMEM+20% FBS (SD-DM), and 4) treated with 40% CCCM in DMEM+20% FBS (CCCM-DM). Expression of pluripotency and germ cells genes were examined in EBs from each group by RT-qPCR at the time of days 0, 4, 7 and 14. In addition, immunofluorescent (IF) staining was done for pluripotency and germ cells markers.

Results: RT-qPCR data revealed that CCCM-EB and CCCM-DM had a significant increase in differentiation of female germ cell from hESCs than SD-EB and SD-DM. On the other hand, comparison between basal media revealed that EB medium is a better medium than DMEM+20% FBS for female germ cell development from hESCs. Localization of the germ cells within the cultures was detected using IF for TRA-2-49, SSEA1 and VASA antibodies in all groups.

Conclusion: Cumulus cells conditioned medium supports female germ cell development from hESCs assessed by gene expression profile. Also, EB medium as basal medium has better impact on differentiation induction.

Key words: *Cumulus cells, Conditioned medium, In vitro gametogenesis, Human embryonic stem cells, Female germ cells.*

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Oral Presentations

9th Yazd International Congress and Student Award on Reproductive Medicine

O-1

The relationship between coronavirus disease 2019 in pregnancy with maternal and fetal outcomes: An analytical cohort study

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Background: Coronavirus disease 2019 (COVID-19) is a type of pneumonia, which is rapidly increased reports of death and confirmed complications. Limited data were available about COVID-19 during pregnancy.

Objective: To assess the relationship between epidemiological and clinical features of coronavirus disease 2019 in pregnancy with maternal and fetal outcomes.

Materials and Methods: This analytical and retrospective cohort study, conducted on all pregnant women who confirmed cases of COVID-19 in Nekouei-Hedayati-Forghani Hospital in Qom, from February 1, 2019, to September 15, 2020. All epidemiological and clinical features collected from pregnant women's medical records. A logistic regression model used to determine covid-19 in pregnancy associated with maternal and neonatal outcomes.

Results: The most common symptoms reported by pregnant women with COVID-19 were shortness of breath 60%, dry cough 59% and fever 42%. After adjusting adjusted by the potential confounding factors, COVID-19 in pregnancy was associated with a significantly higher risk of admission to the intensive care unit (OR = 6.16, 95% CI = 1.23-31), cesarean section (OR = 0.45, 95 CI = 0.25-1.03); preterm birth (OR = 3.01, 95% CI = 1.4-6.54), fetal distress (OR = 5.7, 95% CI = 2.13-15.59), and the neonatal intensive care unit admissions(OR = 3.04, 95% CI = 1.21-7.70).

Conclusion: The results show that COVID-19 associated with adverse maternal and fetal outcomes such as admission to the Intensive care unit, cesarean section, fetal distress, preterm birth and neonatal intensive care unit admissions.

Key words: *Coronavirus pneumonia, Epidemiological characteristics, Maternal outcomes, Fetal outcomes, Retrospective study.*

O-2

Evaluation of the effects of human bone marrow mesenchymal stem cells conditioned medium on growth and maturation of mouse ovarian follicle after vitrification

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Background: In vitro culture of isolated follicles of cryopreserved ovaries can be proposed as a fertility preservation method. MSCs secreting various levels of growth factors and are an appropriate option for enriching the follicle culture media.

Objective: The purpose of this study was to evaluate the effect of human bone marrow mesenchymal stem cells derived conditioned medium (hBMSCs-CM) on growth and maturation of mouse ovarian follicle, and embryonic development after vitrification.

Materials and Methods: hBMSCs were cultured and the collected conditioned medium was concentrated and stored. The collected ovaries from Two-wk-old mice were divided randomly into vitrified and non-vitrified groups. Preantral follicles of both groups were isolated and cultured in α-MEM enriched with ITS and FBS supplemented with different concentrations of CM (2.5, 5, and 7.5%) for 12 days. During the culture period, survival rate, follicular maturation, follicular diameter, and levels of 17 βestradiol secretion were evaluated. In vitro fertilization and embryonic development were observed after culture.

Results: The survival rate, antrum formation, and oocyte maturation were higher in 7.5% CM subgroups than 2.5 and 5% CM in both vitrified and non-vitrified groups. Also, the follicle diameter in 7.5% CM was higher than other subgroups of both groups on day 4. Higher percentages of fertilization and embryo development were seen in 7.5% CM subgroups of the non-vitrified and vitrified group. Also, higher hormone secretion was observed in 7.5% CM subgroup in both vitrified and non-vitrified groups.

Conclusion: The present study suggests that the addition of 7.5% CM to mice ovarian preantral follicle culture media enhances follicle growth and oocyte maturation.

Key words: *Vitrification, Human bone marrow mesenchymal stem cells, Conditioned medium, Follicle.*

O-3

The effect of Diazinon on cell proliferation and apoptosis in testicular tissue of rats and the protective effect of vitamin E

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Background: Diazinon (DZN) is an organophosphate pesticide, and nowadays this pesticide is mostly used in agriculture. In this study, we analyzed the effects of DZN and vitamin E (Vit E) on apoptosis and the proliferation of germ cells in rat testis.
Objective: This study aimed to examine the effect of diazinon on apoptosis and the proliferation of germ cells in rat testis and the protective effect of vit E.
Materials and Methods: In this experimental study, 30 male Wistar rats were divided into five groups (n = 6 per group) consisting of control, sham (received olive oil), experimental group I (60 mg/kg DZN), experimental group II (60 mg/kg DZN and 200 mg/kg Vit E), and experimental group III (200 mg/kg Vit E). After six wks, left testis of rats was removed for the detection of proliferative cell nuclear antigen (PCNA) and terminal deoxynucleotidyl transferase end-labeling (TUNEL).
Results: Compared with the control group, DZN in the experimental group I decreased the number of PCNA positive cells and increased the number of TUNEL-positive cells ($p < 0.001$). Vit E improved detrimental changes by the decrease in the rate of apoptosis and the increase in the proliferation of testicular germ cells ($p < 0.001$).
Conclusion: Vit E can decrease the number of TUNEL-positive cells and increase the number of PCNA-positive cells by the neutralization of the toxicity caused by DZN in the testicular tissue.
Key words: *Apoptosis, Diazinon, Proliferation, Testis, Vitamin E.*

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O-4
The effect of platelet lysate on mouse ovarian tissue following auto- transplantation: A biochemical analysis

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Background: Platelet lysate (PL) has an increasing role in tissue engineering and regenerative medicine. During the early stages of ovarian transplantation, free radical production and inflammation can cause the loss of large numbers of immature follicles. PL as a condensed collection of platelet growth factors and cytokines, is obtained by lysing the platelet through temperature-shock and contains antioxidant and anti-inflammatory factors that are useful for improving ovarian graft survival.
Objective: We investigated the effect of intraperitoneal injection of PL on the transplanted mouse ovarian tissue.
Materials and Methods: 18 Naval Medical Research Institute (NMRI) mice (4-5 wk old) were divided into 3 groups (n = 6): Control, autograft and autograft + PL (5 ml/kg daily intrapritoneal injections). After 7 days, serum concentrations of total antioxidant capacity, malondialdehyde (MDA), tumor necrosis factor alpha (TNF-α), IL-6, and IL-10 were evaluated. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's test, differences were considered significant at $p < 0.05$.
Results: The serum concentrations of IL-6, TNF-α and MDA increased significantly in the autograft group compared to the control group whereas these parameters reduced significantly in the autograft + PL group. Total antioxidant capacity and the serum level of IL-10 also reduced significantly in the autograft group when compared to the control while it significantly increased in the autograft + PL group.
Conclusion: Our study provides the first evidence that treatment with PL induces protective responses through reducing oxidative stress and inflammation after transplantation of mouse ovarian tissue.
Key words: *Platelet lysate, Ovarian tissue, Transplantation, Oxidative stress, Inflammation.*

O-5
Genetics and transcriptome profile of cryopreserved human sperm associated antigens (SPAGs)

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Background: Human sperm associated antigens (SPAGs), formerly known as sperm membrane protein, are eighteen-type proteins that some types of them, have a momentous role in various biological functions especially fertility outcome. The molecular weight

range allotted to SPAG proteins is between 24-71 k Da. The role of 8 types of these SPAGs (SPAG 1, 2, 6, 8, 9, 12, 13, 15) has been confirmed in infertility. Thus, any damage in quoted SPAGs can lead to infertility. In spite of favorable aspect of cryopreserved sperm for assisted reproductive technology, detrimental impact of freezing on cells has been quoted in many studies. In this regard, cryopreservation has an unfavorable effect on sperm quality perhaps via perturbation of SPAGs expression.

Objective: This study aimed to appraise the impact of rapid freezing on the gene expression of SPAGs in normal human spermatozoa.

Materials and Methods: The semen samples were collected from 12 normospermic individuals. All twelve normo-ejaculated samples were prepared via density gradient centrifuge and thereupon, the aliquots of motile sperms were divided into two fresh and freeze groups. Afterwards, sperm samples were mixed (1:0.7) with spermfreeze® cryoprotectant for 10 minutes. Then the mixture was loaded into cryotube and freezed with rapid freezing procedure. After three days of freezing at -196°C, the specimen were thawed in tap water for 5 min and incubated for 2 hr at recovery time in a CO_2 incubator. RNA from sperm was extracted with TRIzol. After synthesis of *cDNA*, *SPAGs* gene expression analysis was performed using Real-time PCR.

Results: The results of statistical analysis showed a decrease in the gene expression of SPAG5, SPAG7, SPAG12 (SNU13/ NHP2L1) during rapid freezing procedure. The results are significant at the $p \leq 0.05$ level. No significant reduction in the expression level between fresh and freeze group was found in remained SPAGs.

Conclusion: The results pointed out that cryopreservation procedure could negatively affect gene expression of some SPAGs in human spermatozoa. Hence, alteration of SPAGs expression could offer new suggestions to evaluate probable molecular correlations between freezing and increased failure rate of in vitro fertilization and intracytoplasmic sperm injection.

Key words: *Antigen, Cryopreservation, Fertility, Human, Sperm.*

O-6

Production of recombinant human leukemia inhibitory factor protein and its immunologic and anti-fertility impacts as a contraceptive candidate vaccine in mice model

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Background: Contraceptive vaccines are one of the methods studied to prevent fertility in mammals. Various factors are involved in the establishment and maintenance of the pregnancy and can be targeted for antifertility vaccine design. The human leukemia inhibitory factor (hLIF) is considered as a cytokine of the interleukin-6 family. LIF is also involved in the embryo implantation process.

Objective: The production and functional competence of the LIF, as the immunocontraceptive vaccine in Balb/c mice, was investigated in this experimental study.

Materials and Methods: Recombinant hLIF (rhLIF) was generated in a variety of host-vectors system. The protein expression rate and functional activity of rhLIF were assessed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and tetrazolium reduction assay, respectively. The production and characterization of Rabbit polyclonal antibody (pAb) to rhLIF was performed applying enzyme-linked immunosorbent assay and western blot techniques. The Balb/c mice were classified into two study groups. In group 1, each mouse was intraperitoneally inoculated by purified rabbit anti-rhLIF in 3th day and day 4 following vaginal plaque observation; after sacrificing on day 7, the number of implantation sites was quantified. Mice in second group were subdivided into two vaccinated and controls groups The rhLIF protein as well as phosphate buffer saline was emulsified with Freund's adjuvant and injected into both vaccinated and control groups, respectively. The inhibitory rate of implantation was investigated in the uterine of mice. The secreted levels of interferon-γ and interleukin-4 were determined in cultured splenocyte of mice induced by rhLIF. Also, the mRNA levels of *immune responsive gene 1* (*IRG-1*), *cochlin* (*COCH*), *amphiregulin* (*Ar*), and heparin-binding EGF-like growth factor (*HB-EGF*) genes were evaluated. The inhibition of fertility after delivery, reversibility of immune response against rhLIF, and survival rate of mice were assessed.

Results: Our data showed that pET32b/hLIF and pColdI/hLIF vectors could successfully express rhLIF in all hosts. The produced rhLIF was functionally active and the produced anti-rhLIF pAb could specifically bind to commercial rhLIF and native LIF extracted from mouse uterus. Passive immunization outcomes indicated that anti-rhLIF antibody entirely inhibited the fertility potential in all vaccinated mice compared to controls. Active immunization of Balb/c mice with rhLIF led to the implantation and fertility reduction rate up to 80.49% and 75%, respectively. All mice produced a high amount of anti-rhLIF antibodies in both serums and vaginal fluids wash after 16 weeks; while, these antibodies were disappeared from vaginal fluid washes six months later. The findings of splenocyte stimulation with hLIF demonstrated a significant increased level of both cytokines in vaccinated mice compared to the controls. A significant decreased gene expression of *IRG-1*, *Ar*, and *HB-EGF* was observed in vaccinated group compared to control group; however, the mRNA

level of *COCH* gene showed no significant change.
Conclusion: rhLIF could inhibit pregnancy in a high rate of female mice. The immunization of female Balb/c mice with rhLIF prevented fertility and the gene expression associated with rhLIF. To investigate the side effects of this vaccine in a wide range, further studies are needed.
Key words: *Leukemia inhibitory factor, Contraception, Vaccine, Active immunization, Mice.*

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O-7
Vitamin C and E supplementation effects on secretory and molecular aspects of vascular endothelial growth factor derived from peritoneal fluids of patients with endometriosis

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Background: Endometriosis is an extremely heterogeneous disease and affects about 10% of the female population during their reproductive years. Recent studies showed that endometriosis is an angiogenesis-dependent disease. Peritoneal macrophages are a well-characterised source of vascular endothelial growth factor (VEGF).
Objective: The aim of this study was to determine the *VEGF* gene expression and production in peritoneal macrophages of patients with endometriosis under the effects of vitamins C and E in comparison with control.
Materials and Methods: The lab trial study carried out on 50 patients undergoing laparoscopy and peritoneal fluid samples were collected from them. We compared the *VEGF* gene expression and production in peritoneal macrophages among groups by using real-time polymerase chain reaction and enzyme-linked immunosorbent assay methods, respectively.
Results: Our results showed that gene expressions influenced by vitamin C increased in different concentrations and incubation times, except for the incubation time after 48 h. In the case of vitamin E, this was evident with the exception of vitamin E 50 μM after 24 h and vitamin E 100 μM after 48 h.
Conclusion: Our findings indicated that vitamin C and E in different concentrations and incubation times altered *VEGF* gene expression in the peritoneal macrophages but they had not affected on *VEGF* productions.
Key words: *Endometriosis, Vascular endothelial growth factor, Vitamin C, Vitamin E.*

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O-8
LncRNA XIST/ miR-132/ HMGA2 axis modulate Insulin Resistance in PCOS: A molecular signature for prediction

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Background: Polycystic ovary syndrome (PCOS) is a common heterogeneous endocrine disorder. Studies showed that insulin resistance (IR) appeared in only 60% women with PCOS and seems to be independent of obesity.
Objective: It was hypothesized that dysregulation of HMGA2/miR-132-3p/ lncRNA XIST axis may correlate with IR.
Materials and Methods: In this case-control study, four groups participated including 20 healthy controls, 30 having only PCOS, 20 only IR+ and 30 PCOS+/IR+. None of them suffered from any syndroms, no pregnancy and no history of hormonal therapy. Real-Time PCR, ELISA and chemilumenisce recruited to assess the level of studied factors.
Results: The 87% and 63% reduction in level of lncRNA XIST and HMGA2 observed in IR+, but interestingly, both showed significant increase more than 3.3 fold in groups with PCOS+. Conversely, miR-132 expression levels increased about 3.3 and 4.0 fold in groups of PCO+/IR+ and IR+, respectively. The expression of miR-132 in PCOS+ group was significantly reduced by 98% compared to the normal group. HMGA2 is post-trancrptionally targeted and controlled by miR-132, which can explain down-regulation HMGA2 in IR. In the other side HMGA2 function in cell proliferation and its over-expresion can justify in PCOS. Taken together, these results

introduced another molecular mechanism involved in onset of IR in PCOS. ROC curve analysis showed that HMGA2/miR-132/lnc-XIST have 100% sensitivity and specificity to predicate IR in PCOS patients.

Conclusion: lncRNA XIST/miR-132/HMGA2 axis can be candidated as a panel of differentiative signature to predict IR not only in women with PCOS, but also could be applicable even in healthy individuals.

Key words: *PCOS, IR, HMGA2, miR-132, lncRNA XIST.*

O-9

The correlation between varicocele and unfolded protein response occurred in ER stress

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Background: Excessive reactive oxygen species generation plays a crucial role in male infertility, especially varicocele. One of the most cardinal pathways that defend cells against this destructive situation is the unfolded protein response (the so-called UPR/ER stress response). The UPR/ER is triggered by aggregation of unfolded/misfolded proteins in the Endoplasmic Reticulum (ER) lumen, leading to detach ER chaperons from ER membrane including inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and the PKR-like endoplasmic reticulum kinase (PERK). In the face of stress conditions, BiP is detached from membrane sensors and the three mentioned proteins are transiently activated to modify cell survival signals. Eventually, should the stress condition prolong, apoptosis is prompted by specific inducers such as the Jun-kinase/caspase-3 pathway.

Objective: The assessment of UPR/ER pathways in a VCL-induced rat model to find out the plausible role of UPR/ER stress response in varicocele condition.

Materials and Methods: Varicocele induction was surgically performed on ten 8-wk-old adult male Wistar rats, as varicocele group, and ten rats were considered as a control-sham group. After conducting sperm function tests, the expression of BiP, Caspase-3, Bax, Bak, Bim, Bcl2, XBP1, and NRF2 using Real-time PCR, and expression of p-JNK, CHOP, and NRF2 using Western blot were assessed. The data between the two groups were compared with the Independent *t* test, and p-value lower than 0.05 was considered statistically significant between the two groups.

Results: To assess the activation of UPR/ER pathways in VCL testis, the BiP/GRP78/HSAP5 protein level was evaluated, and no difference in the expression of BiP in VCL testis tissue compared with control group was indicated. By prolonging UPR response, IRE1 pathway induces apoptosis by activation of ER-associated protein degradation pathway (ERAD), which is accomplished by XBP1s, and stimulation of JNK/p-JNK pathway by downregulation of Bcl2 and upregulation of Bax and Bak, leading to activation of Caspase-3. Increased level of XBP1s mRNA, phospho-JNK ($p = 0.04$) and caspase-3 transcript (4.84 ± 0.64 versus 1.14 ± 0.14, $p = 0:03$) in the VCL testis tissue, was a sign of activation of the JNK pathway.

Conclusion: Ample evidence has shown that in the UPR/ER stress response, the first pathway to be activated is PERK, then ATF6, and finally IRE1. As CHOP and NRF2 protein content were no higher in VCL testicular extracts compared to control testis, it is clear that late apoptosis pathway, PERK/ATF4/NRF2/CHOP, has not activated. Activation of the p-JNK-induced Caspase-3 apoptotic signal is also suggested that we are in the late stages of the UPR/ER stress response. The UPR/ER response is certainly activated in the VCL testis by activation of the IRE1/JNK pathway.

Key words: *Varicocele, Endoplasmic reticulum stress, Unfolded protein response, ROS.*

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O-10

A detailed study in adenomyosis and endometriosis; evaluation of the rate of coexistence between uterine adenomyosis and DIE according to imaging and histopathology findings

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Background: Endometriosis and adenomayosis are common gynecological disorders and in this work we want to evaluate the co-existence of these two diseases.
Objective: The current study was designed to evaluate the relationship between adenomyosis and its subtypes with endometriotic lesions ovarian endometrioma (OMAs) along with the posterior deep infiltrative endometriosis (DIE). We also examined the the accuracy, sensitivity, and specificity of both transvaginal sonography (TVS) and magnetic resonance imaging (MRI) in diagnosis of adenomyotic uterus.
Materials and Methods: In this retrospective cross-sectional study, we selected 154 women with coexistence of endometriosis and adenomyosis according to their imaging, intra operative or pathological findings who were nominated for laparoscopic surgery. Eighty-six patients undergoing DIE resection without laparoscopic hysterectomy (group 1), and 68 patients with laparoscopic hysterectomy plus DIE resection (group 2).
Results: The accuracy, sensitivity and specificity of ultrasonographic and MRI findings for adenomyosis diagnosis were 72.1%, 77.6%, 40.0% and 49.2%, 41.5%, 90.0% respectively. Therefore TVS was more sensitive diagnostic tool for detecting adenomyosis, however, MRI was more specific than TVS in diagnosis of diffuse adenomyosis especially with simultaneous presence of uterine leiomyoma. Regarding the association of different types of adenomyosis (focal and diffuse) with different endometriosis lesions (OMA and posterior compartment DIE), we found that diffuse type of adenomyosis is more frequent in the absence of rectal and rectovaginal septum DIE ($p \leq 0.05$).
Conclusion: In addition to the questionable different nature of rectal and rectovaginal septum DIE lesion, there is no relationship between adenomyosis subtypes and endometriotic lesions.
Key words: *Adenomyosis, Endometriosis, MRI.*

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O-11
Testing the vulnerability-stress-adaptation model of marriage in infertile couples

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Background: In all cultures, achieving parental role is a fundamental condition of individual perfection and sexual identity. Inability to have a child is stressful and causes disturbances in marital quality, satisfaction and stability of couples.
Objective: The purpose of this study was to test the vulnerability-stress model of marriage in infertile couples referring infertility centers in Tehran.
Materials and Methods: The present cross-sectional study was performed in two stages on 200 infertile couples (400 individual) in infertility centers in Tehran. In the first step, based on the questionnaires and the relationships between endogenous, exogenous variables; a conceptual model was designed. The predictor variables such as personality trait, infertility related stress and coping strategies, quality of life as a mediator variable and marital stability as an outcme variable, were tested. Data were collected using Norton Questionnaire, ENRICH marital satisfaction, Neo personality traits, Newton fertility problem inventory, marital instability scale and Rahim's conflict resolution strategies questionnaire. The conceptual model was tested after evaluating the data and analyzing with LISREL software.
Results: Marital satisfaction and marital quality affected marital stability in both men and women directly, while the infertility-related stress affected marital stability only in women indirectly. In men, coping strategies directly affected marital quality. Also, in both men and women, marital satisfaction directly affected the quality of marriage. In women, coping strategies directly affected marital satisfaction. Infertility-related stress in men and women directly affected marital satisfaction. Also, after examining the fit indices, the conceptual model tested in infertile couples had a good fit.
Conclusion: Infertility-related stress, coping strategies, and marital satisfaction; are the important predictors of marital quality. Also, marital satisfaction and marital quality are the predictors of marital stability.
Key words: *Marriage, Couples, Infertility, Model.*

O-12
Effect of crocin and metformin on the reproductive system dysfunction of diabetic male mice induced by methylglyoxal

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Background: Diabetes has recently been a serious problem in the world. Sexual and reproductive disorders

are one of the most important secondary complications in patients with diabetes.

Objective: The effect of crocin on methylglyoxal (MGO)-induced diabetes in the male reproductive system has not been studied yet; so this study performed on MGO-induced diabetic male mice.

Materials and Methods: 70 male NMRI mice, one-month-old, weighing 20-25 g were divided into 7 groups (n = 10): sham, MGO (600 mg/Kg/d), MGO+crocin15, 30 and 60 mg/kg/d, MGO+Metformin (200 mg/kg/d), and crocin 60 mg/kg/d. Methylglyoxal administered orally in 30 days. In 14st day, after proving hyperglycemia, Metformin and crocin administered orally. On the 31st day of the study, plasma and tissue samples prepared for experimental assessments.

Results: Blood glucose and insulin levels in the MGO group are higher than the sham group ($p < 0.001$), and decreased with Metformin ($p < 0.001$) and crocin treatment (not in all doses). Testis width and volume decreased in the MGO receiving mice, and improved in crocin treated mice ($p < 0.05$), but not in the metformin group. Superoxide dismutase decreased in diabetic mice ($p < 0.05$) and Malondialdehyde enhanced ($p < 0.001$). Crocin and Metformin improved MDA and SOD. Testosterone ($p < 0.001$), and sperm count ($p < 0.05$) decreased in diabetic mice, treatment in all doses recovered these variables. Luteinizing hormone increased in diabetic mice ($p < 0.001$) and crocin treatment (but not metformin) decreased it. Seminiferous diameter and height decreased in diabetic mice and increased in treatment groups. Vacuoles and ruptures have been seen in diabetic testicular tissue, crocin improved testicular morphology ($p < 0.01$).

Conclusion: MGO increases oxidative stress, reduces sex hormones, and induces histological problems in male reproductive organ. Crocin and metformin improved the reproductive damage caused by MGO induced diabetes.

Key words: *Crocin, Diabetes mellitus, Methylglyoxal, Oxidative stress, Reproductive system.*

O-13

Evaluation the effect of human sperm incubation time in polyvinylpyrrolidone on sperm structure reactive oxyen species, acrosome reaction, and mitochondorial membrane potential

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Background: Polyvinylpyrrolidone (PVP) is a chemical used in intracytoplasmic sperm injection for sperm immobilization. In human sperm, PVP has been shown to damage sperm membranes, DNA integrity, mitochondrial membrane, and destroy axonal tubules and fibrous sheaths.

Objective: The aim of this study was to investigate the ideal time that sperm can be safely incubated in PVP with less possible damage.

Materials and Methods: Twenty-five normospermic samples were used. Sperm samples were prepared by swim-up method. Sperm samples incubated in 10% PVP at different time intervals (0, 15, 30, and 60 min). The effect of PVP was assessed on sperm structure, reactive oxyen species, acrosome reaction, Mitochondorial Membrane potential at different time intervals.

Results: Sperm parameters, DNA integrity and chromatin quality in 15, 30 and 60 min after incubation sperm with PVP were significantly changed compared to the 0 min. Moreover, in 30 and 60 min after incubation with PVP, above parameters were significantly changed compared to the 15 min. 60 min after incubation sperm with PVP, these parameters were significantly changed compared to the 30 min.

Conclusion: Sperm samples could be incubated with PVP for 15 min with less possible damage. While, prolonged incubation may damage the sperm parameters, DNA integrity and chromatin quality significantly.

Key words: *Polyvinylpyrrolidone, Sperm mithokondria, Sperm ROS.*

O-14

What is the accurate culture system for in vitro culture of cryopreserved 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.

Objective: In this study we pursue three goals to achieve the accurate culture system for in-vitro culture of cryopreserved human ovarian tissue.

Materials and Methods: First, comparing agar as a cultivation substrate with matrigel-coated insert in order to attain a suitable culture substrate. Afterwards, investigating the effect of basic fibroblast growth factor (bFGF) and/or kit ligand (KL) in the culture medium. Third, evaluating the effect of Phosphatase and TENsin homolog (PTEN) inhibitor (Bpv (HOpic)) and/or mTOR activators, phosphatidic acid (PA) and propranolol (PP), on the activation and subsequent development of in-situ culture of human primordial follicles. All 7-day cultures

were performed with slow frozen-thawed human ovarian cortical tissues obtained from transsexual women. At first the ovarian fragments were cultured on either matrigel-coated inserts or agar-soaked substrates. In the second phase, four different groups were examined: 1) control (base medium; BM), 2) KL (BM+100 ng/ml KL), 3) bFGF (BM+100 ng/ml bFGF) and 4) bFGF+KL (BM+100 ng/ml KL+100 ng/ml bFGF). In the third phase, control (without stimulators), Bpv (100 µM BpV (HOpic)), PA (200 µM), PA+PP (50 µM), and Bpv+PA+PP groups were compared. The incubation of ovarian cortical fragments was conducted for 24 hours with different stimulators and then for 6 days without stimulators. Follicular growth, proliferative, apoptotic and developmental gene expression, hormone secretion and PI3K/mTOR pathway protein expression were evaluated.

Results: In the first phase, no significant difference was found for follicular growth. The apoptotic index was lower in the agar cultured group and *Ki67* gene expression showed a significantly higher expression in agar cultured group. In the second phase, the proportion of growing follicles had no significant difference between cultured groups. The level of estradiol hormone had significantly increased in the bFGF+KL group. The expression of *Ki67* gene indicated a significant increase in the bFGF+KL group. In the third phase, the proportion of primordial and growing follicles were not significantly different after 24 hours of incubation among experimental groups. Western blot analyses indicated a significant reduction of FOXO3a in the PA+PP or Bpv+PA+PP groups compared to the control group. After 7-days of culture, the proportion of transitional follicles were significantly higher in the PA group compared to other groups. The estradiol level was significantly higher at the last day of culture compared to day 1, except for the Bpv group. Hormonal secretion was significantly higher in the PA and PA+PP groups and lower in the Bpv and Bpv+PA+PP groups compared to the control group.

Conclusion: Agar is similar to matrigel-coated inserts for culturing human ovarian tissue and it is an inexpensive substrate too. The combination of KL and bFGF positively influences steroidogenesis in the granulosa cells without increasing the total number of growing follicles. Temporary treatment of human ovarian tissue with mTOR activators, enhance the initiation of primordial follicle development and positively influence steroidogenesis, while Bpv (HOpic) has a potentially negative effect on follicular activation and function.

Key words: *In-situ ovarian culture, Agar substrate, Kit ligand, Basic fibroblast growth factor, mTOR, PI3K pathways.*

O-15

Investigation of signaling pathways to understanding Carob function for inducing spermatogenesis in an in-vitro platform

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Background: Impairment in the spermatogenesis process is the main cause of male infertility. Recently, scientists tried to improve the efficiency of male fertility treatment through the use of herbal nutraceuticals extract. Carob is being traditionally used for male infertility treatments. However, there is no scientific evidence for the principal mechanism effect of Carob on spermatogenesis-related signaling pathways.

Objective: Herein we evaluate 3 main spermatogenesis-related signaling pathways in mouse testicular cells-enrich for spermatogonial stem cells following treatment with Carob whole extract.

Materials and Methods: To evaluate the spermatogenesis-related TGF-β, BMP4, GDNF (MEK related) Signaling Pathways following treatment with Carob whole extract, after finding non-toxically Carob concentration for testicular cell culture by pi staining (2 mg/ml), isolated cells are treated by the medium containing Carob extract and one of the following small molecules: SB431542, LDN193189 and PD0325901 respectively. Cells were collected for gene expression analysis after 9 days of treatment.

Results: Our primary results suggested that by inhibiting the BMP4 signaling pathway using LDN193189 at the presence of Carob, all of the examined genes (Plzf, Gfr-α1, Bcl-6b, Dazl, Stra8) were significantly decreased compared to Carob treat. Gene expression profiles had different patterns on inhibition of other signaling pathways.

Conclusion: It seems that the BMP4 signaling pathway is the master effector upon Carob function. Activation of this signaling pathway, directly and indirectly, effect on differentiation and self-renewal of spermatogonial stem cells to promote spermatogenesis. However, the carob contains a set of effective compounds that promote spermatogenesis by the effect on most spermatogenesis related signaling pathways.

Key words: *Spermatogenesis, Spermatogonial stem cells, Carob, Signaling pathways.*

O-16

Beneficial effects of minocycline on the ovary of polycystic ovary syndrome mouse model: Molecular docking analysis and evaluation of *TNF-α, TNFR2, TLR-4* gene expression

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Background: Polycystic ovary syndrome (PCOS) is the most common cause of ovulatory infertility. Inflammation may be involved in the pathogenesis and development of PCOS.
Objective: We investigated the anti-inflammatory effect of minocycline on tumor necrosis factor-α (TNF-α), tumor necrosis factor receptor 2 (TNFR2), and toll-like receptor 4 (TLR4) expression levels and the key features of PCOS in a mouse model.
Materials and Methods: Molecular docking was performed by Molecular Operating Environment software. PCOS was induced by estradiol valerate injection (2 mg/kg/day) in 40 mice. After 28 days, the mice were divided into five groups, including control, PCOS, minocycline control, minocycline PCOS model (50 mg/kg), and letrozole PCOS (0.5 mg/kg). The Levels of follicle-stimulating hormone, luteinizing hormone, estradiol (E2), and testosterone were determined by ELISA. H&E staining was used for histological analysis in the ovarian tissues.
Results: Docking scores were -10.35, -10.57, and -12.45 kcal/mol for TNFα, TLR-4, and TNFR2, respectively. The expression levels of TNF-α, TNFR2, and TLR4 were detected by Real-Time PCR. PCOS models exhibited acyclicity, a significant increase in E2 levels ($p < 0.01$), and no difference in follicle-stimulating hormone, luteinizing hormone, and testosterone. The expression levels of TNF-α, TNFR2, and TLR-4 significantly increased in PCOS (2.70, 7.90, and 14.83-fold, respectively). Estradiol valerate treatment significantly increased graafian follicles ($p < 0.001$) and decreased corpus luteum (CL) ($p < 0.01$). Minocycline treatment in PCOS led to a significant decrease in E2 ($p < 0.01$) and graafian follicles ($p < 0.001$) and a significant increase in the CL numbers ($p < 0.05$).
Conclusion: Our findings showed the positive effects of minocycline on E2 level, CL and graafian follicles counts, suggesting that minocycline might inhibit these proteins and improve ovulation in our mouse model of PCOS.
Key words: *PCOS, Minocycline, TNF-α, TNFR2, TLR-4.*

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O-17
Expression of *CALM1*, *PSMD6*, and *AK124742 LncRNA* genes in cumulus cells of infertile PCO women: A good predictor of successful fertilization

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Background: In human assisted reproductive technology (ART), selection of high-quality embryos to transfer usually is based on morphological criteria but it cannot be always a good predictor of successful fertilization. Analyzing gene expression of cumulus cells (CCs) might lead to some important molecular information about the embryo quality. Calmodulin 1 (*CALM1*), Proteasome 26S Subunit, Non-ATPase 6 (*PSMD6*), and AK124742 expression in the CCs of pregnant patients were more significant compared to the non-pregnant ones. One of the well-known causes of female infertility is polycystic ovary syndrome (PCOS) and the number of retrieved oocytes with a higher implantation potential is limited, so the process of selecting good embryos in PCOS patients is very important.
Objective: The aim of this study was to compare the expression of *CALM1*, *PSMD6*, and *AK124742* genes in the CCs of infertile PCO patients with control fertile group.
Materials and Methods: Samples were the CCs from 33 fertile egg donor women and 33 infertile PCO women. They undergo ART and the CCs were collected and frizzed till real time PCR (RT-PCR) was performed. The expression of *CALM1*, *PSMD6*, and *AK124742* genes was detected by RT-PCR. Chemical pregnancy rates were used to assess the success of ART.
Results: Clinical pregnancy was observed in 38 of the 66 patients. Expression of all three genes *CALM1*, *PSMD6*, and *AK124742* in the pregnant group were higher than the non-pregnant group. This increase was not significant for the *CALM1* gene but for two genes *PSMD6* ($p < 0.001$) and *AK124742* ($p < 0.05$) were significant. The expression of *CALM1* and *ak124274* gene increased significantly and the expression of psmd6 decreased significantly in PCOs group compared to the control group ($p < 0.05$).
Conclusion: All three genes are proper markers for predicting embryo competence due to increased expression levels in pregnant groups.
Key words: *CALM1, Infertility, lncRNA, PCO, PSMD6.*

O-18
Culture in perfusion mini bioreactor can enhance in vitro spermatogenesis

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Background: In vitro spermatogenesis is one of the main aim of male infertility treatment, which proves critical in cancer patients who undergo treatment with gonadotoxic drugs and methods. Conventional methods of culture cannot support organs or tissues removed from body for a long time. A biomimetic system is achieved by a bioreactor capable of culturing tissues under in vivo-like conditions. Overall, the controlled parameters are fluid flow, pH, temperature, waste removal, nutrition flow. Application of a perfusion flow is for mimicking native testicular microenvironment.
Objective: In this study, we intend to evaluate the progression of spermatogenesis after in vitro transplantation (IVT) of spermatogonial stem cells (SSCs) isolated from mouse fresh testis tissue in mini perfusion bioreactor.
Materials and Methods: Adult mouse azoospermia model was used to remove testis tissue. SSCs isolation was carried out using two enzymatic digestion methods. The cell identification was confirmed via detection of promyelocytic leukaemia zinc finger (PLZF) protein. After being labeled with DiI, the cells were transplanted into azoospermic adult mice. After being fragmented, host testes were incubated for two and eight weeks in a bioreactor. Histological, molecular and immunohistochemical assessments were done after two and eight weeks. Data were statitically analyzed using analysis of variance (ANOVA) test and significancy was considered at ($p < 0.05$).
Results: Histological analysis suggested successful maintenance of spermatogenesis in host testis tissues grown in the bioreactor. Molecular analysis indicated that PLZF, Tekt1 and Tnp1 genes were expressed and that their expression in the experimental IVT group was significantly more than the control group (without transplantation) and 0-day cell suspension ($p < 0.05$). Immunohistochemical evaluation of host testis fragments in the experimental group showed that PLZF, synaptonemal complex protein (SCP3) and acrosin binding protein (ACRBP) proteins were expressed in spermatogonial cells, spermatocytes and spermatozoa, respectively.
Conclusion: Dynamic culturing methods appear to be capable of enhancing spermatogenesis in vitro. Such three- dimensional (3D) culturing system is able to give rise to haploid cells and can offer conditions similar to those of native like physiological microenvironment of testicular tissue. The current bioreactor, thus, can potentially provide an enhanced culturing system for testicular organ culture. Our findings reveal that following two weeks of SSCs transplantation in vitro, and 3D dynamic organ culture, these cells had migrated to basement membrane of seminiferous tubules and settled down through homing and initiated the spermatogenesis process. Perfusion bioreactor dynamic culturing system fosters spermatogenesis induction to generate haploid cells, in which long term (56 days) culturing host testicular tissue segments of the IVT group permitted spermatogenesis completion, giving rise to morphologically intact and mature spermatozoa.
Key words: *Spermatogonial stem cells, Mouse, Transplantation, Azoospermia, Perfusion bioreactor.*

O-19
Protective effect of crocin on electromagnetic field-induced testicular damage and heat shock protein A2 expression in male BALB/c mice

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Background: Exposure to electromagnetic fields (EMF) emitted from mobile phones may cause a deleterious effect on human health and may affect the male reproductive system. Crocin, a carotenoid isolated from Crocus Sativus L. (Saffron), is a phar macologically active component of saffron.
Objective: This study was conducted to investigate the protective effect of crocin on the male reproductive system of 60 day old mice after EMF exposure.
Materials and Methods: Twenty-four male BALB/c mice were randomly divided into 4 groups: 1) electromagnetic (EM) group (2100 MHZ); 2) Crocin (Cr) group (50 mg/kg); 3) Em+Cr group (2100 MHZ+50 mg/kg), and 4) Control group. Sperm parameters (count, and abnormal percent), testis weight index, testis volume, seminiferous tubule diameter, germinal epithelium thickness, luteinizing hormone, follicle-stimulating hormone and testosterone serum level, testicular Heat shock protein A2 (HspA2) immunoreactivity, and apoptosis were evaluated.
Results: HspA2 immunoreactivity, apoptosis in the germinal epithelium and abnormal sperm were increased in EM group compared with the control group ($p < 0.05$). Sperm count, luteinizing hormone, and testosterone serum level were decreased in the EM group compared with the control group ($p < 0.05$). These parameters were improved in the EM+Cr group compared with EM group significantly ($p < 0.05$).
Conclusion: Our findings revealed that EMF exposure leads to harmful impressions on the male reproductive system, while crocin can attenuate EMF-induced destructive effects.
Key words: *Apoptosis, Electromagnetic field, Crocin, Heat shock protein, Testis.*

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O-20

Annexin-V MACS sperm selection method could be effective on separation of sperm with high expression of *PLCZ1* gene and development of high blastocyst rate in male factor patients with high DNA fragment

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Background: Sperm selection according to morphology and motility in assisted reproduction technologies (ART), is not sufficient for selecting the best sperm especially in patients who have male factor problems. Apoptotic sperm and non-apoptotic ones are distinguished from each other by negative selection in Annexin-V magnetic-activated cell sorting (MACS) technique. So, compaction rates in embryo quality are enhanced in this method. PLCZ1, which is one of the oocyte activating factors, starts oscillations of Ca^{2+} in oocyte and it has a significant impact on the fertilization and implantation process.
Objective: In this study, hypostasis of sperm selection relying on motility and morphology in ART techniques, not be sufficient for selecting the most qualified sperm especially in male factor patients are shown. Apoptotic sperm cells and non-apoptotic ones are distinguished from each other in the Annexin-V MACS-DGC technique. Therefore, this method enhanced the quality of embryo compaction rate.
Materials and Methods: Semen samples of 30 infertile couples who have male factor problem with DNA fragmentation index (DFI) above 30% were selected for this study. The samples of each patient were divided into two groups, control and experimental. The control group was washed with the routine density gradient centrifugation (DGC) method and the experimental group was selected by magnetic-activated cell sorting combined isolate density gradient centrifugation (MACS-DGC). Similarly, eggs in each female of patients were divided into 2 mentioned groups. Control was injected by DGC, on the other hand, the experimental group was injected by MACS-DGC. On both before and after processing sperm parameters were evaluated. DFI was reported based on the halo sperm method both before and after processing. Embryo quality, blastocyst formation rate, and fertilization rate were estimated, after ICSI. Expression of *PLCZ1* was evaluated by real-time PCR. Comparison between results of two groups was determined by SPSS software.
Results: The research reported that sperm morphology and motility after the MACS-DGC method (1.7%, 45%) were significantly higher in comparison with the DGC method (1.1%, 40%) and before washing (0.9%, 35%). The percent of DFI in the MACS-DGC group (36%) was significantly reduced in comparison to DGC (45%) and primitive group (55%). The number of oocytes injected was 93 and 111 in DGC and MACS-DGC group, respectively. The fertilization rate in both groups was approximately equal (73.11 in DGC versus 72.07% in MACS-DGC). The rate of day 3 embryos with good grade was significantly higher than in the MACS-DGC group (72.5%) in comparison to the DGC group (51.47%) ($p < 0.05$). The blastocyst rate in the MACS-DGC group (69.69%) was significantly higher than the DGC group (48%). PLCZ1 gene expression in MACS-DGC was significantly increased compared to the DGC group ($p = 0.046$).
Conclusion: Results show that sperm selection based on the MACS-DGC method can enhance morphology, motility, and decrease sperm DFI. No significant difference was observed in fertilization rate, but the percent of the high-quality embryo on days 3 and 5 was significantly higher by this method. According to the mechanism of the MACS-DGC method, it can be suggested as a good choice for patients with high DFI.
Key words: *MACS, PLCZ1, High DFI, Male factor.*

O-21

Viability of isolated preantral follicles using decellularized ovarian tissue after grafting under the kidney capsule

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Background: nowadays scientist use decellularized tissue as a novel technique in regenerative medicine. Recently the application of decellularized tissue in the field of human fertility preservation has been studied. Due to the high resemblance of decellularized ovarian tissue to the main organ, it can be used as a scaffold for follicle growth and development.
Objective: Mice ovarian follicles can be viable and seed into the acellular scaffold after 7 days of grafting.
Materials and Methods: In this study fragmented bovine ovarian cortex (2 × 2 mm) were decellularized by Sodium dodecyl sulfate, Triton X100 and Ammonium. 120 primordial follicles of NMRI mice were isolated and put into the decellularized scaffold (n = 30/ 4 scaffold) then transplanted under the kidney capsule for 7 days. H&E staining was used to determine follicle morphology after transplantation. Follicular proliferation was measured by Ki-67 antibody. Apoptosis (TUNEL) and vessel formation (CD31) were analyzed.

Results: According to the H&E staining results, after 7 days of grafting, 38/120 follicles were viable (31.6%) and seeded into the scaffold. Ki67-positive OCs was found in 15.2% of cells of the grafted scaffold. While TUNEL-positive cells were in 13.7% of granulosa cells. According to H&E staining results and CD34-staining, vessels were found inside the scaffold after 7 days of grafting.

Conclusion: This study shows that follicles can survive and seed into the acellular matrix after 7 days of grafting.

Key words: *Fertility preservation, Ovary, Scaffold.*

O-22

Application of auto-crosslinked hyaluronic acid hydrogel loaded with the bone marrow mesenchymal stem cell-extracellular vesicles to prevent the formation of intrauterine adhesions in a rat model

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Background: Severe trauma of uterus may cause damage in the basal layer of endometrium leading to intrauterine adhesion (IUA) or Asherman's syndrome (AS), and eventually infertility. Nowadays, conventional approaches including surgical adhesiolysis and following hormone therapy are used to treat AS in clinics. However, the high recurrence after these procedures reveals the importance and superiority of IUA prevention instead of treatment. As a preventive agent, auto-crosslinked hyaluronic acid (HA) hydrogels can provide an anti-adhesive barrier to decrease the incidence of IUA. In addition, MSC-derived extracellular vesicles (EVs) have been recently introduced as an effective and novel therapeutic agent to reduce inflammation and fibrosis. This study has been designed to investigate whether combining the auto-crosslinked HA hydrogel with mesenchymal stem cell-extracellular vesicles (MSC-EVs) could improve the efficiency of HA in endometrial regeneration in the rat model of AS.

Objective: To evaluate whether the combination of HA hydrogel and MSC-EVs could facilitates functional regeneration of injured uterus in experimental rats.

Materials and Methods: Forty eight-week-old female Wistar rats weighting 200–250 g were randomly assigned into 5 groups (n = 8/each): I) Intact group: without any intervention, II) AS Model group: model was established by three surgical steps of mechanical injury (incision, curettage, and suture), III) Sham surgery group: Subjected to the abdominal surgery, incisions and suturing, but not the curettage procedure, IV) HA + MSC-EVs: A mixture of 400 µl HA hydrogel + 160 µg/kg/dose MSC-EVs (around 200 µl hydrogel containing 20 µg EV per each horn) was injected into the uterine horn immediately after making the AS model and V) HA: Receiving an intrauterine injection of only 400 µl HA hydrogel immediately after making the AS model. Two weeks after the transplantation, four rats from each group sacrificed and uterine samples were harvested to be evaluated histologically by H&E and Masson's trichrome staining. The remaining four animals in each group were coupled with fertile males (female: male = 2:1) 1 week after modeling for 3 months. The number of deliveries and the cumulative number of pups were assessed at the end of this time to survey reproductive function.

Results: Histology examination revealed significantly thicker endometrium, increased gland numbers and fewer fibrotic areas in the HA + MSC-EVs and HA transplantation groups compared with the model group. The cumulative number of pups and number of deliveries also showed a significant increase in the HA group compare to the model group. But our results displayed no significant differences between the HA + MSC-EVs and HA groups in terms of morphometric parameters and mating test outcomes.

Conclusion: MSC-EVs cannot amplify preventive properties of HA on the rat model of AS.

Key words: *Intrauterine adhesions, Asherman's syndrome, Hyaluronic acid hydrogel, Extracellular vesicle.*

O-23

In vitro cytotoxicity of zinc oxide nanoparticles in mouse ovarian germ cells

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Background: Recently, metal oxide nanoparticles such as zinc oxide nanoparticles (ZnO-NPs) have received

considerable attention and humans are exposed to them in everyday life. The increasing use of ZnO-NPs may lead to human health issues. However, little is known about their effects on female reproductive systems, particularly on female germ cells. Germ cells differentiation is a complex biological process that is sensitive to environmental insults and any negative effect on germ cells development may inhibit fertility.

Objective: The purpose of this study was to assess the effects of ZnO-NPs on mouse ovarian germ cells (OGCs) as an in vitro model for the assessment of nanotoxicity in the OGCs. To the best of our knowledge, no study has been conducted to determine the effects of ZNO-NPs on female OGCs. Our study provides a sensitive in vitro model to assess the toxic effects of ZNO-NPs and other NPs in the female OGCs. This study aimed to determine the impact of ZnO-NPs on mouse OGCs in an in vitro system.

Materials and Methods: Briefly, after isolation and culture of OGCs, the effects of ZnO-NPs on these cells were evaluated using light microscopy, cell proliferation assessment, reactive oxygen species (ROS) level determination, standard cytotoxicity assessment (cell viability assessed by PI staining) and gene expression analysis.

Results: Our results demonstrated that ZnO-NPs have cytotoxic effects in a concentration- and time-dependent manner in mouse OGCs. Exposure of cells to ZnO-NPs concentration-dependently enhanced ROS generation. Furthermore, molecular analysis of ZnO-NPs-treated cells showed a significant increase in expression of premeiotic germ cells markers but a decrease in meiotic and post-meiotic markers compared to un-treated cells.

Conclusion: Our data provides a preliminary insight into possible adverse effects of ZnO-NPs on mouse OGCs differentiation even at low concentrations.

Key words: *Nanoparticle, Zinc oxide, Ovarian germ cell, Infertility.*

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O-24

Oxidative stress-dependent toxicity of dextran-coated superparamagnetic iron oxide nanoparticles on mouse embryo produced by in vitro fertilization

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Background: Superparamagnetic iron oxide nanoparticles (SPIONs) are capable to penetrate the placenta. Also, small nanoparticles can cross the blood-testis barrier and aggregate in the testes. Thus, SPIONs might have adverse impacts on reproduction systems.

Objective: The influence of adding dextran-coated SPIONs (D-SPIONs) into the fertilization medium was investigated in a dose-dependent manner on gene expression of oxidative stress enzymes in the resultant blastocysts in a mouse model.

Materials and Methods: Mature oocytes were collected from superovulated female BALB/c mice and randomly divided into three groups (0, 50, and 250 µg/ml of D-SPIONs). These concentrations were mixed into fertilization medium as control, low, and high dose groups, respectively. The toxic effects of D-SPIONs on murine in vitro fertilization (IVF) were investigated by developmental competence and alterations in gene expression of antioxidant enzymes were assessed for glutathione peroxidase 1, superoxide dismutase 1, and catalase in the blastocysts derived from IVF. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (SPSS 20) and presented as mean ± standard deviation.

Results: Mature oocytes were collected from superovulated female BALB/c mice and randomly divided into three groups (0, 50, and 250 µg/ml of D-SPIONs). These concentrations were mixed into fertilization medium as control, low, and high dose groups, respectively. The toxic effects of D-SPIONs on murine IVF were investigated by developmental competence and alterations in gene expression of antioxidant enzymes were assessed for glutathione peroxidase 1, superoxide dismutase 1, and catalase in the blastocysts derived from IVF. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (SPSS 20) and presented as mean ± standard deviation.

Conclusion: Our results may suggest that increasing these antioxidant enzyme genes, as reactive oxygen species scavengers, meaningfully promoted overtime to protect the resultant blastocysts from oxidative damage. Despite considerable usage of D-SPIONs in numerous fields of science and technology, this study presented extensive worries about their toxicity towards IVF. Therefore, it is important to perform further studies to detect the potential risks of this nanoparticle in various areas of nanotechnology.

Key words: *Blastocyst, Catalase, Developmental competence, Oxidatiove stress.*

O-25

Evaluation of the effect of granulocyte-macrophage colony stimulating factor on sperm quality in oligoasthenoteratospermia men

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Background: Oligoasthenoteratospermia (OAT) is characterized by abnormalities in sperm count, motility and morphology. Poor sperm quality can adversely affect the results of assisted reproductive technique. Development of sperm media is necessary to improve the sperm parameters of these patients. Granulocyte-macrophage colony stimulating factor (GM-CSF) is a natural growth factor produced by the reproductive organs, previous studies show that this growth factor in the semen of infertile men is lower than that of fertile men. However, there is no study to assess the effect of GM-CSF on sperm quality.
Objective: The aim of this study is to evaluate the effect of GM-CSF as a sperm medium supplement on sperm quality in OAT patients.
Materials and Methods: In the present study, semen specimens were collected from 20 OAT patients who have male infertility factors, according to WHO criteria. After the swim-up washing procedure, each of the samples was divided into two groups; experiment, and control. In the experimental group, samples were incubated with a medium containing 2 ng/ml GM-CSF for one hour, yet, in the control group, the sperms were incubated without GM-CSF for the same time. The sperm motility was examined with phase-contrast microscopy, Eosin-nigrosin staining method was used to assess sperm viability, and DNA fragmentation were evaluated by TUNEL test. The expression of sperm glucose transporters (GLUT 1, 3) was determined using Immunofluorescent staining, the phospho-Akt/total Akt ratio was assessed by the Western blotting method. Data were analyzed by SPSS software and P-value < 0.05 was considered statistically significant.
Results: As compared to the control group, supplementation with GM-CSF improved sperm progressive motility, enhanced GLUT 1 and 3, and phospho-Akt/total Akt expression ($p < 0.05$). In GM-CSF treated groups, DNA fragmentation was lower than control ones ($p < 0.05$). There was no significant difference between the viability of the control and experimental groups.
Conclusion: Our results showed that GM-CSF can improve sperm quality by influencing motility and energy metabolism in spermatozoa which can be affected by increasing the phosphorylation of AKT for the first time. This growth factor could be an appropriate supplement in sperm media for OAT patients.
Key words: *GM-CSF, Oligoasthenoteratospermia, Sperm quality.*

O-26
Alternation of interleukins expression from fallopian tube epithelial cells after co-incubation with spermatozoa

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Background: Fallopian tubes are important places in which spermatozoa are saved until fertilization with oocytes occurs. The immune system in the reproductive tract has an important role in overcoming pathogens while preparing a safe environment for allogenic spermatozoa. Interleukins are among the most important variables of the immune system which provide effective response in normal and pathological conditions. Therefore, many scientists are interested in better understanding of the role of interleukins in reproductive immunology.
Objective: The aim of this investigation was to find out more details about the role of interleukins in the interaction between spermatozoa and fallopian tube epithelial cells. Therefore, the expression of different interleukins from the fallopian tube cell line (OE-E6/E7) which had co-incubated with spermatozoa was investigated by PCR array.
Materials and Methods: We collected sperm samples from 10 healthy men. All samples were checked to ensure they have normal features according to WHO guidelines. Simultaneously, we cultured epithelial cell line in 6-well plates and incubated until 70% of each well was covered by the epithelial cells. Cells without spermatozoa were analyzed as the control group. The cells and the spermatozoa were co-cultured for 24 hr. Then, the cells were washed and followed by RNA extraction and cDNA synthesis. PCR array was performed to evaluate the transcriptomic changes of different interleukins. To confirm our results, the concentrations of IL-10 and IL-1β were also analyzed by ELISA.
Results: The results of our investigation indicated that the expression of some interleukins in the vicinity of sperm significantly changes. It has been shown that anti-inflammatory interleukins including IL-9 ($p = 0.02$) and IL-10 ($p \leq 0.01$) from fallopian tube epithelial cells were significantly upregulated in the presence of spermatozoa. However, the expression of pro inflammatory interleukins such as IL-16, IL-17, IL-1A, and IL-1B was significantly ($p < 0.05$) lower in the case group than the control group. Moreover, the concentration of IL-10 in the case group was higher

than the control. Although, the concentration of IL-1B in the case group was lower than the controls.

Conclusion: This study indicates that spermatozoa modulate the expression of interleukins from OE-E6/E7. Moreover, altered genes expression might have increasing survival chance of spermatozoa in fallopian tubes' microenvironment.

Key words: *Fallopian tube, Spermatozoa, Interleukins, PCR array.*

O-27
Effects of knockout serum replacement on the quality of frozen-thawed human spermatozoa

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Background: Cryopreservation of human spermatozoa is an important technique in the treatment of male infertility. This procedure is regularly used for preserving the fertility potential of young male patients with cancer before chemo- or radiotherapy. It is well known that the freeze-thaw practice leads to the functional and structural damages to human spermatozoa, mainly due to the formation of ice crystals, oxidative stress, and osmotic imbalance. In order to decrease sperm cryo-damages and improve the quality of post-thawed spermatozoa, factors, such as the composition of the freezing medium, methods of freezing (i.e., slow, rapid, and vitrification), and the way of packaging the samples (cryotube or straw) have been largely investigated. Knockout serum replacement has been recently used as an effective serum substitute for culture of several mammalian cells and cryopreservation of some cell types. It is a rich source of amino acids, antioxidants, vitamins, and some trace elements and would be preferable due to the components' reliability, no varies between batches and the absence of probably microbial agents.

Objective: In this study, we used KSR as a serum substitute in the freezing medium for sperm cryopreservation. For this purpose, we designed a simple handmade freezing medium that consisted of sucrose and different concentration of HSA or KSR for the rapid freezing of human sperm cells and compared them with commercial freezing medium.

Materials and Methods: Twenty semen samples were taken from normozoospermic men who referred to the Royan Institute. After the swim-up process and the evaluation of fresh sample, supernatants were divided into five groups. The four aliquots were diluted with a sucrose solution containing different percent (5%, 10%) of HSA and KSR. The other aliquot was diluted with a freezing medium containing (Sperm-Freeze) as a control group (CON). The samples were hold in liquid nitrogen vapor for 15 min and then plunged into the liquid nitrogen and cryopreserved for 1 month. After thawing, sperm parameters including motility characteristics, viability, acrosome integrity and DNA intactness were assessed.

Results: All of sperm parameters were significantly decreased in cryopreserved samples compared to the fresh group. There were no significant differences in the motion characteristics of spermatozoa between the control and experimental groups. However, the highest sperm viability, acrosome integrity and DNA intactness was achieved by the addition of 10% KSR to the freezing medium, which was statistically significant when compared with other experimental groups.

Conclusion: In conclusion, our results demonstrated that the addition of 10% KSR to the sucrose-based freezing solution improves the quality of post-thawed human spermatozoa, including motility, viability, acrosome integrity, and DNA integrity and may have potential to develop chemically defined freezing medium.

Key words: *Human sperm, Cryopreservation, Knockout serum replacement, Human serum albumin.*

O-28
Reconstruction of the mouse uterine tissue using polycaprolacton/ gelatin/ polydimethylsiloxane hybrib scaffolds: In vitro and in vivo study

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Background: Serious endometrial damage in women of fertility age is often associated with the formation of uterine scars and a lack of functional endometrium prone to infertility or miscarriage. Uterine tissue engineering using biological materials and stem cells may replace the need for surrogacy and may prevent the necessary immune suppression therapy. However tissue engineering structures were also used as laboratory models to study the mechanisms of endometrial invasion.

Objective: Reconstruction of the mouse uterine tissue using polycaprolacton/ gelatin/ polydimethylsiloxane hybrib scaffolds (in vitro and in vivo study).

Materials and Methods: In the present study, according to the structure of mouse uterine tissue, a tubular nanofiber scaffold was designed and fabricated. Mouse cells were cultured on target scaffold. 3- (4،5-dimethylthiazoyl-2-yl) 2، 5-diphenyltetrazolium

bromide (MTT) test was performed to evaluate cell viability on the scaffold. Hematoxylin and eosin staining examined cell growth and proliferation on the scaffold. Mouse embryos were cultured on the target scaffold and examined with immunofluorescents staining. Finally, a tubular scaffold replaced one of the branches of the rat uterus and was evaluated 30 days after surgery by hematoxylin and eosin staining and immunohistochemistry for tissue formation.

Results: The tubular scaffold designed in this study showed that due to the location of the cells between the scaffold layers, cell infiltration between the nanofibers was good due to the small porosity of nanofibers. It also had a higher performance than similar tubular scaffolds. In the present study, the mouse embryo was hatched on the scaffold and attached to the scaffold. This indicates that the embryo was compatible with the scaffold. Also, after tubular scaffold transplantation instead of the mouse uterine horn, many cells close to the injured site migrate inward and the tubular tissue of the mouse uterus was formed on the scaffold.

Conclusion: In the present study, we developed a scaffold with the standards needed for whole uterine tissue engineering. It also could be useful for multiple tissue engineering applications. In these scaffolds, cell proliferation and migration occured well while enhancing angiogenesis to regenerate new uterine horns. Electrospun polycaprolacton/ gelatin/ polydimethylsiloxane fibrous scaffolds were developed to use as promising uterine tissue engineering.

Key words: *Mouse uterus, Tubular scaffold, Embryo, Nanofiber.*

O-29

Designing a new delivery system containing quercetin and edible oils to treat male infertility induced by nonalcoholic fatty liver in rats

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Background: Recent molecular and physiological studies have shown that adverse effects of nonalcoholic fatty liver diseases (NAFLD) extend far beyond the liver. NAFLD can impair male reproductive function by increasing reactive oxidative stress levels, reducing the expression of antioxidant genes and inducing damage in testes immune privilege. Antioxidant therapy and its effectiveness depend on whether the exogenous antioxidant will be readily absorbed to reach high enough that are required to decrease the pathological damages. Quercetin (Quer), as an antioxidant, is able to ameliorate oxidative stress but has low bioavailability in the body. Therefore designing new drug delivery systems are needed to reach the best effects.

Objective: We aimed to prepare a new delivery system containing edible oils for quercetin entrapment to slow release.

Materials and Methods: Bigels were prepared using cottonseed oil/cannabis oil/alginate/ferula gum. Sprague-Dawley rats were housed for 2 wk, then NAFLD was induced by 58% of dietary calorie as lard and 42 g/L fructose for 16 wk. The experimental protocol was approved by the ethical committee of Zanjan University of Medical Sciences, Zanjan, Iran. After confirming the NAFLD induction, animals were divided into five groups: Control, control NAFLD, received 2 mg/kg Quer loaded on bigels, free bigels, and free Quer for 45 days as daily gavage. Semen parameters (count, motility, and morphology), viability (Eosin-nigrosine staining) and serum testosterone levels were analyzed. In addition, histological sections of testicular tissues were investigated by Hematoxylin-Eosin staining method. In situ detection of apoptosis was performed using terminal deoxynucleotidyl-transferase dUTP nick end labeling (TUNEL) assay.

Results: The sperm count, sperm motility, normal morphology and testosterone level were significantly lower in the NAFLD group than those the controls. Moreover, higher head and tail abnormality percentages were seen in the sperm of these groups. Bigel-Quer significantly improved the serum testosterone level, sperm count, motility, and morphology compared with the NAFLD group. Spermatogenic cells in all stages of differentiation (spermatogonia, primary spermatocytes, early spermatids, late spermatids) are observed and preserved normally in the testicular tubules and lumen filled with mature sperms in the control group. Interestingly, atrophic changes in the testicular tubule architecture with swelling in spermatogonia cells, detachment from tubule membrane, reduced number of mature sperm, and reduced lumen thickness were seen in the NAFLD. In the Quer, bigel and bigel-Quer-treated groups, swelling and vacuolation rate of germ cells decreased. The testicular morphology, and tubule structure were significantly normalized, especially in the bigel-Quer-treated group. Serum testosterone levels significantly increased and reached the healthy control group in the bigel-Quer group. TUNEL-positive cells in testes increased significantly after NAFLD induction. Quantitative analysis showed a significant decrease in testicular TUNEL-positive cells following bigel-Quer treatment, but not in other groups.

Conclusion: The bigel showed synergistic effects with Quer for treating infertility in rats with NAFLD. Stability and bio-availability of Quer are important aspects that should be considered to justify its supplementation. Empowering antioxidant shield of

NAFLD patients by Quer supplementation can improve various damage effects and clinical status of diseases.

Key words: *Quercetin, Non-alcoholic fatty liver, Semen parameters, Bigel.*

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O-30
Debates on COVID-19 presence in semen: A systematic review

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Background: Coronavirus disease 2019 (COVID-19) is a challenge not only for survival and being alive, but probably for ability to have a child. This concern is more exacerbated for men, because sex differences susceptibility has been reported for men and men are more prone to COVID-19. Also, orchitis is reported in autopsy of men, which may be due to the highly expression of ACE2 in testicular cells.

Objective: Some studies have reported the real time polymerase chain reaction (RT-PCR) detection of virus in semen of men, affected by COVID-19; while, others did not confirm these data. So, the aim of this study was a systematic review of published data regarding the detection of SARS-CoV-2 virus in semen.

Materials and Methods: The literature search was performed in PubMed, Scopus, and Google scholar based on the following key words: ["severe acute respiratory syndrome–coronavirus 2" OR "COVID-19", OR "2019-nCoV", OR "SARS-CoV-2", OR "severe acute respiratory syndrome coronavirus 2" OR "SARS CoV2" OR "SARS CoV 2"] AND ["semen" OR "sperm" OR "testis" OR "testicles" OR "seminal" OR "testes" OR "male reproduction" OR "orchitis" OR "testicular" OR "male fertility" OR "male infertility" OR "epididymis" OR "prostate" OR "testosterone" OR "DHT" OR "dihydrotestosterone" OR "azoospermia". All published papers were screened from December 2019-december 2020. The literature search was conducted by manual screening of the titles and abstract. The full text of studies that detected the virus in semen by RT-PCR and RT-qPCR were reviewed.

Results: 10 papers published from April-December 2020 were suitable. one paper reported that 6.66% of cases (6 of 38) tested positive for virus in semen. Among them, 2 recovered and 4 patients (15 ≥ years) in acute phase were positive. Nine other studies reported that the semen samples were negative for detection of virus RNA in semen which were as follows. Song, Wang colleague tested semen and testicular biopsy of 12 young recovered patients (11 patients with mild symptoms, 1 asymptomatic and 1 patient in acute phase) and testis biopsy achieved from 1 dead patient. Xiao colleague collected semen samples from 34 Chinese men (18-57 yr) with generally mild symptoms and 6 patients (19%) developed scrotal discomfort. Pallotti colleague checked one 31-yr-old man, 8 days after positive pharyngeal swap. Ning, Li colleague also analyzed 17 patients with aged 23-46 yr. They showed symptoms or signs related to male reproductive system. Zhao colleague evaluated 23 patients in recovery phase. Nora, Philippos colleague tested 18 patients, 8-54 days after absence of symptoms and 2 samples from patients with active infection. Temiz, Dincer colleague tested 55 patients, 18 to 60-yr old. Li, Xiao colleague tested 23 patients, aged above 18 yr. Ruan, Hu colleague analyzed 74 men, aged 20-50 yr. All these 9 studies reported negative results in different age categories and different COVID-19 phases.

Conclusion: The detection of CIVID-19 in semen was noted and the possibility of male factor infertility and sexual transmission should not be neglected.

Key words: *COVID-19, SARS-CoV-2, Testis, Sperm, Semen.*

O-31
Impact of sperm parameters on mRNA level of AnnexinA2, Sp17, SerpinA5, Prdx2, oxidative stress, and sperm DNA fragmentation

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Background: Today, it has been shown that having the normal sperm parameters cannot only represent fertility status of male partners of infertile couples. The potential role of several molecular and cellular factors associated with fertilization and embryo failure is not clearly identified.

Objective: The aim of this study was to investigate the association between sperm DNA fragmentation, oxidative stress as well as some sperm functional genes and the sperm parameters among both men of infertile couples with a history of recurrent early pregnancy loss and fertile men.

Materials and Methods: The mRNA levels of the *AnnexinA2*, *Sperm protein 17* (*Sp17*), SerpinA5, and *Peroxiredoxin-2* (*Prdx2*) genes were comparatively evaluated between sperm samples of infertile men with abnormal parameters (n = 25), male partners of infertile

couples with normal parameters (n = 25), and the fertile men with normal sperm parameters (n = 25) as experimental group I, II and control, respectively by using quantitative real time polymerase chain reaction. The sperm DNA fragmentation (SDF) was assessed using Chromomycin A3 (CMA3), acridine orange (AO), annexin V (ANXV) staining and Propidium iodide (PI). Sperm maturity was evaluated by acrosom reaction test. To determining the stress oxidative, malondialdehyde (MDA) and total antioxidant capacity (TAC) levels were measured in seminal plasma.

Results: The gene expression profile of *SP17* showed a significant down-regulation between experimental I as well as experimental II and control group ($p < 0.005$ and $p < 0.0007$, respectively). In contrast, *SerpinA5* mRNA level was significantly down-regulated in experimental groups I ($p < 0.05$). Both experimental groups showed an increase in *PRDX2* mRNA level. However, there was a significant association between experimental group II and controls based on *PRDX2* gene expression. Also, there was no significant difference between three groups in accordance of *AnnexinA2* gene expression levels. The results demonstrated significant higher rates of CMA3+ and AO+ sperm cells in both experimental group I and II compared to the controls. The most numbers of necrotic sperm cells were detected in experimental group I based on PI staining. However, we found no significant change in early apoptotic rates (ANXV+) of sperm specimen between all study groups. There was a significant decrease in acrosome-reacted spermatozoa in experimental group I in comparison with controls. Furthermore, a significant positive correlation was seen between seminal MDA and TAC concentration.

Conclusion: The data indicates that *Sp17* not only has potential functions in the fertilization process, but also in the developing embryo at stages of implantation and pregnancy maintenance. *SerpinA5* gene expression is strongly associated with abnormal sperm morphology. SDF plays a role as a major cause of male infertility independent of the sperm parameters.

Key words: *Sperm parameter, Gene expression, DNA fragmentation, Reactive oxygen species.*

O-32

Morphological evaluation of oocytes with image processing methods in patients undergoing intracytoplasmic sperm injection

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Background: Morphological assessment of oocyte quality is one of the most essential and sensitive steps in infertility treatment and deciding on treatment type. Oocytes abnormalities can be seen and detected under a microscope by embryologists. Diagnosis of the type and severity of abnormalities in in vitro fertilization centers is done by embryologists. Diagnosis is based on the appearance of the egg and according to scientific standards. Factors such as fatigue, inexperience and taste can cause differences in the outcome. Using image processing and morphological detection techniques, the egg and its cytoplasm can be identified and its features extracted. Finally, with the help of the decision tree, the normal egg can be distinguished from abnormal.

Objective: This study aimed to evaluate human oocyte abnormalities by image processing. Our goal was to develop a diagnostic tool that can analyze microscope images of human oocytes and derive a detection of the oocyte cytoplasm and zona-pellucida that is functional for quality evaluation in assisted insemination.

Materials and Methods: The approach of the present study includes four main phases: 1) segmentation, 2) feature extraction, 3) learning model (decision tree), and 4) model assessment. In the segmentation phase we use two algorithms, first, the oocyte was identified, and then the required features are extracted with the help of the second algorithm using the Hough transform. In the second phase, the extracted features are used to diagnose oocytes according to 11 different, including cytoplasmic and zona-pellucida abnormalities. This approach is made by wavelet transforming and Fourier-conversion. To this aim, we evaluate some statistics in the Haar wavelet transform domain. In the third phase, the normal oocyte was distinguished using decision tree model learning from the abnormal. Finally, a program was written using the Python programming language that has the ability to distinguish normal from abnormal oocyte.

Results: In this study, using innovative oocyte and cytoplasm algorithms, it was identified with great accuracy. 700 photos were received from Mehr Infertility Center Rasht. In all cases, the designed algorithms succeeded in distinguishing normal from abnormal oocyte. Software was developed using the Python programming language to distinguish normal from abnormal oocyte.

Conclusion: This study reported experimental results on a collection of microscope images of oocytes Indicated the proposed approach's effectiveness. It seems that, measuring the quality of oocytes with image processing helps classify oocytes into normal and abnormal without human intervention.

Key words: *Biomedical image processing, Decision tree, Human oocyte, Intracytoplasmic sperm injection.*

O-33

Does dual trigger with human chorionic gonadotropin and gonadotropin releasing hormone agonist improve the outcome of IVF in poor responder women?

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Background: Poor responder has lower number of follicle and mature oocytes after ovarian stimulation. There are various protocols that have been performed to improve In vitro fertilization (IVF) outcome in poor responders. Dual trigger is one of the triggering protocol to increase the number of retrieved oocytes, the number of mature oocytes, fertilized embryos, implantation and pregnancy rates in normal and high responder women. Several studies have investigated the efficacy of dual triggers in poor responders and the results are still controversial.
Objective: To investigate the efficacy of dual trigger consisted of human chorionic gonadotropin (hCG) plus gonadotropin-releasing hormone agonist (GnRH-a) for final oocyte maturation in increasing number of oocytes retrieved, number of mature oocyte and cleavage embryo in poor responder women.
Materials and Methods: A retrospective analytic study was performed in 260 cycles fulfilling the POSEIDON group 3 and group 4 criteria from January 2018 until October 2019 in Halim Fertility Center IVF Clinic. All poor ovarian responder women underwent modified natural cycle protocol for IVF cycles. Final maturation of oocytes was divided into two groups: group I (114 cycles) received 250 µg of recombinant hCG alone as the single trigger and group II (146 cycles) triggering was done with coadministration of 250 µg of recombinant hCG plus 1 mg GnRH-a simultaneous as dual-trigger. Baseline characteristics and cycle parameters, as well as IVF outcomes of two groups were compared.
Results: In this study, there was no significant difference in the number of retrieved oocytes between the two groups but the number of mature oocytes (MII) was higher in the dual trigger group than single trigger but there was no significant differences between them ($p > 0.05$). The number of fertilized oocytes (2PN) and the number of cleavage embryo were higher in the dual trigger than single trigger groups but there were no significant differences between the two groups ($p > 0.05$).
Conclusion: Dual trigger for final oocyte maturation might improve the outcome of IVF cycles in poor responder women.
Key words: *Dual trigger, Poor responder, In vitro fertilization.*

O-34
The experience and needs of puerperal women who have had a child following assisted reproductive technologies (ART): A qualitative study

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Background: There is a steadily increasing number of newborns born following assisted reproductive technologies (ART), in Europe. Women who became pregnant ART in the Netherlands are under the care of a primary midwife. It is known that during pregnancy, these formal infertile women might have specific experiences such as anxiety and insecurity and paradoxical needs in maternity care. Little is known about how they experience the first few weeks after birth (puerperium).
Objective: The aim of this research is to investigate how women who have had a child after ART experience puerperium and what care needs these women have.
Materials and Methods: From 2017 till 2020, we interviewed sixteen women were interviewed who had a child after fertility treatment by in vitro fertilization or intracytoplasmic sperm injection. This explorative, qualitative study was based on the constructivist paradigm, using a comparison/grounded theory design.
Results: The three themes that emerged from the analysis were 1) the puerperal woman, 2) the caregiver and 3) parenting. The main need of the he puerperal women was to be able to talk about their experiences "when the baby arrived, I just couldn't believe it was my child". From the care provider, they needed understanding "She only had to say one sentence 'so I know it's different for you" coordinated information and continuity of care. The processes that underlie this are the transition to parenthood, insecurities "Oh I think it's all scary", the unreality "I never learned how to take care of a child because I did not believe that a child would come" and gratitude in having a child.
Conclusion: Fertility treatment and the additional uncertainties are mentioned as reasons whether or not to prepare for the puerperium and to have little expectations regarding puerperium. It is important for care providers to be aware of the experiences of the women, to make space for emotions, show understanding and give tailored information and care. In further research, we would like to explore the views of the partners and couples with different ethnic and cultural backgrounds.
Key words: *Intracytoplasmic sperm injection, Puerperium, ART.*

O-35
Immunology and immunotherapy in RIF and recurrent miscarriage

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The most RSA is mainly due to immune factors and Mainly affects 30~40 years old women. Three main players of immune system that affecting implantation are 1) TH1–TH2 balance 2) natural killer cells, and 3) autoantibodies. In pregnancy we have shift toward TH2 dominance and Th2 cells act as an antagonist against

embryo cytotoxicity of Th1 cells. There is no screening tests for Th1 and Th2 and rising progesterone levels can stimulate Th2 and inhibit Th1 secretions. Association between autoantibodies and miscarriage is clear but association between autoantibodies ART failure is inconclusive. In RIF, ACLA and anti-β2glycoprotein1 antibodies are not detect but LAC is more often detected and some evidence suggest that it is reasonable to test these antibodies in RIF. Implantation is interaction between maternal killer immunoglobulin-like receptors (KIRs) (expressed by (uNKs)) and fetal human leukocyte antigen (HLA) (expressed by extravillous trophoblasts). There is different haplotypes for KIR and HLA and if there is haplotype KIR AA and HLA-C2C2, they may lead to RIF, recurrent miscarriage, preeclampsia, and (IUGR). Excessive inhibition of uNK cell may lead to pre-eclampsia and low birth weight. And strong activation of uNK cells can lead to macrosomia. uNK measurement is possible directly by uterine biopsy but it is inaccurate and indirectly by assessment of peripheral blood NK cells (CD56 (majority of these cells)-CD16-CD38). There is dramatic increase in the mid-secretory phase starting 6-7 days after the LH surge but routine testing in RIF is not advised. There is different chimeric fetal cells during pregnancy and H-Y antigene is one of them and is male-specific minor antigens and H-Y antibody produce against it in patients with secondary RPL who had a firstborn male. HLA class II alleles and HLA-G polymorphisms in conjunction with H-Y Antibodies may lead to RPL and RIF (in male and female fetuses) and homosexuality in male fetuses (due to inhibitory effect on the masculinization of the brain). Due to different immunological problems there is different Immunotherapies like Intravenous human immunoglobulin, steroids, anti-TNF drugs, intra lipid, immunosuppressant drugs and Immunization with lymphocytes that is the most studied immunologic treatment for RM and it consiste of PBMCs that were isolated by centrifugation of patients' husband`s blood and administered intradermally. And increase blocking antibody (kind of IgG) that Inhibits lymphocyte reaction and deters the immune system's attack on embryos. And also Increase concentration of TGF-β1 and Restoring balance in the Th1/Th2 and Treg cells. It should use fresh, intradermally, befor, and during pregnancy with low dose (less than 1×10^8 lymphocytesc) and paternal source is better than other sources.

O-36
The serum levels of insulin-like growth factor-1 as a prognostic and diagnostic tool in IVF

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Insulin-like growth factor 1 (IGF-1), is a small single-chain polypeptide, secreted by liver in response to GH. The IGF-1 is expressed in most tissues but has a specific role in amplification of gonadotropin hormonal action during follicular growth and development.we want to discuss about some possible benefit of checking IGF-1 serum level in ART.

Measuring GH levels in the serum may not reflect true GH status as the hormone is released in a pulsatile manner, mainly during the night. We can check IGF-1 serum level in order to determine which women may benefit from GH as an adjuvant therapy. It would also be useful to know whether the baseline serum IGF-1 level has any predictive value in women with normal ovarian reserve. High levels of IGF-1 in day 2 in these patients predict a poorer response than expected based on traditional ovarian reserve markers so, this marker could be used to guide the starting dose and protocol selected for these patients. The most important uses of checking IGF-1 serum level is in poor responders group. The poor responder group demonstrated more than two fold increase in the mean serum level of IGF-1 in cycle day 2 compared with normal responders, and a three fold increase compared with the high responder group . IGF-1>72 ng/ml in day in the poor responder group had 70% sensitivity and 78% specificity for a negative outcome. Cycle day 2 IGF-1 serum levels are predictive for a negative outcome to COH in the poor responders group. We can check IGF-1 serum level for improving IVF outcome and this parameter could be used to: 1) reflect true GH status and AGHD diagnosis to determine which women may benefit from GH as an adjuvant therapy. 2) to predict a poorer response than expected based on traditional ovarian reserve markers. 3) to guide the starting dose and protocol selected for patients. 4) to predict negative outcome to COH in the poor responders group. 5) to determine which women benefit from LE pretreatment.

O-37
Use of menstural blood drived-mesenchymal stem cells in infertility field

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Mesenchymal stem cells (MSC) are highly important in regenerative medicine because of their inherent regenerative properties. They can be harvested from several adult tissues, such as the bone marrow, umbilical cord, peripheral adipose tissue, placenta, menstrual blood, fluid, and amniotic fluid. They are an excellent source of growth factors/ cytokines. Menstural blood-mesenchymal stem cells (MB-MSCs) can be isolated from menstrual blood. These cells have high proliferative, self-renewal, and multiple differentiation potentials. MB-MSCs expressed surface markers CD9, CD29, CD41a, CD44, CD59, CD73, CD90, and CD105; human telomerase reverse transcriptase, etc. It has been

demonstrated that MB-MSCs could differentiate into ovarian tissue-like cell and differentiation of MB-MSCs into germ cells. MB-MSCs were easy to access compared with bonemarrow-MSCs and umbilical-MSCs. Using of HMB-MSC in field of infertility such as POF, endometriosis, and Asherman syndrome has been investigated. Appliance of MSC to treat female infertility such as a Asherman syndrome, endometriosis, premature ovarian failure and poor ovarian responder studied in some article and the researchers believed that MB-MSC might improve infertility in the disease mentioned above by gene expression.

O-38

The effect of autologous platelet-rich plasma on in vitro maturation of immature human oocytes

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Background: Elements in the culture medium can have an increasing effect on the growth and development of follicles and oocytes in the laboratory. In this study, the effect of autologous platelet enriched plasma on the maturation of immature human oocytes in vitro was investigated.

Objective: Blood contains many platelets that can be used enriched. Platelet-rich plasma (PRP) has been used for more than a decade now in a variety of fields, such as surgical wound healing or unhealed fractures. The use of autologous PRP has the advantage that it does not cause allergic reactions in the patient and can be easily used.

Materials and Methods: The follicles were cultured in culture medium for 12 days and the medium contained 5 and 10% of platelet extract .The culture medium was changed every other day and finally, the percentage of survival and growth of oocytes was examined under a microscope at the end of culture. After 12 days of culture, oocytes showed significant growth in environments containing platelet extract and were able to reach the size of mature oocytes in the control environment, but at the end of 12 days of culture, the highest survival belonged to the experimental medium containing 5% platelet and the number of oocytes in other groups showed a significant decrease compared to this experimental group.

Results: As a result of this study, it was shown that PRP improves the culture medium of immature oocytes and is effective in its growth and survival rate.

Conclusion: At the end of the culture, the best survival was related to 5% platelet-rich plasma, but using different doses of PRP and other tests with more immature oocytes are recommended. It seems that platelet can be used as a supplement or a suitable alternative to regular serums.

Key words: *Immature oocytes, In vitro maturation, Plated-rich Plasma.*

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O-39

A novel biallelic missense variant in cyclin B3 is associated with failure of oocyte meiosis II and recurrent fetus triploidy

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Background: Recurrent pregnancy loss (RPL) is an important infertility-related complication affecting up to 5% of clinical gestations. The both parental and embryonal factors are associated with RPL. Triploidy is one of the common chromosomal abnormalities affecting pregnancy and accounts for an important portion of first-trimester abortions. Triploidy has been reported in some cases of RPL but its underlying molecular mechanism remains unknown.

Objective: The aim of this study was to determine the genetic causes of RPL associated with fetus triploidy in an Iranian family.

Materials and Methods: We examined the status of genomic imprinting, short tandem repeat (STR) markers and performed the whole exome sequencing in a family including two sisters with RPL history. Additionally, we assessed oocyte maturation in vivo and in vitro and effect of the candidate protein variant in silico.

Results: Triploidy of maternal origin was confirmed in the aborted fetuses by STR markers genotyping. All the maternally inherited pericentromeric STR alleles were homozygous in the fetuses and oocytes maturation was deficient. A new deleterious missense variant

(c.T4050A, p.V1251D) of the cyclin B3 gene (*CCNB3*) was identified by whole exome sequencing. The homozygous mutation affecting a residue conserved in placental mammals and located in a region that can interact with the cyclin-dependent kinases co-segregated in homozygosity with RPL.

Conclusion: Here, we report a family in which a novel damaging variant in cyclin B3 is associated with the failure of meiosis II in oocyte and recurrent fetus triploidy, implicating a rationale for *CCNB3* testing in RPL patients.

Key words: *Recurrent pregnancy loss, Triploidy, Whole exome sequencing, CCNB3.*

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O-40
Challenges in herbal personalized medicine

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Traditional medicines have been used for years. They are the oldest and most diverse forms of healing that form the foundation of medical systems in many regions of the world. Every continent has its version of traditional medicine. Although modern medicine is the foundation of treatment nowadays, herbal and traditional medicine can help this system in various ways farther than it is estimated. WHO has been studying and working on traditional and herbal medicine for years and defines traditional medicine as "the sum total knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness." and is trying to help the world benefit from its different potentials. The positive benefits of T&M medicine are that they may not be as costly as modern therapies and medications, they are accessible for local communities, making them a vital part of well-being and the belief systems in these parts of the world. The practitioners use plants and herbal elements to treat a wide variety of ailments and diseases. Now it should be noted that the key element in medication in traditional and herbal medicine is that it is a personal based therapy. Because of the lack of genetics knowledge in the older ages the factor interfered to dissociate patients for receiving the proper therapy they need was their phenotypical properties from skin color to height, weight, body mass, and etc. Herbal and traditional medicine does not have a specific pathway to cure an ailment because of the multicomponent structure of the drugs and therapies that are usually built up of a few herbal plants. Therefore comparing it to precision medicine is unsustainable. Precision drugs are medicines that trigger a reaction in the body that is easily measurable and identical. Modern practitioners although using traditional and herbal medicine in their personal life usually don't have the confidence and knowledge needed to interact with the medical situations they confront. In Iranian traditional medicine, ''Mezaj" is a key concept in defining human health and disease. In this view, just as the fingerprints of no two people are the same, the "Mezaj" and composition of no two people are the same, and also in many diseases, certain changes occur in the "Mezaj" of the individual. It is believed that by dividing patients based on the type of disease and considering the individual "Mezaj" and the "Mezaj" of the disease and combining this issue with the specific "Mezaj" of drugs it can be more successful in predicting the effectiveness of the drug or the possibility of side effects. In other words, it is possible to shorten the path to pharmacogenetic goals based on the "Mezaj" phenotype.

Key words: *Herbal and traditional medicine, Pharmacogenomics, Mezaj.*

O-41
Investigating the expressions of miRNA-125b and TP53 in endometriosis: Does it underlie cancer-like features of endometriosis?

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Background: Endometriosis is generally considered as a benign condition, but there is a possibility for it to become cancerous. miR-125b was upregulated in both endometriotic tissues and serum samples of women with endometriosis but its potential targets in endometriosis are still not fully understood.

Objective: The role of miR-125b in the regulation of TP53 expression in endometriosis was tested with a bioinformatics approach. In addition, the expression of miR-125b and TP53 in both eutopic endometrium (Eu-

p) and ectopic endometrium (Ec-p) in endometrium tissues of patients with endometriosis was compared to these in the normal endometrium tissues of controls (Normal).

Materials and Methods: In this case-control study, the eutopic and ectopic samples were collected from 20 patients who underwent laparoscopic surgery and the normal endometrium tissues were collected from 20 controls with no evidence of endometriosis. For bioinformatics approach a protein-protein interaction network was constructed based on co-expressed potential targets of miR-125b. Quantitative PCR technique was used for measurement of miR125b and TP53 expression.

Results: Our results showed that miR-125b was significantly overexpressed in Ec-p. In addition, there was a significant TP53 underexpression in both Ec-p and Eu-p samples compared with normal tissues.

Conclusion: There was a negative correlation between miR-125b and TP53. In addition we observed a noticeable decreased expression of TP53 in both Ec-p and Eu-p samples. These findings may be interpreted as the roles of miR-125b/TP53 axis in the pathogenesis of endometriosis. With the help of bioinformatics analyses we conclude that there is a possible role of miR-125b in cancer-like features of endometriosis.

Key words: *Endometriosis, TP53, miR-125b, Ectopic endometrium.*

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O-42

Evaluation of the miR-144 and its candidate target gene expression in cumulus cells and its impact on in vitro maturation of oocyte in patients with polycystic ovary syndrome (PCOS)

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Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. One of the problems in IVF cycles in PCOS women is predisposing to develop ovarian hyperstimulation syndrome. Therefore, in vitro maturation (IVM) of the oocytes has grown as an alternative treatment. Transcriptomic signatures of cumulus cells (CC) have the potential to serve as valuable non-invasive biomarkers for oocyte competence. Recent studies suggest miRNA involvement in regulating follicular growth, differentiation and development. miR-144 is one of the miRNAs that has been shown to involve in oocyte maturation.

Objective: In this study, the expression level of miR-144 and cyclooxygenase-2 (COX-2) as its candidate target gene was examined in women with PCOS, then its impact on IVM outcome of oocyte was evaluated.

Materials and Methods: A total of 30 cumulus-oocyte complexes with oocyte at GV stage were retrieved from 20 women with PCOS during IVF cycles and cultured in IVM medium for 24 hr at 37°C. After IVM, maturity of oocytes was assessed through morphological criteria and the samples were divided into two groups: matured and unmatured oocytes. The expression level of miR-144 and COX-2 in CCs of each group were detected by qRT-PCR and the relation between the expression level of them and IVM of oocytes was evaluated.

Results: In the 30 retrieved GV oocytes, 18 oocytes (60%) were matured after IVM and placed in matured group, whereas 12 oocytes (40%) could not mature and placed in U group. We found that the expression level of miR-144 was lower (P-value: 0.0008) and the COX-2 mRNA level was higher (P-value: 0.005) in CCs of matured group than in CCs of unmatured group. So, the selected miRNA was related to oocyte nuclear maturation in PCOS women.

Conclusion: We determined that the expression profile of miR-144 and COX-2 were different in CCs isolated from oocytes that could mature after IVM compared with those that could not in PCOS women. Since oocyte competence has an important role in formation of normal zygote and blastocyst, the expression level of this miRNA can be used for predicting oocyte quality before IVM process.

Key words: *Polycystic ovary syndrome, In vitro maturation, miR-144, COX-2, Cumulus cells.*

O-43

Evaluating senescence of amniotic fluid mesenchymal stem cell in different passages by Q-PCR analysis of *FoxM1* gene

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Background: Stem cells are undifferentiated cells that have the ability to transform and differentiate into different cell types. These cells have two important characteristics that differentiate them from others. They have the ability to reproduce unlimited and remain in the undifferentiated state. The most important mature types of these cells are mesenchymal cells, which become more susceptible to accumulation of cell damage with passing time and increase longevity. These damages can help to improving recovery, senescence and finally death of cells. Various factors cause the intrinsic and harmful process of senescence such as internal factors as genetics, the expression of some genes as *P53*, Nuclear factor NF-kappa-B and Forkhead Box M1 (*FoxM1).* free radicals and external factors such as environmental changes that affect the function of the cell. FoxM1 is a member of the Forkhead transcription family, which has been actively involved in regulating organism growth, differentiation and cell proliferation and is important for the expression of cell cycle-dependent genes in the G2 phase.
Objective: The main goal of this study was to evaluate the expression level of FoxM1 gene as a marker of senescence in mesenchymal stem cells isolated from human amniotic fluid at different passages.
Materials and Methods: Totally 37 amniotic fluid samples were obtained from pregnant mothers referred to the PND Department of Yazd Reproductive Sciences Institute. After culturing successive passages and examining the cells morphologically and characterizing them by flow cytometry, their aging status was evaluated in several passages by using beta-galactosidase (X-gal Cinna Gene) staining. After RNA extraction by Tripure kit and cDNA synthesis by Thermo Fisher kit, 10 samples in passages 4 and 7 were evaluated for FoxM1 gene expression change as a marker of aging and GAPDH gene as internal reference using (quantitative PCR) technique. The data were analyzed using GraphPad Prism and SPSS version16 software.
Results: Microscopic examination and staining of beta-galactosidase showed that mesenchymal stem cells isolated from amniotic fluid enter the aging stage in different passages. Comparing the results of *FoxM1* gene expression in different passages (2, 4, and 7), showed a statistical meaningful increase of expression in old cells compared to young cells ($F = 10.43$; $P < 0.001$). Despite the increased expression of *FoxM1* gene in the passage 4 compared to the young cells in passage 2, indicated that there was no significant difference between two groups ($t = 1.134$, $p<0.05$). Comparison of *FoxM1* gene expression in aging cells of passage 7 compared with young cells of passage 2, showed that the increase was statistically significant ($t = 3.758$; $p < 0.003$).
Conclusion: *FoxM1* gene expression in cellular aging has an effective role in preventing cellular aging, and control of aging-related traits includes reducing cell doubling time, regenerative power, and differentiation potential.
Key words: *Mesenchymal stem cells, Amniotic fluid, Cellular senescence, FoxM1 gene expression, Q-PCR.*

O-44
Comparison of polymorphism 139 C> A (rs737008) of protamine 1 gene in infertile men with diagnosis of oligospermia and asthenospermia referred to Gerash Infertility Treatment Center from 2016 and 2017

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Background: The male infertility accounts for about half of infertility in couples. The idiotypic asthenospermia and oligospermia, which mostly occur as a result of genetic mutations, are among the main causes of male infertility.
Objective: Until now, the relationship between different SNPs in the protamine1 (*PRM1*) gene and male infertility has been reported. In this study, we evaluated the possible correlation between 139 C> A (rs737008) SNP in the *PRM1* gene and asthenospermia/oligospermia in patients who referred to the Gerash Infertility Center.
Materials and Methods: Three groups were considered in this study including healthy fertile males, asthenospermia patients, and the patients suffering from oligospermia. After DNA extraction from their blood samples, the PCR was carried out to amplify a 558 bp *PRM1* gene fragment. Then, the RFLP technique was performed to identify the SNP in the PCR products.
Results: Our results showed that the frequency of the 139 C> A (rs737008) SNP in the population study was 41%. We found no significant differences between the SNP and asthenospermia/oligospermia in the current study. According to the demographic data, no significant differences were also found between smoking or alcohol consumption and male infertility in this study.
Conclusion: In this study, no significant relationship between male infertility and the frequency of the rs737008 polymorphism was observed. It seems that a wider investigation on the other SNPs within the protamin gene will help us to provide more reliable information in this context.
Key words: *Polymorphism, Protamin, Asthenospermia, Oligospermia, Male infertility.*

O-45

Exploring the dysregulated mRNAs–miRNAs–lncRNAs interactions associated to idiopathic non-obstructive azoospermia

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Background: Non-obstructive azoospermia (NOA) is the most clinical problem in case of infertility. About 70% of NOA patients are idiopathic with uncharacterized molecular mechanisms.

Objective: This study aimed to analyze the possible pathogenic miRNA-target gene interaction and lncRNA-miRNA association involved in NOA.

Materials and Methods: In the current study, differentially expressed (DE) nRNAs, miRNAs and lncRNAs were determined using the microarray dataset and statistical software R. miRNAs–mRNA and miRNA-lncRNA interactions were identified and the base-pair binding between the seed region of miRNAs and complementary nucleotides in 30 UTR of mRNAs were analyzed. The influence of the validated single nucleotide polymorphisms was described by calculating the minimum free energy (MFE) of the interaction.

Results: A total of 74 mRNAs, 14 miRNAs, and 10 lncRNAs were identified to have significant differential expression in testicular tissue between patients and the fertile group. Four of the DE-mRNAs and all of the reported DE-miRNAs were upregulated. In addition, all of the represented DE-lncRNAs were showed to be downregulated. miR-509-5p and miR-27b-3p were found to interact with target gene polo-like kinase 1 (PLK1) and Cysteine-rich secretory protein2 (CRISP2), respectively. Rs550967205 (A > G) positioned at 30 UTR CRISP2 and rs544604911 (T > C) located at 30 UTR PLK1, with lowest MFE in miRNA-mRNA interaction, were assumed to have possible pathogenic roles linked to spermatogenesis arrest.

Conclusion: The results of the study provide new clues to understand the regulatory roles of miRNAs and lncRNAs in the pathogenesis and diagnosis of idiopathic azoospermia.

Key words: *Azoospermia, mRNA, miRNA, lncRNA, Gene expression.*

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O-46

Testicular expression of TDRD1, TDRD5, TDRD9, and TDRD12 in azoospermia

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Background: Tudor domain-containing proteins (TDRDs) play a critical role in piRNA biogenesis and germ cell development.

Objective: piRNAs, small regulatory RNAs, act by silencing of transposons during germline development and it has recently been shown in animal model studies that defects in *TDRD* genes can lead to sterility in males.

Materials and Methods: Here we evaluate gene and protein expression levels of four key TDRDs (TDRD1, TDRD5, TDRD9 and TDRD12) in testicular biopsy samples obtained from men with obstructive azoospermia (OA, n = 29), as controls, and various types of non-obstructive azoospermia containing hypospermatogenesis (HP, 28), maturation arrest (MA, n = 30), and Sertoli cell-only syndrome (SCOS, n = 32) as cases. One-way ANOVA test followed by Dunnett's multiple comparison post-test was used to determine inter-group differences in *TDRD* gene expression among cases and controls.

Results: The results showed very low expression of *TDRD* genes in SCOS specimens. Also, the expression of TDRD1 and *TDRD9* genes were lower in MA samples compared to OA samples. The expression of TDRD5 significantly reduced in SCOS, MA and HP specimens than the OA specimens. Indeed, TDRD12 exhibited a very low expression in HP specimens in comparison to OA specimens. All these results were confirmed by Western blot technique.

Conclusion: TDRDs could be very important in male infertility, which should be express in certain stages of spermatogenesis.

Key words: *Spermatogenesis, Non-Obstructive Azoospermia, piRNAs, TDRD genes.*

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O-47
Correlation between long non-coding RNA *MALAT1* and *HOTAIR* expression with sperm parameters and MDA level in infertile men

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Background: Infertility is a common complete disorder, which can be caused by oxidative stress. Accumulating evidence suggest that long non-coding RNA (lncRNA) metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1)* and HOX transcript antisense RNA (*HOTAIR)* is involved in the regulation of the oxidative stress responses.
Objective: We aimed to investigate the possible expression status of *MALAT1* and *HOTAIR* in the sperm and its correlation between sperm parameters and malondialdehyde (MDA) levels.
Materials and Methods: Specimens were obtained randomly from 25 fertile men and 25 infertile men, aged between 25-55 yr old. Sperm parameters were evaluated by computer-aided sperm analysis. Sperm chromatin quality were assessed by acridine orange staining method. Seminal MDA levels were determined by thiobarbituric acid reaction method. The expression of *MALAT1* and *HOTAIR* was detected by RT-PCR.
Results: A decreased level of *MALAT1* and *HOTAIR* expression was observed to be associated with the infertile patients ($p < 0.001$). The relative expression level of *MALAT1* and *HOTAIR* were positively correlated with motility and morphology ($p < 0.001$). Meanwhile we found the expression levels of genes were negatively correlated with sperm chromatin damage and MDA levels ($p < 0.001$).
Conclusion: The decreased expression of *MALAT1* and *HOTAIR* resulted in high level of MDA, DNA denaturation and abnormal semen parameters. These findings exhibited the important implications of lncRNAs serving as a potential therapeutic indicator to assess male infertility in assisted reproductive procedures.

Key words: *LncRNA, MALAT1, HOTAIR, Sperm, Infertile.*

O-48
Y chromosome microdeletion in azoospermia fctor region in globozoospermic man

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Background: Thin long tail deletion of Y chromosome is the most common molecular genetic cause of infertility. It is considered to be severe in men which occurs in the three region of the azoospermic factor; AZFa, AZFb and AZFc. These region contain multiple genes involved in spermatogenesis.
Objective: The aim of this study was to investigate the Y chromosome deletion pattern among infertile men with globozoospermic referring to Yazd Infertility Treatment Center.
Materials and Methods: 19 infertile men referred to Yazd Reproductive Science Institute with globozoospermia (from 2014 to 2016) were studied considering microdeletions in Y chromosome. Using multiplex PCR and six different STS (Sequence-Tagged Site) markers microdeletions of Y chromosome in AZFa, AZFb and AZFc regions was analysed.
Results: In our samples, the deletion of AZF regions of the Y chromosomes was not abserved in any blood sample of globozoospermic man.
Conclusion: In 19 samples, no defect was observed in the AZF regions of the Y chromosomes was not the cause of globozoospermia.

Key words: *Male infertility, Globozospermia, Y chromosome deletion, Azoospermic factor, multiplex PCR.*

O-49
Evaluation of the expression level of miR-1271 and its association with the *GRB2* gene expression in tissue samples of patients with endometriosis

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Background: Endometriosis, a relatively prevalent gynecologic disorder, affecting 6 to 10 percent of women in reproductive ages around the globe. Primary recognition can help to decrease its progression and morbidity. Many studies demonstrated that microRNA has a vital role in the pathogenesis of endometriosis. miR-1271 and its direct target gene, *GRB2*, expression have been studied in gynecologic cancers and found to be involved in cell proliferation, migration, and metastasis, while their role in endometriosis has not been studied.

Objective: In this study, we measured *miR-1271* and *GRB2* genes expression in the endometrial tissues of patients (eutopic and ectopic tissues) compared to the control samples.

Materials and Methods: In our study, the endometriosis tissue samples of 15 patients with endometriosis and 15 women without endometriosis were collected. We used quantitative polymerase chain reaction to check the level of *miR-1271* and *GRB2* genes expression in these samples.

Results: We observed a significant decrease in *miR-1271* expression level in both ectopic and eutopic samples of patients with endometriosis compared with control samples, while there was a noticeable increase in the expression level of its target gene, *GRB2*, in tissues of endometriosis patients compared with normal control samples.

Conclusion: We discovered an inverse relationship between the reduction of *miR-1271* expression level and increase in the expression level of *GRB2*. Therefore, increased *GRB2* expression in endometriosis tissues can be due to decreased expression of this microRNA. Our findings suggested that miR-1271 maybe play the role as a biomarker in the diagnosis of patients with endometriosis.

Key words: *Biomarker, Endometriosis, miR-1271, GRB2.*

O-50
Evaluation of the expression level of miR-337-3p and its association with the *RAP1A* gene expression in tissue samples of patients with endometriosis

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Background: Endometriosis, a common and multifactorial disease in women, has different symptoms such as pelvic pain and infertility. Recent studies demonstrated that genetic factors have an important role in its pathogenesis so that dysregulation of many genes and microRNAs have been reported in this disease. Based on previous studies, we know that decreased expression level of miR-337-3p in ovarian and cervical cancers can lead to increase its target genes like *RAP1A*, which plays role in the pathogenesis of these diseases. miR-337-3p expression also downregulated in serum samples of endometriosis patients. However, the role of miR-337-3p and its direct target gene *RAP1A* in endometriosis tissues have not been investigated.

Objective: The goal of this study was to compare the expression level of miR-337-3p and its direct target gene, *RAP1A*, in endometriosis tissues and control samples to find their relationship with pathogenesis of endometriosis.

Materials and Methods: We measured miR-337-3p and *RAP1A* expression levels by quantitative polymerase chain reaction (qRT-PCR) in 15 ectopic and eutopic tissue samples from patients with endometriosis and 15 normal endometrium tissue samples from women without endometriosis.

Results: The results showed a significant increase in the expression level of *RAP1A* gene in the endometriosis tissue samples (both of ectopic and eutopic tissues), while miR-337-3p expression level decreased significantly in these tissue samples compared with the normal endometrium samples.

Conclusion: In this study, we found an opposite relationship between miR-337-3p and *RAP1A* gene expression in endometriosis so that decrease in miR-337-3p expression can lead to increase in *RAP1A* gene expression in endometriosis tissues. Changes in the expression of these genes in our study can also interpret as the role of them in the pathogenesis and progression of endometriosis.

Key words: *Endometriosis, microRNA, miR-337-3p, RAP1A.*

O-51
GM3-synthase (*hST3Gal V*) gene expression in endometriotic tissues

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Background: Endometriosis is a gynecological disease, affects 10%-15% of women in their reproductive ages under the influence of hormonal, genetic, epigenetic, and environmental factors. According to Sampson's theory, endometrial cells are implanted and proliferated outside the uterine cavity, attacking the pelvic structures and causing chronic inflammation. Hence endometriosis can be considered benign cancer. Changes in the cell surface glycosylation is a common phenotype observed during cell differentiation, tissue development cancers, and oncogenesis, a key feature associated with the potentiality of cancer cells for metastasis and invasion. Studies are indicative of changes in the expression of the human ST3 beta-galactoside alpha-2,3-sialyltransferase 5 *(hST3Gal V)* gene, which encodes the GM3 synthase enzyme (the producer of the GM3 ganglioside).
Objective: In this study, we examined changes of *hST3gal V* gene expression in ectopic and eutopic endometrial tissues of women with endometriosis compared with the control group.
Materials and Methods: Samples were collected from 20 women with endometriosis (10 eutopic and 10 ectopic samples) and also 10 normal endometrium samples were enrolled as the control group. Ectopic biopsies were obtained with the use of the laparoscopic procedure, eutopic and control biopsies were obtained with the use of pipelle. RNA extraction and cDNA synthesis were performed for all samples and then gene expression levels were measured by real time-PCR, using designed primers for *hST3Gal V* and also *GAPDH* as the housekeeping gene. Data analysis performed using One-way ANOVA as the statical method. Values were expressed as mean ± SEM and the results were considered significant at the level of $p < 0.05$.
Results: Results showed that the *hST3Gal V* gene expression was reduced in eutopic samples than control group ($p = 0.538$) gene and *hST3Gal V* gene expression in ectopic samples was reduced than both eutopic and control groups ($p = 0.696$ and $p = 0.153$, respectively).
Conclusion: Results shows a decrease in the gene expression profile of *hST3Gal V* in endometriotic samples. Since GM3 ganglioside is a substrate for the extension and branching of other gangliosides, it seems that the lower expression of the *hST3Gal V* gene can be involved in the etiology of the disease. This study is limited by the number of samples in each group. Further studies with larger samples numbers can provide more accurate results in this regard.
Key words: *Endometriosis, hST3Gal V, Gm3 Synthase, Ganglioside, Gene expression.*

O-52
Endometrial scratching affects gene expression of *NLRP3* in patients with unexplained repeated implantation failure: A randomized control trial

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Background: Alternative strategies have been used to augment success rate of implantation in IVF/ICSI cycles in unexplained repeated implantation failure patients. Endometrial scratching is one of these procedures. It seems scratching can affect *NLRP3* gene expression which has an important role on receptivity of endometrium. NLRP3 is an intracellular sensor that detects a broad range of harmful sterile or infectious stimuli, resulting in the formation and activation of the *NLRP3* inflammasome
Objective: In the present study, we investigated whether gene expression of *NLRP3* (*NOD-*, *LRR-* and *pyrin domain-containing protein 3*) is affected by endometrial injury during proliferative phase of menstrual cycle before embryo transfer.
Materials and Methods: Twenty women with unexplained repeated implantation failure who failed to conceive during three or more IVF/ICSI cycles and embryo transfer were selected. The patients randomly classified into two study groups (N = 10 in each group). In the intervention group (not in the control group), endometrial scratching was done on day 9-13 in the proliferative phase of the preceding menstrual cycle. Then, endometrial biopsies of the intervention and control groups were performed in the luteal phase (on 19-21 day). The RNA of all samples was extracted and cDNA synthesis was performed. The expression of *NLRP3* was quantified by quantitative real-time PCR.
Results:*NLRP3* gene expression from all samples was investigated. Relative expression of *NLRP3* was lower in the intervention samples compared to the controls.
Conclusion: The inflammasome components are suggested as a novel family of endometrial biomarkers. This result is in consistent with other studies that showed dysregulated inflammasome activation has involved in the disruption of maternal–fetal immune-tolerance and in pregnancy complications.
Key words: *Endometrial scratching, NLRP3, Unexplained repeated implantation failure.*

O-53
The influence of single blastomere biopsy on human embryo expansion and pregnancy result

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Background: The use of preimplantation genetic testing (PGT) in fresh and frozen intracytoplasmic sperm injection cycles and the possible damage is still unclear. Studies on aspects of this method, such as the prevalence of expansion on day 5 and pregnancy rate, are limited.
Objective: This study aimed to assess the rate of embryo expansion on day 5 in PGT patients and particular developmental components (expansion stage, inner cell mass, and trophectoderm) of euploid blastocysts influence on pregnancy outcomes.
Materials and Methods: A total of 433 embryos from 115 patients from intracytoplasmic sperm injection with or without PGT using fluorescence in situ hybridization method for X, Y, 13,18, and 21 chromosomes in fresh or freeze cycles between february 2018 and June 2020 was evaluated. The zona pellucida of fresh embryo transfer patients as a control group was untreated. In the PGT group, 6-8 cell embryos on the day 3 with grade A were hatched by laser, and extract one blastomere for PGT. Following evaluation, embryo transfer was done on day 5. Statistical analyzes were performed using SPSS 23 and $p < 0.05$ was considered statistically significant.
Results: In embryos that screened with X, Y, 13,18, and 21 probes in the fresh and freeze PGT cycles, more euploid embryos reached blastocyst with expansion 3, 4, and 5 ($p < 0.001$). Single blastomere biopsy (SBB) in PGT groups increases blastocyst expansion grade, and pregnancy outcomes compare with blastocyst embryos without blastomere biopsy and PGT ($p < 0.01$). Embryos with an expansion grade A compared with C had a higher pregnancy rate ($p < 0.01$). Blastocysts with a trophectoderm and inner cell mass grade of A or B compared with C had a higher likelihood of pregnancy rate ($p < 0.01$).
Conclusion: Among euploid embryos, expansion grade is the best predictor of sustained implantation; however, a composite score of embryo morphology on day 5 may provide additional guidance. Therefore, this investigation shows that the laser zona hatching may positively affect embryo expansion grade and pregnancy rates.
Key words: *Preimplantation genetic testing, Zona pellucida, Fluorescence in situ hybridization.*

O-54
Investigation of immunosuppressive-immunomodulatory markers in amniotic fluid-derived mesenchymal stem cells from women who experienced recurrent pregnancy loss

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Background: The amniotic fluid contains a heterogeneous population of different cells that are produced prior to the gastrulation process. Therefore, it is expected that mesenchymal stem cells derived from the amniotic fluid will have high plasticity between mature and pluripotent stem cells. Due to unique features of these cells such as high cloning potential, high self-renewal capacity along with chromosomal stability and low immunogenicity as well as anti-inflammatory and immune-modulating properties, it has attracted more and more attention from researchers.
Objective: The aim of this study was to investigate the immunosuppressive genes in mesenchymal stem cells isolated from amniotic fluid of women with a history of recurrent pregnancy loss (RPL) and the effect of gamma interferon as an immunological stimulus on the expression of these genes.
Materials and Methods: The study group included pregnant women with a history of unexplained RPL. The control group consisted of pregnant women with at least one healthy child, no history of miscarriage, and normal hormonal and immunologic profiles. In this study, mesenchymal stem cells (MSCs) isolated from amniotic fluid from RPL and non-RPL women. On the other hand, each cell line was examined under 5 different treatment groups, control and 4 groups with 20 and 100 IU IFN-γ per ml of medium over two periods of 24 h and 72 h. Finally, the relative mRNA expression level of immune-suppressive/modulator gene including two indole amine-2 and 3-dioxygenase 1 and 2 in AF-MSCs in both groups were evaluated and compared using Q-PCR.
Results: The average expression of candidate gene *IDO1* and *IDO2* showed a significant increase in the RPL group rather than non-RPL, specially under treatment with 100 IU IFN-γ and after 24 h. Interestingly, expression of both genes *IDO1* and *IDO2* decrease after 72 h in RPL and non-RPL groups ($p = 0.05$).
Conclusion: Immunosuppression by MSCs, which is currently recognized as a powerful tool in preventing acute rejection, graft therapy, and regenerative medicine, is not an inherent potential but is induced by environmental factors. Various studies have identified that some potential causes of unexplained RPL are due to immunological factors. The results of this study,

especially for indole amine-2 and 3-dioxygenase genes, do not rule out such a possibility. Despite the unknown role of AF-MSCs in the abortion mechanism, the results of this study suggest that there is a significant difference between the mRNA level of understudied genes between AF-MSCs in the RPL and non-RPL group. Due to the absence of such a similar study, it cannot be fully interpreted, however, these cells appear to represent genetic compartments of couples with a history of RPL that may defective in immunological factors. However, planning for further investigation of these uncertain immunological mechanisms appears to be valuable in the future.

Key words: *Recurrent pregnancy loss, Immunosuppressive gene, Mesenchymal stem cells, Amniotic fluid, Quantitative gene expression.*

O-55
Evaluating cell free DNA in spent embryo culture media in cleavage and blastocyst stage

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Background: Chromosomal abnormalities are one of the most important causes of failure in in vitro fertilization. Preimplantation genetic testing can be a way to prevent the transfer of aneuploid embryos. It entails the use of invasive techniques to obtain embryonic DNA, with major technical limitations and ethical issues today. Therefore, the use of new non-invasive methods is a suitable solution to this problem. One of the non-invasive methods is to use the embryo spent culture medium. The origin of cell free DNA in embryo spent culture medium is trophectoderm cells and the internal cell mass.

Objective: Cell-free genomic DNA in the embryonic culture medium can be a non-invasive method for genetic assessment.

Materials and Methods: This study reviewed 25 spent embryo culture mediums. The spent culture medium used between day 3 and day 5 of embryonic development. Patients were undergoing intracytoplasmic sperm injection, and each embryo was in one drop of culture medium. We had two control samples: the culture medium contaminated with purified DNA from human blood and the culture medium without embryonic development. All samples were evaluated with nanodrop for dsDNA and ssDNA concentration. Among the collected medium, ten samples (group 1) concentrated by heating, then evaluating *SRY* and *FMR1* genes with real-time polymerase chain reaction (RT-PCR) (group 1). Six samples were three days, and four samples were five days. The rest of the samples were classified into three groups. The cell-free DNA from the medium was purified with the blood DNA extraction kit. in group 2 with Genet bio kit, group 3 with YTzol pure DNA kit (yekta Tajhiz), and group 4 with High Pure Viral Nucleic Acid extraction kit (Roche). They evaluated by RT-PCR. Nine samples were three days, and six samples were five days.

Results: Although cell-free DNA was confirmed in the samples using nanodrop (with a range of 160 to 225 ng per microliter), the cycle of threshold did not observe in the RT-PCR product of group 1. The Purified samples were amplified in group 2,3 and 4 for *SRY* and *FMR1* genes with RT-PCR and observed only acceptable cycle of threshold in the fourth group.

Conclusion: The high protein and solutes in the culture medium and the low amount and quality of DNA are restrictive. For better results, it is necessary to purify the genomic DNA and amplify it with precise kits. Our research is underway to improve DNA collection, amplification, and testing to isolate genomic DNA.

Key words: *Cell free DNA, Spent embryo culture media, Preimplantation genetic testing.*

O-56
In vitro implantation of euploid and aneuploid embryos

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Background: Although embryo selection for transfer is usually based on morphology, 70% of embryos with high morphological quality have chromosomal abnormalities. The results of implantation and pregnancy rate assessments following preimplantation genetic screening (PGS) are controversial. There is still no in vitro study to compare the implantation of human euploid and aneuploid embryos.

Objective: This study was designed to compare the ability of aneuploid embryos to attach to endometrial cells with euploid embryos by simulating the human endometrium using a three-dimensional scaffold.
Materials and Methods: After informed consent, 10 endometrial biopsies were taken from fertile women. Endometrial cells were isolated and expanded in 2D cultures to achieve enough cells. The fibrin-agarose scaffold was made and stromal cells were cultured into the scaffold, after 24 hr, the epithelial cells were seeded on the scaffold. Cell culture continued for 5 days to reach the appropriate confluence. Then, cell proliferation was assessed by MTT assay. The simulated endometrial construct was confirmed by H&E and immunohistochemistry (IHC). The embryos were also examined by performing PGS following conventional comparative genomic hybridization array. 10 euploid and 10 aneuploid blastocysts were selected for co-culturing. Partial hatching of blastocysts was performed using a laser system. Blastocysts were co-cultured with the 3D structure of human endometrial cells for 72 hr. The blastocyst's attachment to the endometrial-like structure was examined under a phase-contrast microscope and scanning electron microscopy.
Results: The MTT OD of scaffolds increased during 5 days of cell culture ($p < 0.05$). The histological evaluation of the co-culture systems was done under light microscopy by H&E staining. On the top of the 3D culture system, epithelial cells shaped a constricted cell monolayer. Stromal cells combined with the fibrin-agarose scaffold got lengthened and expanded, displaying that the 3D culture systems supplied a suitable environment for the growth of endometrial cells. In the 3D culture, the origins and locations of epithelial and stromal cells were defined by cytokeratin and vimentin immunostaining, respectively. IHC for cytokeratin was only positive for epithelial cells in the surface epithelium. IHC for the vimentin was positive for the stromal cells located in the 3D matrix. These results showed that fibrin-agarose scaffold could simulate the human endometrial structure. Using scanning electron microscopy and phase-contrast microscopy, it was found that only euploid embryos were able to attach to the endometrial construct while aneuploid embryos weren't.
Conclusion: Our findings determined that PGS allows us to transfer top-quality embryos with higher implantation potential. It improves implantation and pregnancy rate during assisted reproductive technologies cycles, especially in patients with recurrent implantation failure.
Key words: *Three-dimensional culture, Implantation, Human endometrial cells, Aneuploid and euploid embryos, CGH array.*

O-57
Non-invasive preimplantation genetic diagnosis (PGD) for X-linked disease by sex determination through cell-free DNA

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Background: Preimplantation genetic diagnosis (PGD) is a useful clinical tool to identify embryos with or at risk of specific genetic malady before embryo implantation. Current procedures for embryo chromosomal screening require an invasive biopsy of the embryo. Blastomere biopsy has a potential lesion to the embryos may result in developmental defects or abortion. Thus, a non- invasive PGD is needed.This study hypothesized that embryonic DNA is present in the spent culture medium. We focused on X-linked disorders, these single-gene diseases due to the presence of defective genes on the X chromosome are dominant in males.
Objective: Therefore, the objective of this study was to discriminate between female (XX) and male (XY) embryos by detecting Y chromosome- specific genes in cell-free DNA and comparing to PGD results. It opens a new window for the development of a non-invasive PGD method.
Materials and Methods: Embryo´s spent media from day 3 and day 5 embryos development were collected. The modified phenol-chloroform solution was used for DNA extraction from spent media. DNA from spent media was evaluated using SRY, TSPY, and AMELOGENIN as targets using the qPCR method. IBM SPSS and Medcals were used for statistical analyses, to compare sex determination of embryos using spent medium with PGD results.
Results: Yield and purity of the extracted DNA as well as repeatability of the method were performed well using the modified phenol-chloroform solution. The amount of DNA at day 5 embryo culture medium was significantly higher than day 3. Results of sex determination using spent medium by Q-PCR were consistent with the results of PGD and 12th wk sonography. This invasive PGD method using a spent culture medium gave a sensitivity of 66.7%,

specificity of 100%, positive predictive value of 100%, and negative predictive value of 67.6 (N = 56, Nxx = 23, Nxy = 33).

Conclusion: This investigation provides a potentially effective procedure that can help to avoid the invasive preimplantation genetic diagnosis, especially about X-linked diseases. Results of sex determination using spent medium by Q-PCR were consistent with the results of PGD. Improvements in DNA collection, amplification, and testing may allow for PGD without biopsy in the Future.

Key words: *PGD, X-Link diseases, cfDNA.*

Poster Presentations

P-1

Effects of maternal voluntary wheel running during pregnancy on the neonatal rat ovary

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Background: Regular maternal exercise in pregnancy enhance the physiological, metabolic, and psychological health of mother and fetus. Probing the effects of maternal exercise in gestation, on the developmental programming of pups all over intrauterine and postnatal life, is known as a novel and favorable research field.

Objective: The purpose of the present study was to evaluate the effects of maternal voluntary wheel running during mid or late gestation on rat neonatal estrogen and progesterone plasma concentration; ovarian development and its angiogenesis; and development of the primary oocyte, primordial follicle, and their apoptosis.

Materials and Methods: 21 female Wistar rats were accidentally distributed into experimental groups (doing exercises during the 2nd and 3rd wks of pregnancy, n = 14) and control (n = 7). In the exercise groups, each rat had access to a running wheel (diameter = 34.5 cm, width = 9.5 cm) that was embedded in their cage and during the 2nd and 3rd wk of pregnancy, it rotated freely during the resistance of 100 g. In this regard, it is notable that each wheel was linked to a counter that recorded its rotations. Pregnant rats in the control group were put in the cages without any access to a running wheel. After birth, the neonate's blood was obtained and the estrogen and progesterone concentration were evaluated. Thereafter, the ovaries were removed and used for histological investigations and apoptic assessment.

Results: A significant increase was found in estrogen and progesterone concentration in neonates of experimental groups ($p = 0.001$). The experimental groups had an increased ovarian diameter ($2^{nd}W$: $p = 0.044$ and $3^{rd}W$: $p = 0.005$) and angiogenesis ($2^{nd}W$: $p = 0.003$ and $3^{rd}W$: $p = 0.001$). In addition, significant enhances were seen in the number ($2^{nd}W$: $p = 0.017$ and $3^{rd}W$: $p = 0.035$) and diameter ($2^{nd}W$: $p = 0.046$ and $3^{rd}W$: $p = 0.004$) of primordial follicles as well as in the diameter of primary oocytes ($2^{nd}W$: $p = 0.037$ and $3^{rd}W$: $p = 0.019$) of the experimental groups compared to the control group. Moreover, maternal voluntary wheel running reduced the number ($2^{nd}W$: $p = 0.001$ and $3^{rd}W$: $p = 0.001$) of apoptotic primordial follicle in the experimental groups compared to the control group.

Conclusion: It was shown that maternal voluntary wheel running of the pregnant rats during mid or late gestation increase estrogen and progesterone plasma concentrations, and ovarian size and its angiogenesis in neonates. Furthermore, this type of exercise increases the primordial follicle/primary oocyte numbers and diameters as well as oocyte nuclei, while inversely decreases the numbers of apoptotic primordial follicles.

Key words: *Apoptosis, Exercise, Neonatal, Ovary, Rat.*

P-2

The effect of cysteine and glutamine on human sperm functional parameters during vitrification

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Background: Assuming the adverse effects of reactive oxygen species (ROS) on sperm function, this study was conducted to assess the effects of cysteine and glutamine as effective antioxidants on human sperm parameters under vitrification.

Objective: The present study aimed to investigate the protective effect of cysteine and glutamine on motility parameters, plasma membrane potential, mitochondrial membrane potential, DNA damage and human sperm intracellular ROS during vitrification.

Materials and Methods: Twenty normozoospermic samples were used. The samples were subjected to a vitrification process and cysteine (5 and 10 mM) and glutamine (10 and 15 mM). The sperm motility parameters, mitochondrial membrane potential (MMP), plasma membrane integrity (PMI), DNA damage and intracellular ROS damage were assessed for each sample.

Results: Statistical analyses showed that motility, mitochondrial membrane potential and DNA damage decreased in the vitrified groups with cysteine 5, 10 mM and glutamine 10, 15 mM separately. Also intracellular ROS increased significantly compared to the fresh group ($p < 0.05$). No significant differences were observed for PMI compared with the fresh group ($p > 0.05$). Supplementation of cysteine and glutamine in both concentrations separately decreased intracellular ROS and DNA damage of spermatozoa with significant increase in PMI, MMP and progressive motility compared to vitrified control group ($p < 0.05$).

Conclusion: The results showed no significant effect of a specific concentration in cysteine and glutamine on sperm parameters compared to other concentrations. Both amino acids have the potential to improve the

harmful effects of freezing on sperm parameters.

Keywords: *Cysteine, Glutamine, Human, Sperm parameters, Vitrification.*

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P-3
Bone morphogenetic protein 15 induces differentiation of mesenchymal stem cells derived from human follicular fluid to oocyte-like cell

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Background: Follicular fluid (FF) is essential for developing ovarian follicles. Besides the oocytes, FF has abundant undifferentiated somatic cells containing stem cell properties, which are discarded in daily medical procedures. Earlier studies have shown that FF cells could differentiate into primordial germ cells via forming embryoid bodies, which produced oocyte-like cells (OLC).

Objective: This study aimed at isolating mesenchymal stem cells (MSC) from FF and evaluating the impacts of bone morphogenetic protein 15 (BMP15) on the differentiation of these cells into OLCs.

Materials and Methods: Human FF-derived cells were collected from 78 women in the assisted fertilization program and cultured in human recombinant BMP15 medium for 21 days. Real-time polymerase chain reaction and immunocytochemistry staining characterized MSCs and OLCs.

Results: MSCs expressed germline stem cell (GSC) markers, such as OCT4 and Nanog. In the control group, after 15 days, OLCs were formed and expressed zona pellucida markers (ZP2 and ZP3), and reached 20-30 μm in diameter. Ten days after induction with BMP15, round cells developed, and the size of OLCs reached 115 μm. A decrease ranged from 0.04 to 4.5 in the expression of pluripotency and oocytespecific markers observed in the cells cultured in a BMP15-supplemented medium.

Conclusion: FF-derived MSCs have an innate potency to differentiate into OLCs, and BMP15 is effective in promoting the differentiation of these cells, which may give an in vitro model to examine germ cell development.

Key words: *OLC, Follicular fluid, Bone morphogenetic protein 15, Mesenchymal stem cell.*

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P-4
Exploration of couple's experiences of long-term marital satisfaction: A qualitative study

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Background: Marital satisfaction is a multidimensional phenomenon, which refers to the quality of marital relationship, or the general view of marriage status and reflection of happiness and marital performance. Repetition of certain positive behaviors can make a big difference in the success of continued married life, and that awareness of such behaviors seems to be critical to recognizing certain warnings.

Objective: This study with qualitative approach conducted to promoting long term marital satisfaction by exploring couple`s experiences.

Materials and Methods: This study was conducted using descriptive phenomenology method. The participants were 12 person (six couples) with a history of 20-30 yr of married life expectancy and a marital satisfaction score of above 65. The data were collected by purposeful sampling and semi-structured interviews, analyzed using Colaizzi method. By categorizing the codes, subcategories, and main categories were extracted.

Results: An analysis of the experiences of the participants resulted in emergence of eight subcategories, and three main categories: "strong foundation for living together", "mutual commitment to protecting marital cohesion", and "striving to improve sexual relations".

Conclusion: A long-term marriage associated with a variety of variables, including a strong foundation for living together, a mutual commitment to protect marital cohesion, and an effort to improve sexuality. And the results showed that the type of relationship will change during the years after marriage in a way that takes on certain meanings and concepts and can be interpreted in physiological, cultural and other specific contexts.

Key words: *Marital satisfaction, Long married life, Phenomenology.*

P-5

Potential therapeutic effect of bee pollen and metformin combination on testosterone and estradiol levels, apoptotic markers and total antioxidant capacity in a rat model of polycystic ovary syndrome

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Background: Polycystic ovary syndrome (PCOS) is associated with metabolic disorders as well as infertility. Many traditional remedies have been reported to show estrogenic and antioxidant potential. Bee pollen (BP) is a natural compound, reported as one such remedy.

Objective: The present study aimed to investigate the effects of BP extract (BP) and metformin (MET) on estradiol (E2) and testosterone (T) levels, apoptotic markers, and total antioxidant capacity (TAC) in a rat model of PCOS.

Materials and Methods: In this experimental study, 54 female Wistar (n = 6/group) rats received 2 mg of estradiol valerate (EV) intramuscularly and 6 additional rats were considered the control without EV injection. The rats were treated with BP (50, 100, and 200 mg/kg), MET (300 mg/kg) and BP+MET (50 BP+300 MET, 100 BP+300 MET, and 200 BP+300 MET mg/kg). Serum levels of E2 and T were assessed by the ELISA method. TAC of serum was also determined. The expressions of *Bcl2*, *Bax*, *Caspase3 (Cas-3)*, and *Sirt-1* genes were evaluated by real-time polymerase chain reaction (PCR). Data were statistically analyzed using one-way ANOVA.

Results: In the untreated PCOS group E2 and T levels ($p < 0.01$), and Bcl2 ($p = 0.007$) expression were increased, but TAC ($p = 0.002$) and expression of Bax ($p = 0.001$), Cas-3 and Sirt-1 ($p < 0.01$) were decreased significantly. The levels of E2 and T, as well as the expressions of Bcl2, were decreased in all treated groups compared to the untreated PCOS group ($p < 0.01$). On the other hand, TAC and expression of Bax, Cas-3and Sirt-1 were increased in the BP- and MET-treated groups ($p < 0.05$).

Conclusion: BP and MET synergistically improved serum E2, T, and TAC levels, and expression of apoptotic genes.

Key words: *Metformin, Apoptosis, Bee pollen, Estradiol, Polycystic ovarian syndrome.*

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P-6

Prevention of uterine fibrosis in rabbit model by intrauterine stem cell conditioned media injection immediately after endometrial curettage

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Background: Uterine fibrosis or Asherman's syndrome is a uterine acquired disorder with symptoms of implantation disturbances, menstrual irregularities and abortion.

Objective: The main goal of this study was evaluation of the effects of conditioned media of bone marrow-derived mesenchymal stromal/stem cells (BM-MSCs) in prevention of uterine fibrosis immediately after uterine curettage in rabbit.

Materials and Methods: This study included 12 female rabbits (24 uterine horns in total) were randomly divided into four groups of intact negative control, curettage positive control, stem cell therapy, and stem cell conditioned media injection in the way that two corresponding uteri from a rabbit were assigned in different groups.

Results: The BM-MSC-conditioned media treated uterus showed regenerated endometrial layer compared with lower diameter of endometrium in the control group. It showed that BM-MSC-conditioned media play a positive role in the regeneration of the uterine wall. Area of fibrotic tissue in the treated groups was lower than the control groups.

Conclusion: Injected stem cell conditioned media had preventing effect on occurrence of uterine fibrosis. Therefore, BM-MSC-conditioned media can be suggested to be injected immediately after endometrial curettage.

Key words: *Mesenchymal stromal/stem cell, Conditioned media, Uterine fibrosis.*

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P-7

Evaluation of the effect of folic acid and nicotinic acid on malondialdehyde levels of semen in Oligospermia men after cryopreservation

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Background: Reactive oxygen species and free radicals are one of the most important detrimental factors on sperm quality, especially during the freezing process. One of the important factor is reactive oxygen species which increases the lipid proxidation of cell membranes.
Objective: In this study, an attempt was made to measure the concentration of malondialdehyde levels in semen in oligospermia men before and after cryopreservation process and to evaluate the effect of folic acid and nicotinic acid on the malondialdehyde concentration after freezing.
Materials and Methods: For this purpose, semen fluid sample was collected from 25 oligospermia men in the age range of 25 to 45 yr. Each sample was divided into 5 groups: fresh group, freeze group without antioxidants (control), freeze group with nicotinic acid (10 mM), freeze group with folic acid (50 nM) and freeze group with a combination of nicotinic acid (10 mm) + folic acid (50 nM). The concentration of malondialdehyde was measured in nmol/ml in each group.
Results: Our study showed that the concentration of malondialdehyde in the semen increased after freezing compared to before freezing ($p > 0.001$). Also, the concentration of malondialdehyde in the group of folic acid + nicotinic acid was lower compared to other groups after freezing ($p > 0.001$).
Conclusion: The combination of folic acid and nicotinic acid antioxidants with sperm freezing medium reduced the level of Malondialdehyde and lipid peroxidation of sperm membrane during the freezing process and thereby maintains the fertility potential in oligospermia men.
Key words: *Cryopreservation, Sperm, MDA, Nicotinic acid, Folic acid.*

P-8

Effects of nicotinic acid and Folic acid on sperm motility, Viability and DNA integrity in oligospermia men during cryopreservation

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Background: Sperm freezing is an important technique for treating infertility and maintaining sperm, but because of the increased production of reactive oxygen species (ROS), threats such as chromatin damage and sperm DNA, threaten sperm motility and consequently reduce fertility. Antioxidants are important compounds to minimize the deleterious effects of freezing sperm and maintaining fertility potential.
Objective: The aim of this study was to investigate the effect of nicotinic acid and folic acid antioxidants on the sperm motility, survival and deoxyribonucleic acid of oligospermia men during cryopreservation.
Materials and Methods: For this purpose, 25 semen samples of Oligospermia men who referred to the Fertility and Infertility Center of Shahid Beheshti Hospital in Isfahan province were randomly taken into sterile containers and after fluidization, sperm parameters including morphology, motility, and concentration, life and quality of chromatin and their DNA were Measure according to WHO criteria. Then, each sample was divided into 4 parts with freeze-dried medium for freezing: antioxidant-free (control group), 50 nM folic acid, 10 mM nicotinic acid, 50 nM folic acid + 10 mM nicotinic acid. After freezing, the samples were thawed and reexamined for sperm parameters.
Results: Our study showed that the process of freezing sperm by damaging the spermatozoa and cell membrane, resulted in decreased motility and competence of sperm to fertilize with oocytes and decrease the fertility potential of males ($p < 0.001$).
Conclusion: The use of folic acid and nicotinic acid antioxidants during sperm freezing reduces the harmful effects of free radicals created during the freezing process and helps preserve the fertility potential of Oligospermia men ($p < 0.001$).
Key words: *Folic Acid, Nicotinic Acid, Deoxyribonucleic Acid, Oligospermia, Cryopreservation.*

P-9

Effects of monosodium glutamate on apoptosis of germ cells in testicular tissue of adult rat: An experimental study

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Background: Monosodium glutamate (MSG) is used as a flavoring and food seasoning. Some studies have reported the oxidative effects of using this substance on various tissues.
Objective: This study has investigated the effects of MSG and the protective effect of vitamin C (Vit C) on apoptosis of testicular germ cells and biochemical factors.
Materials and Methods: In this experimental study, 24 adult male Wistar rats were randomly divided into four groups: control (received distilled water), Vit C group (150 mg/kg), experimental group 1 (MSG 3 gr/kg), experimental group 2 (MSG 3 gr/kg + Vit C 150 mg/kg). The rats were gavaged for 30 days and then were sacrificed, the right testis was isolated for

biochemical examinations for the glutathione, malondialdehyde, and left testis used in histological experiments. Tunnel staining was used to determine the number of apoptotic cells.

Results: The results showed that apoptotic cells in the MSG group had a significant increase compared to the control group (p = 0.001), but the number of these cells in the MSG co-administered with Vit C and Vit C groups was significantly lower than the MSG group. Germinal epithelial thickness also decreased in the MSG group compared to the control group.

Conclusion: MSG can lead to increase apoptotic changes in the germinal epithelial of the testicle, and Vit C as an antioxidant can modify the pathological and biochemical changes induced by MSG.

Key words: *Apoptosis, Monosodium glutamate, Rat, Testis, Vitamin.*

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P-10

Cryoprotective effect of pentoxifylline on spermatogonial stem cell during transplantation into azoospermic torsion mouse model

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Background: Preserving the spermatogonial stem cells (SSCs) in a long periods of time during the treatment of male infertility using stem cell banking systems and transplantation is an important issue.

Objective: This study was conducted to develop an optimal cryopreservation protocol for SSCs using 10 mM pentoxifylline (PTX) as an antioxidant in basal freezing medium.

Materials and Methods: Testicular torsion - a mouse model for long-term infertility- was used to transplant fresh SSCs (n = 6), fresh SSCs treated PTX (n = 6), cryopreserved SSCs with basal freezing medium (n = 6) and cryopreserved SSCs treated PTX (n = 6). 8 wk after transplantation, samples were assessed for proliferation (through evaluation of MVH and ID4 markers) and differentiation (via evaluation of c-Kit and SCP3, Tnp1, Tnp2, and Prm1 markers).

Results: Morphological and flow cytometry results showed that the SSCs were the population of cells able to form colonies and to express ID4, α6-integrin and β1-integrin markers, respectively. We found positive influence from PTX on proliferative and differentiative markers in SSCs transplanted to azoospermic mice.

Conclusion: In the recipient testis, donor SSCs formed normal spermatogenic colonies and sperm. These data indicate that adding the PTX is an effective way to efficiently cryopreserve germ cells enriched for SSCs in cryopreservation, and this procedure could become an efficient method to restore fertility in a clinical setup, but more studies are needed to ensure its safety in the long term.

Key words: *Male infertility, Testicular torsion, Spermatogonial stem cells, Transplantation, Pentoxifyllin.*

P-11

The effect of low-dose aspirin on the pregnancy rate in frozen-thawed embryo transfer cycles: A randomized clinical trial

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Background: The results of previous studies on the effect of low-dose aspirin in frozenthawed embryo transfer (FET) cycles are limited and controversial.

Objective: To evaluate the effect of low-dose aspirin on the clinical pregnancy in the FET cycles.

Materials and Methods: This study was performed as a randomized clinical trial from May 2018 to February 2019; 128 women who were candidates for the FET were randomly assigned to two groups receiving either 80 mg oral aspirin (n = 64) or no treatment. The primary outcome was clinical pregnancy rate and secondary outcome measures were the implantation rate, miscarriage rate, and endometrial thickness.

Results: The endometrial thickness was lower in patients who received aspirin in comparison to the control group. There were statistically significant differences between the two groups (p = 0.018). Chemical and clinical pregnancy rates and abortion rate was similar in the two groups and there was no statistically significant difference.

Conclusion: The administration of aspirin in FET cycles had no positive effect on the implantation and the chemical and clinical pregnancy rates, which is in accordance with current Cochrane review that does not recommend aspirin administration as a routine in assisted reproductive technology cycles.

Key words: *Aspirin, Embryo transfer, Pregnancy rates.*

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P-12

The ameliorating effect of hydroalcoholic extract of date palm (Phoenix Dactilifera L.) fruit on formaldehyde reproductive toxicity of male NMRI mice

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Background: Formaldehyde (FA) is one of the most widely used materials in industrial and sciences. Prolonged contact with FA might have harmful effects on fertility due to increasing the reactive oxygen species level. On one hand, date palm (Phoenix Dactilifera L.) fruit extract (DPFE) contains a high concentration of natural antioxidants that could scavenge free radicals.

Objective: The aim was to investigate the prophylactic effects of DPFE, with strong antioxidant properties, on FA-induced testicular toxicity in male mice.

Materials and Methods: Thirty-two adult NMRI male mice were randomly divided into four groups: control group (CTL; distilled water, orally, 35 days), FA group (FA; 0.25 mg/kg intraperitoneally (i.p.), 20 days), treatment group (DT+FA; DPFE, 4 mg/kg, for 35 days followed by FA administration, 0.25 mg/kg, i.p., 20 days), and date fruit extract group (DT; DPFE, 4 mg/kg, orally, 35 days). After this period, the blood collected and left epididymis and testis tissues were isolated to evaluate the sperm parameters and histological examination, respectively.

Results: The FA administration increased the sperm morphological anomalies and reduced the sperm count, viability and motility and also testosterone versus the control group ($p < 0.001$). In addition, histological studies of the testes showed that FA causes changes in the testis seminiferous tubules such as destruction of germinal epithelium and vacuolization of the tubules. The DPFE consumption before FA administration could partially ameliorate the reduced testosterone, sperm and testicular parameters due to FA.

Conclusion: The date palm fruit extract use might have discount effects on FA-induced testicular toxicity.

Key words: *Formaldehyde, Date fruit, Testis, Sperm, Testosterone.*

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P-13

The role of HLA-G in recurrent pregnancy loss: A case-control study

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Background: Human leukocyte antigen (HLA)-G is the main molecule for maternal acceptance of the semi-allogenic fetus by adjusting the maternal immune system in the pregnancy.

Objective: The aim of this study was to determine the role of sHLA-G in recurrent pregnancy loss (RPL) in North of Iran.

Materials and Methods: This case-control study was done on two different groups including 40 women with recurrent miscarriage, and 40 non-pregnant healthy women. Soluble HLA-G (sHLA-G) levels were measured using a BioVendor sHLA-G ELISA kit.

Results: Findings show that women with recurrent abortion had significantly higher sHLA-G concentrations than fertile women (mean ± SD: 220.62 ± 223.48 u/ml and 87.77 ± 91.65 u/ml, respectively $p < 0.0001$, Mann-Whitney test).

Conclusion: There is many argumentation about the role of HLA-G in the pregnancy and RPL. Therefore document in this context remains obscure. So it can be concluded that sHLA-G may not act in the implantation of the embryo, but its role in the preservation of maternal tolerance to fetus, because serum sHLA-G level increased in the after abortion and postpartum in both women who had recurrent spontaneous abortion and normal vaginal delivery.

Key words: *HLA-G, Recurrent pregnancy loss, Early pregnancy, Reproduction.*

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P-14

Protective effect of the co-administration of testosterone and sodium hydrosulfide on testicular H2S levels and serum testosterone in experimental model of varicocele

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Background: Androgen secretion is reduced in varicocele. Hydrogen sulfide (H2S), is known as an antioxidant and antiapoptotic molecule.
Objective: This study aimed to assess the effects of co-administration of testosterone and NaHS on sperm count, H2S levels in testicular tissues and serum testosterone in varicocele-induced male rats.
Materials and Methods: Adult male rats were randomly assigned to 5 groups: sham, varicocele, varicocele+testosterone, varicocele+NaHS, varicocele+tetstosterone+NaHS. In the varicocele groups, the left renal vein was partially ligated. In treatment groups, five wk after the induction of varicocele, testosterone (200 µg/kg, subeffective dose) was given subcutaneously for four wk and NaHS (15 µmol/L in drinking water, subeffective dose) were given for four wk. The Left testis tissue samples resected for evaluation H2S levels. The left epididymis tissue also resected for sperm count. blood samples were taken from the inferior vena cava.
Results: Varicocele caused significant reduction in sperm count, testicular H2S levels and serum testosterone compared with the sham group. Administration of testosterone+NaHS significantly increased these parameters compared with varicocele group. But there were no significant changes in these parameters in varicocele+NaHS and varicocele+testosterone group compared with the varicocele group. However, there was a significant enhancement in serum testosterone levels in varicocele+testosterone group compared with the varicocele group but this enhancement was lesser than varicocele+tetstosterone+NaHS group that may due to synergistic effect of NaHS and testosterone.
Conclusion: This study suggested that long term testosterone and NaHS co-administration could improve testicular H2S levels and serum testosterone in varicocele male rats. Therefore, testosterone+NaHS appears to be a useful treatment against varicocele.
Key words: *Varicocele, Testosterone, Hydrogen sulfide, Testicular H2S levels, Serum testosterone.*

P-15
The effectiveness of mindfulness-based cognitive therapy on sexual function in reproductive age

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Background: Sexuality is an important part of the human life.
Objective: The aim of this study was to investigate the effectiveness of group counseling mindfulness-based cognitive therapy on sexual function of reproductive age women.
Materials and Methods: This study is a parallel randomized controlled trial with pre-test, post-test and follow-up. Fifty reproductive age women in randomly allocated in two intervention and control group. For intervention group (25 persons) 8 sessions of mindfulness intervention (90 minutes weekly) was done and control group received routine clinic services. FSFI questionnaire were complete by two groups before, after and one month after intervention. Data analysis was performed using SPSS 24 and $p < 0.05$.
Results: In intervention group main score of FSFI were reported 22.43 ± 5.66, 26.43 ± 4.96 and 26.26 ± 4.57 before, after and one month after intervention respectively and in control group were 24.00 ± 5.66, 18.50 ± 5.46 and 18.83 ± 5.35 before, after and one month after intervention respectively. The results of the study show that group counseling mindfulness-based cognitive therapy has a meaningful effect on sexual function of on women of reproductive age.
Conclusion: Mindfulness counseling significantly improve sexual function of reproductive age women.
Key words: *Sexual function, Mindfulness, Reproductive age women, FSFI.*

P-16
Antioxidant effects of royal jelly on lead induced sperm DNA damage and sperm abnormality in male mice

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Background: Royal jelly, a secretion vintage of the salivary glands of worker bees, is an extremely impressive antioxidant and possesses eminent free radical scavenging trait. It has been shown to have anti-tumor, antibiotic, anti-inflammatory, immunomodulatory and as well as antioxidant properties. Royal jelly has remarkable positive effects on reproductive system and fertility. On the other hand, lead, which is widely used in industry, can be detected in foods, drinking water, ambient air, dust, and various cosmetics products. Its toxicity causes excess levels of reactive oxygen species (ROS) and cellular oxidative stress. More importantly, our knowledge of the effects of lead on sperm DNA integrity is significantly limited.
Objective: We investigated the effect of Royal jelly against reproductive toxicity caused by lead exposure in mice.
Materials and Methods: Male mice received lead acetate (50 mg/kg) and plus Royal jelly (25, 50 or 100 mg/kg) via oral gavage for 28 days. Adult male mice were divided into five groups (n = 5). Caudal

epididymal sperm characteristics, lipid peroxidation and in vitro fertilizing capacity were evaluated after 4 weeks.

Results: Lead acetate-intoxicated mice exhibited testicular tissue injury and decreased serum levels of SOD, TAC, testosterone and increased serum level of MDA and nitric oxide. The count, viability, motility and normal morphology of the sperms were decreased in lead-induced group. Royal jelly prevented testicular injury, increased serum levels of SOD, TAC and improved the semen quality and decreased serum level of MDA and nitric oxide. However, Royal Jelly can reduce the regulation of Bax and Caspase-3 pro-inflammatory factors in lead-treated mice by reducing oxidative stress.

Conclusion: Our findings showed that Royal jelly with its antioxidant effects reduces inflammation and cell death in testis and sperm DNA structure following exposure to lead. However, further studies are needed to illuminate other mechanisms of Royal jelly's effect on testis function.

Key words: *Royal jelly, Lead acetate, Sperm, Caspase3, Bax.*

P-17

Correlation between serum TGF-ß levels and recurrent implantation failure during implantation window in women undergoing IVF treatment

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Background: Frequent implantation failure is a common problem among women who underwent in vitro fertilization (IVF) procedure. Therefore, it is necessary to know the factors affecting recurrent implantation failure following IVF treatment.

Objective: The aim of present study was to investigate the relationship between serum TGF-ß levels and recurrent implantation failure during the implantation window in women undergoing IVF.

Materials and Methods: This study was performed on 39 patients including 20 women with recurrent implantation failure (case group) and 19 women with successful pregnancies in the first IVF cycle (control group). Serum TGF-ß levels were measured using enzyme-linked immunosorbent assay (ELISA) method. Demographic information including age, body mass index (BMI) and number of implantation failure were recorded.

Results: The mean serum levels of TGF-ß in the individuals with recurrent implantation failure was significantly lower than the control group (663.48 pg/ml vs. 1028.49 pg/ml). Moreover, the serum TGF-ß levels were significantly different among case and control groups based on the age and BMI grouping. However, there was no relationship between serum TGF-ß levels in the case group and age, BMI and number of implantation failures.

Conclusion: Serum TGF-ß levels may play a crucial role in the physiopathology of recurrent implantation failure. Measurement of this factor in the patients with recurrent implantation failure is recommended which may reduce the incidence of recurrent implantation failure following IVF treatment. However, further randomized clinical studies are required to clarify the definite correlation.

Key words: *Recurrent implantation failure, TGF-ß, In vitro fertilization.*

P-18

L-carnitine reduces inflammation and oxidative stress in mouse ovarian tissue following autotransplantation

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Background: Transplantation of ovarian tissue is a fertility restoration technique in patients undergoing chemotherapy and radiotherapy. A major issue associated with ovarian transplantation is ischemia/reperfusion injury that leads to depletion and apoptosis of follicles. L-carnitine has antioxidant and anti-inflammation properties and can therefore be used to reduce ischemic damages.

Objective: The aim of this study was to investigate the effect of L-carnitine injection on transplanted mouse ovarian tissue.

Materials and Methods: The Naval Medical Research Institute (NMRI) mice at the age of 4-5 weeks, were divided randomly into groups of: Control, autograft and autograft + L-carnitine (200 mg/kg daily intraprotoneal injections). Seven days post ovary autografting, serum levels of Malondialdehyde (MDA), total antioxidant capacity, tumor necrosis factor alpha (TNF-α), interleukin (IL)-6 and IL-10 were measured. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey test, and the means were considered significantly different at $p < 0.05$.

Results: A significant increase was found in the serum level of IL-6, TNF-α and MDA in the autograft group compared to the control counterpart whereas the mentioned parameters reduced significantly in the autograft+L-carnitine group. The Total antioxidant capacity and the serum level of IL-10 also revealed a significant decrease in the autograft group when compared to the control while they significantly increased in the autograft+L-carnitine group.

Conclusion: L-carnitine could reduce oxidative stress and inflammation following mouse ovarian tissue transplantation.

Key words: *Ovary transplantation, L-Carnitine, Ischemia-reperfusion, Inflammation.*

P-19

Assessment of sperm parameters in type 1 and 2 diabetes mellitus male mice C57

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Background: Diabetes mellitus could have multiple effects on various organs of the body. One of the organs that are sensitive to these effects is testis and spermatogenesis process.
Objective: Aim of this study was to compare the effects diabetes type 1 (DM1) and type 2 (DM2) on sperm parameters.
Materials and Methods: Forty male mice C57 (8 weeks; 22 gr) were divided into 4 groups (n = 10/each). Mice were fed with standard-chow diet except DM2 group that was fed with a 60%-kcal high-fat diet for 8 weeks. Furthermore, sham group received a single dose of sodium citrate buffer (0.005 mg/kg) as soluble of streptozotocin (STZ), DM1 group was induced by multiple low-dose injections of STZ (45 mg/kg/day for 5 consecutive days), and DM2 group after four weeks was given a single dose of STZ (110 mg/kg). After eight weeks, the mice were sacrificed and sperm was extorted from the cauda epididymis for tests on sperm parameters.
Results: This study showed that the effects of diabetes on sperm parameters were compared between groups. The mean percentage of sperm non progressive motility significantly was higher in DM2 group than control group ($p = 0.05$), however sperm total motility difference between groups wasn't remarkable. Moreover, the mean percentage of sperm concentration was lower in DM1 group compared to other groups ($p < 0.02$).
Conclusion: Sperm parameters in type 1 and 2 diabetes mellitus male mice C57 could effect on reproductive system. This result showed that reduction of sperm concentration and progressive motility of sperm in DM1 and DM2 model mice were lower compared to control group.
Key words: *Diabetes mellitus, Sperm parameters, Infertility.*

P-20

Comparison the scores of couples' sexual knowledge and attitude before and after sexual education

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Background: Sexual health education is an essential human need. This education enriches their sexual knowledge and information, and help them to understand their responsibilities, commitments and rights in order to be able to achieve an enjoyable life.
Objective: The purpose of this study was to assess the effect of educational package on couples' sexual knowledge and attitude.
Materials and Methods: An interventional study was performed on a sample of 160 couples referred to premarital counseling center in Kashan City, Iran. Couples were divided randomly into the intervention and control groups (n = 80/each couples). Intervention group received an educational program in ten sessions on weekends for three consecutive weeks and control group received routine program. Sexual knowledge and attitude scale was completed before, after intervention, and in 6 months follow up. The data were analyzed by independent Student's *t* test, Mann Whitney U, Kruska-wallis, Chi-square, and ANOVA in SPSS V16.
Results: Results showed no significant differences in demographic characteristics and sexual knowledge and attitudes between two groups before intervention. The results demonstrated that after intervention and six months follow up, the scores of sexual knowledge increased significantly in the intervention group compared to control group. Mean score of sexual knowledge in intervention and control groups after intervention was 21.72 ± 7.73 and 14.08 ± 7.16 ($p < 0.001$) and after six months follow up was 21.89 ± 7.01 and 15.52 ± 7.73 ($p < 0.001$), respectively. In addition, the results demonstrated after intervention and six months follow up scores of sexual attitudes increased significantly in the intervention group compared to controls. Mean score of sexual attitudes in intervention and control groups after intervention was 134.90 ± 12.84 and 122.62 ± 14.15 ($p = 0.001$) and after six months follow-up was 134.22 ± 13.43 and 124.78 ± 13.48 ($p = 0.001$), respectively.
Conclusion: Sexual educational programs can be effective on couple's sexual knowledge and attitude.
Key words: *Couples, Sexual health, Educational program, Marriage, Attitude.*

P-21

Correlation between sexual knowledge and couple relationship among cardiovascular patients

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Background: Lack of good couple relationship can lead to reduced health, lifespan, satisfaction with marital life, and disruption of growth and excellence of couples.
Objective: The study aimed to evaluate correlation

between sexual knowledge and couple relationship quality among cardiovascular patients.

Materials and Methods: This correlational study was conducted on 200 cardiovascular patients with myocardial infarction or patients undergoing coronary artery bypass grafting during the past two to five months. They referred to the Department of Rehabilitation of Shahid Beheshti Hospital of Kashan, Iran in 2017. Patients who met the inclusion criteria filled the individual characteristics questionnaire, couple quality Relationship scale and sexual knowledge scale. Data analysis was performed in SPSS version 22 using *t* test, analysis of variance, as well as Pearson correlation coefficients. P-value less than 0.05 was considered statistically significant.

Results: In this study, the mean score of participants' age was 52.68 ± 6.88 and 62% of them were male. The score of sexual knowledge scale of the participants was 55.85 ± 11.61 of total score 100 and mean scores of quality relationship scale was 17.82 ± 8.66 of total score 33. According to the results, there was a significant and positive correlation between the score of sexual knowledge scale and the score of quality relationship scale ($r = 0.530$, $p < 0.001$).

Conclusion: Results showed that the sexual knowledge score and couple relationship score in participants was moderate, and promotion of the sexual knowledge in cardiovascular patients can improve their couple relationship.

Key words: *Sexual knowledge, Couple relationship, Cardiovascular patients.*

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P-22
Validity and relibility of the persian version of the sexual quality of life-male questionnaire

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Background: A valid and reliable instrument that can evaluate the impact of sexual dysfunction on quality of life in men is needed.

Objective: The aim of this study was to evaluate the psychometric properties of sexual quality of life-male (SQOL-M) questionnaire in a sample of Iranian men.

Materials and Methods: In this cross-sectional study, using a standard "forward-backward" translation technique, the English language version of the SQOL-M questionnaire was translated into Persian. One hundred and forty eight men (21-57 yr old) that referred to a health center in Kashan city were enrolled in this study. Exploratory and confirmatory factor analysis, convergent and known groups validity by using the international index of erectile function (IIEF) and content validity were assessed. The reliability was evaluated by test re test reliability correlation coefficient (ICC).

Results: Exploratory and confirmatory factor analysis confirmed a one-factor solution with good item-total correlations. Convergent validity showed total score of the international index of erectile function and its subscales were correlated with scores of the the SQOL-M questionnaire. Evaluating known groups validly showed men without erectile dysfunction scored more than men with erectile dysfunction ($p < 0.001$). Content validly was performed by 10 specialists. Reliability evaluation was demonstrated excellent internal consistency and test-retest reliability (Cronbach's alpha and intraclass correlation coefficient were 0.94 and 0.95 respectively).

Conclusion: The results of the study showed the Persian version of SQOL-M instrument has a good structural characteristic and is a valid and reliable tool for measuring the SQOL-M.

Key words: *Sexual quality of life, Men, Reliability, Validity, Erectile dysfunction.*

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P-23
Evaluation the effects of vitamin D supplementation of the extender on sperm quality after freeze-thaw process in normozoospermic and asthenozoospermic Holstein bulls

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Background: Asthenozoopermia is a usual male infertility factor, characterized by decreased sperm motility. There is evidence that vitamin D regulates widespread biological function.

Objective: This study aimed to evaluate the effects of vitamin D on sperm kinematics and apoptosis in normozoospermic and asthenozoospermic bulls' semen after the freeze-thaw process.

Materials and Methods: The effect of vitamin D on sperm quality factors such as sperm kinematic, sperm

plasma integrity, acrosomal membrane integrity, reactive oxygen species (ROS) and apoptosis statues following freezing and thawing process in asthenozoospermic bulls were examined. For this purpose, 32 semen samples of four Holstein bulls (normozoospermic, progressive motility > 70%) and 32 semen samples of four bulls (asthenozoospermic progressive motility < 40%) were collected. Then, the poll semen samples of each group (normozoospermic and asthenozoospermic) were diluted into four equal aliquots of extender containing different vitamin D concentrations (0, 5, 10, and 50 ng/mL) and aspirated into 0.5 mL straw. Semen straws were frozen in liquid nitrogen. After thawing, sperm kinematics parameters, viability, plasma membrane integrity, acrosome integrity, apoptosis statues, and ROS production levels were evaluated.

Results: The percentage of sperm motility and viability were significantly higher in 50 ng/mL of vitamin D in both groups ($p < 0.05$). Normozoospermic bull semen samples had significantly higher curvilinear and average path velocity levels in 50 ng/mL vitamin D groups compared to the control group ($p < 0.05$). However, no significant differences were observed in post-thaw sperm kinematics parameters in asthenozoopsrmic samples. No significant differences were identified in membrane integrity and acrosome integrity in both normozoospermic and asthenozoospermic samples. The percentage of early-apoptosis ($p = 0.049$) and late-apoptosis ($p = 0.005$) in the asthenozoospermic group were significantly higher than the normozoospermic group. Generally, in the asthenozoospermic group, the level of ROS production was significantly higher ($p = 0.049$) compared to the normozoospermic samples.

Conclusion: According to our results, it can be concluded that the vitamin D supplementation of the asthenozoospermic semen extender had no significant effect on the quality of semen after the freeze-thaw process.

Key words: *Vitamin D, Apoptosis, Sperm kinematic, Asthenozoospermic, Normozoospermic.*

P-24

The effect of alpha lipoic acid on human sperm cryopreservation

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Background: Nowadays, infertility problems are dramatically elevating around the world. In this regard, assisted reproductive techniques are developing productively. The cryopreservation technique of sperm cells is one of the common daily processes in infertility centers. However, the freezing-thawing process induces the production of reactive oxygen species (ROS) which is strongly harmful to sperms and reduces their quality post thawing procedure. Accordingly, the addition of some antioxidants to the sperm freeze medium is one way to come over this problem.

Objective: In the current study, considering the therapeutic effect of alpha-lipoic acid (ALA) on improving sperm parameters in the literature, we decided to investigate its impact on preserving freeze-thaw sperms from ROS damages.

Materials and Methods: 20 normozoospermic samples were obtained from the Isfahan Fertility and Infertility Center. Different concentrations of ALA (0, 0.05, 0.1, 0.2, 0.4, 0.8, and 8 mM) were added to the sperm freeze medium to gain the best concentration. With the optimum concentration, its' protective impact on sperm motility and DNA fragmentation was investigated.

Results: 0.2 mM of ALA showed the best effect on sperm motility. Consequently, assessment of sperm DNA damage was carried out before and after the thawing procedure with and without using the optimal concentration of ALA. Our result indicated a significant reduction in DNA damage at the presence of ALA (0.2 mM, $p < 0.05$).

Conclusion: ALA could have a cryoprotective effect on sperm motility and DNA damage through its' antioxidant capacity and its ROS scavenging capacity.

Key words: *Alpha lipoic acid, Freez-thaowing, DNA damage.*

P-25

Anti-cancer properties of ethanol extracts of grey mangrove leaves on a breast cancer cell line

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Background: The breast cancer, as the most incident cancer in women, is rising up the concerns of women mortality worldwide. Herbal medicine showed to be effective in treatment of oncological disorders. Mangrove includes several kinds of phytochemical compounds including terpenoids, alkaloids, flavonoids, saponins, and glycosides which some of them shown to have anti-cancer effects.

Objective: In the current study, the anti-cancer effects of ethanolic (EtOH) extract of the grey mangrove (Avicennia marina) leaves of the Persian Gulf on a breast cancer cell line was evaluated.

Materials and Methods: The leaves of A. marina were collected from Asaluyeh mangrove forest, shores of the Persian Gulf, Iran. The EtOH extract of A. marina leaves were provided according to standard procedures. The phytochemically analysis including total phenolic

and flavonoid contents were measured. In addition, the extracts were analyzed by gas chromatography-mass spectroscopy (GC-MS) and their compounds were analyzed. The extracts were used for in-vitro study. MTT analysis, population doubling time assay, and western blot analysis were performed on MCF-7 breast cancer cell line and Vero cell line. IBM SPSS Statistics 26 and GraphPad prism were used for statistical analysis. The mean differences between groups were analyzed by one-way ANOVA and post hoc Tukey test.

Results: The GC-MS analysis showed that there was 12 potent anti-cancer, anti-proliferation, and anti-oxidant compounds in EtOH extract. Four of these compounds including levoglucosan, 2,4-Di-Tert-Butylphenol, Pentadecanoic acid, and linoleic acid showed to have anti-cancer effects on MCF-7 cell line in previous studies. The MTT proliferation assay showed that the 120 and 160 µg/mL concentrations of ETOH extract had lower value than the control group ($p < 0.05$). Moreover, this result in Vero cell line was only seen in 160 µg/mL concentration ($p < 0.05$). In contrast with other concentrations, total cell counts of the 120 and 160 µg/mL concentrations was significantly lower than control group in all 7 days ($p < 0.01$). Furthermore, in Vero cell line, the 120 µg/mL concentration at day 3, 4, 5 and 7, and the160 µg/mL concentration at day 4 to 7 had lower value than the control group ($p < 0.01$). However, at the MCF-7 cell line, the cell viability rate of 160 µg/mL concentration was only different from control group at day 2 ($p < 0.01$). Moreover, at the Vero cell line, the 120 and 160 µg/mL concentrations had lower value than the control group at day 5 ($p < 0.001$ and $p = 0.041$, respectively). Additionally, the western blot analysis of the EtOH extract showed that the Bax, Cleaved-caspase-1, Cleaved-caspase-3 and Cleaved-caspase-7 proteins' expression were significantly higher than the control group; represented that A. marina EtOH extract induced apoptosis in MCF-7 cell line.

Conclusion: The anti-cancer effects of EtOH extract of A. marina leaves at the concentrations of 120 and 160 µg/mL was shown through anti-proliferation and apoptosis of MCF-7 breast cancer cell line.

Key words: *Mangrove, Avicennia, Anti-cancer, Apoptosis, Ethanol.*

P-26

The comparison of depression and anxiety between fertile and infertile couples: A meta-analysis study

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Background: Depression and anxiety are 2 of the most common reactions in infertile couples. Several studies have been conducted to examine the psychiatric disorders among infertile and fertile couples.

Objective: This meta-analysis was conducted to compare the depression and anxiety in fertile and infertile couples in various studies.

Materials and Methods: The authors searched articles published in multiple databases including World Health Organization, PubMed, Cochrane Library, Scopus, Science Direct, Medline EMBASE and Persian databases including SID and Iran Medx between 2005 and 2017. The main keywords used for searching the databases were: Depression, anxiety, infertility, and fertility. Statistical analyses were performed using comprehensive meta-analysis /2.0 software.

Results: The authors found 42 related articles after searching the databases. 11 articles entered the meta-analysis after considering the inclusion and exclusion criteria. Finally, eight articles were chosen for the comparison of depression and anxiety, two published articles for the comparison of depression, and one published article to compare anxiety in fertile and infertile couples. The results of the heterogeneity test showed a significant heterogeneity among all articles that were analyzed in this meta-analysis in the field of depression and anxiety. The results showed that depression ($p = 0.0001$; Hedges'g = 1.21; 95% CI 0.63-1.78) and anxiety ($p = 0.00001$; Hedges'g = 0.63; 95% CI 0.54-0.73) were higher in infertile couples than fertile couples and that the possibility of a publication bias does not exist in this study.

Conclusion: The analysis of articles used in this meta-analysis showed that depression and anxiety scores in infertile couples were higher than fertile couples.

Key words: *Depression, Anxiety, Infertility, Meta-analysis.*

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P-27

Waiting anxiety in infertile women referring to Yazd Infertility Center

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Background: Infertility has become a serious problem in societies, which may cause inevitable harm to the mental health in individuals. People suffering from their illness may experience increased anxiety if they wait for some time to receive services.
Objective: The present study was conducted to determine the status of waiting anxiety in infertile women.
Materials and Methods: This descriptive-analytic study was conducted on 200 infertile women who consulted the Infertility Center of Yazd for treatment in 2017. The method of sampling was conducted based on convenience sampling (availability sampling). Data were collected with waiting anxiety questionnaire. Statistical analysis was done by using SPSS software (version 16.0). The analysis included: (1) descriptive statistics [mean and standard deviation], (2) chi-square, Student's *t* test, ANOVA, and Pearson correlation coefficient.
Results: The total mean of waiting anxiety in infertile women was 20.69 ± 5.82. Based on the results, the mean of the dimensions were as follows: cognitive dimensions (5.31 ± 2.25), physiologic dimensions (5.24 ± 2.55), emotional dimensions (5.01 ± 2.13) and behavioral dimensions (14.32 ± 2.03). The results also showed that a significant relationship between the total mean of waiting anxiety and cognitive, physiology and behavioral dimensions with duration of infertility exists ($p < 0.05$). In addition, there was a significant relationship between the mean scores of behavioral dimensions with the duration of marriage ($p = 0.04$) and education ($p = 0.015$).
Conclusion: The results of this study showed that infertile women who consulted to the centers were in a moderate condition in terms of waiting anxiety. Therefore, designing and performing effective interventions to reduce the anxiety of infertile women is recommended.
Key words: *Infertility, Women, Waiting anxiety.*

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P-28

Dietary fat and minerals intake are related to semen quantity and quality in men referring to an Iranian Reproductive Sciences Institute: A cross sectional study

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Background: Some epidemiological studies have reported a relationship between infertility and lifestyle patterns including dietary habits.
Objective: Our objective was to identify the relation between sperm parameters and dietary fatty acid and mineral intake among Iranian infertile men.
Materials and Methods: This cross sectional was performed on 400 newly diagnosed infertile men in Yazd Reproductive Sciences Institute from July to December 2019. Men were recruited when their infertility was confirmed by the expert andrologist based on World Health Organization criteria. They delivered a semen sample and answered a 168 items semiquantitative food frequency questionnaire. All data were analyzed using SPSS V. 22 software. P-value less than 0.5 considered as significant.
Results: We found a positive association between poly-unsaturated fatty acid intake, total motility, and normal morphology ($p = 0.03$). Also, there was a significant negative association between the second quartile of sodium and calcium intake and sperm volume (ptrend: 0.04), compared with the first quartile.
Conclusion: We concluded that dietary of poly-unsaturated fatty acid intake, sodium and calcium intake are related to sperm morphology, volume and total motility in Iranian infertile men. However, more research is needed to confirm these relations and provide the evidence needed to exert these findings into clinical practice.
Key words: *Sperm parameters, Male infertility, Fatty acid, Minerals.*

P-29

Food groups intake and sperm variables in men referring to an Iranian Reproductive Sciences Institute: A cross sectional study

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Background: Infertility had an increasing trend between couples in the world. Several factors such as unhealthy dietary habits are associated with sperm abnormality.

Objective: This study was conducted to investigate the association between food groups intake and sperm variables in men referring to an Iranian Reproductive Sciences Institute.
Materials and Methods: 400 infertile Men 20-55 yr of age admitted to an Iranian Reproduction Research Institute, were selected for this cross-sectional study according to the World Health Organization Fifth Edition Laboratory Guidelines. Usual dietary intake was collected by using a 168 items semiquantitative food frequency questionnaire. The relationship between food groups and sperm factors was measured by a multiple linear regression model while other confounding variables were adjusted. All data were analyzed using SPSS V. 22 software. P-value less than 0.5 considered as significant.
Results: According to this study, after adjusting for potential confounders, there was a significant relationship between sperm count with refined grains and soft drink, a significant association between normal morphology with whole grains, low-fat dairy intake and fruit, semen volume is significantly related to red meat intake, low-fat dairy, fruit and tea intake and progressive motility had a significant association between progressive motility with whole grains, low-fat dairy, fruit, soft drink and coffee intake (p-trend < 0.05).
Conclusion: We concluded that there is a relationship between grains, dairy, fruits, meat, caffeine and tea dietary intake with sperm parameters, which are sometimes in line or in contradiction with the results of previous studies.
Key words: *Diet, Male infertility, Food groups, Semen analysis.*

P-30
Upregulation of elafin expression in the fallopian tube of ectopic tubal pregnancies compared to the normal tubes

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Background: Ectopic pregnancy is one of the most important causes of maternal mortality and fallopian tubes are the location of 95% of ectopic pregnancies. Elafin is a natural antimicrobial molecule that plays an important role as an anti-inflammatory agent in mucosal surfaces and has been found in the female reproductive tract.
Objective: The aim of this study was to investigate elafin expression, in the fallopian tube mucosa of ectopic pregnancies compared to the normal tubes using immunohistochemistry techniques and quantitative reverse transcription (qRT-PCR).
Materials and Methods: In this case-control study, uterine tube samples were obtained from patients with an indication for surgical removal of the tubes. The case group (n = 20) consisted of patients who were undergoing salpingectomy due to an ectopic pregnancy, the control group (n = 20) included patients who had a salpingectomy and hysterectomy. Using qRT-PCR and immunohistochemistry, the expression of elafin was investigated in both study groups.
Results: Immunohistochemical expression of elafin in the epithelium and connective tissue was significantly increased in the implantation site of the patients in comparison with the control group ($p < 0.001$). The level of elafin mRNA increased in the mucous membrane of the fallopian tube from patients with the ectopic pregnancy compared to the normal mucosa ($p < 0.001$).
Conclusion: Increasing expression of elafin during an ectopic pregnancy may be a mechanism for enhancing innate immune response and be involved in related pathological conditions such as infection and ectopic implantation.
Key words: *Elafin, Ectopic pregnancy, Immunohistochemistry, Reverse transcriptase quantitative PCR.*

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P-31
Emotions towards potential genetic offspring among oocyte donors: A cross sectional study

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Background: The presence of maternal emotions towards the offspring resulting from assisted reproductive techniques (ART) has been previously reported in oocyte donors. However, there is limited information about the presence of these emotions in oocyte donors during the ART process and before pregnancy.
Objective: The aim of this study was to evaluate these emotions of women towards the potential genetic offspring and tocompare them with women treated with ART by using own oocytes.
Materials and Methods: A cross sectional study was conducted on 150 women who were divided into two

groups of oocyte donors and those treated with ART and using autologous oocyte. At the time of oocyte retrieval and using a validated questionnaire, the emotions toward potential offspring (EPO) resulting from ART and its three dimensions (including imagination, sense of ownership, and importance of treatment outcome) were measured and compared in two groups.

Results: Out of 150 women, the mean score of EPO was 39.34 in oocyte donors and 49.52 in own oocyte women. Comparison of the EPO in the two groups showed that the emotions in all three dimensions were lower in oocyte donors than the other group ($p < 0.0001$). Moreover, in oocyte donors, the mean score of the scale of the importance of treatment outcome dimension was higher than the other two scales ($p < 0.0001$).

Conclusion: The results of the study showed that there is a significant emotion toward the potential offspring in oocyte donors. The presence of these emotions thus should be considered in formulating the ethical principles of ART by using oocyte donation.

Key words: *Oocyte donation, Emotion, Offspring, Assisted reproductive techniques.*

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Reproductive health literacy and its relationship with some demographic factors in men referring to one infertility center in Mashhad, Iran

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Background: Infertility has been one of the most important problems of human societies throughout history, which has always caused many problems for people, depending on the perspective of societies. This disorder, which is defined as infertility after one year of unprotected sex, has directly and indirectly affected about 10% of the world's population. Men have rarely been the focus of research and their knowledge and attitude have been less studied. Researchers must consider the importance of each couple's awareness of infertility issue and also the fact that before any intervention in the field of health literacy and necessity to be aware of the current situation.

Objective: The present study aimed to investigate reproductive health literacy and attitudes toward infertility in men referring to one infertility center in Mashhad in 2019 and its relationship with variables such as age, education, occupation, income, duration of infertility and history of assissted reproductive therapy (ART).

Materials and Methods: In this cross-sectional study, men with infertility who referred to one infertility center in Mashhad in 2019 were entered by convenience sampling. Data collection tools included questionnaires of reproductive health literacy and attitudes toward fertility, as well as a checklist of demographic information. The reproductive health literacy questionnaire includes 20 questions and the attitude questionnaire includes 5 questions. Relationship between age, education, occupation, income, duration of infertility and ART history with reproductive health literacy and attitude was investigated in SPSS V. 20 with *t* test, ANOVA and linear regression with a significance level of less than 0.05. The present study has been approved by the ethics committee of the medical school of the Islamic Azad University of Mashhad with the code IR.IAU.MSHD.REC.1397.020.

Results: Mean and standard deviation of age and duration of infertility in 196 men included 32 ± 6 yr (21 to 60) and 3.11 ± 1.36 yr (1 to 7), respectively. Reproductive health literacy score (3.2 ± 0.3) didn't show significant relationship with age ($p = 0.336$), education ($p = 0.33$), job ($p = 0.493$), income ($p = 0.856$), Infertility duration ($p = 0.136$) and history of ART ($p = 0.057$). All attitude questions were not related to education and history of ART. Attitude about surrogacy ($p = 0.011$) and the possibility of separation of each couple in case of infertility ($p = 0.001$) ($p = 0.015$) was different according to age.

Conclusion: Considering the equality of knowledge level scores of study participants who had different levels of job and university degree and the fact that the average score of most knowledge questions was around 3, it means that their answers to most questions were "I don't know". Therefore, it can be concluded that information about infertility in this group of men is low and the need for educational planning in this regard, especially in infertility treatment centers for specific groups and also through the public media for the general public is felt.

Key words: *Health literacy, Attitude, Infertility, Men, Reproductive.*

P-33

The effect of L-arginine on the menopausal estradiol

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Background: Menopause in women is associated with many complications such as hot flashes, osteoporosis, and infertility that most of them are related to the decrease of estrogen levels in this period. Treatment with high doses of estrogen is common but has side effects.

Objective: In this study, the effect of L-arginine

administration on the level of this hormone in elderly rat was investigated.
Materials and Methods: Elderly Wistar rats were first studied with the help of Papanicolaou smear to identify the stage of female sexual cycle. If confirmed to have diestrus phase, the rats were randomly classified into the following groups: control (saline 1 mL/kg), intraperitoneally, and L-arginine dose groups (5, 25 and 50 mg/kg). They were injected saline or L-arginine over a period of at least three to nine days. At the end, the rats were anesthetized by intraperitoneally injection of ketamine 100 mg/kg and xylazine 20 mg/kg, and the blood samples were collected, and the estrogen levels were measured with enzyme-linked immunosorbent assay kit. The rats' ovaries and uteri were also biometrically examined and fixed in the formalin. They were stained by hematoxylin and eosin and the number of cysts in the ovaries were counted. The data were analyzed by the Analysis of variance (ANOVA).
Results: L-Arginine at all doses (5-25 mg/kg) during all injection periods from three to nine days significantly increased the estradiol levels, but prominently reduced the ovarian cysts at the lowest doses (5 mg/kg).
Conclusion: Low doses of estrogen over short periods of time can relieve menopausal problems in animal, including estrogen levels and ovarian status, and this may be due to the modulatory role of estrogen in the animal's natural processes.
Key words: *Menopause, L-Arginine, Estradiol level, Rat.*

P-34
COVID-19 and male infertility

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Background: Lately in 2019, a new type of coronavirus (Sars-CoV-2) was identified in China. SARS-CoV-2 may damage male reproductive tissues and cause infertility.
Objective: Despite the rare information in the literature on the relation of coronavirus diseases in the productiveness of humans and animals, it is important to be conscious about the effect of the coronavirus disease 2019 (COVID-19) pandemic on male fertility.
Materials and Methods: We searched on PubMed and Google Scholar databases in June 2020 to find papers and studies about COVID-19 and its effects on fertility. The search on PubMed found 98 papers and on Google Scholar found 224 papers. We exclude the papers which their titles or abstracts were not relevant. At last, we select the most related papers to use in this article.
Results: Reports recognized that COVID-19 is closely related to the cells that secrete the angiotensin-converting enzyme 2 (ACE2). ACE2 is one of the enzymes involved in the renin-angiotensin system and is widely secreted in several tissues, for example, testis tissue; and organs that have a high expression of ACE2 are volunteers for infection. Analyses showed that in testicular cells, such as spermatogonia, seminiferous duct cells, Sertoli, and Leydig cells, there is a high expression level of ACE2.
Conclusion: Male infertility is an important problem, so scientists are evaluating if the ACE2 in the Covid-19 pandemic can influence male fertility. To date, there is no evidence if the SARS-CoV-2 virus uses ACE2 receptors in the reproductive system and what, or any affect this can have on human infertility. Although, side effects of COVID-19 pandemic can influence on infertility too, like drugs that use for treatment, chemical disinfectants, and psychological disorders.
Key words: *COVID-19, Sars-CoV-2, ACE2, Infertility.*

P-35
The effect of three-dimensional nanocomposite scaffolds on spermatogonial stem cells differentiation

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Background: Concern of azoospermia is common in male survivors of childhood cancer. Therefore, the culture of spermatogonial stem cells (SSC) for future treatment method is required, because these cells are important in spermatogenesis.
Objective: SSC isolation and differentiation are really important. 3D (Three-Dimensional) scaffolds play important role in cell culture, these scaffolds simulating a microenvironment similar to an extracellular matrix for differentiation of cells. The present study aimed to evaluate the efficiency of spermatogonial cells culture on a 3D microenvironment containing the Chitosan-Alginate (CA) scaffolds that contain graphene oxide (GO) nanocomposite for investigated the differentiation improving.
Materials and Methods: We isolated spermatogonial cells from neonatal 3- to 6-day-old NMRI mice. Then we prepared the scaffolds (Based on our last studies, which we added GO concentrations of 5, 15, 30, 45, 75 µg/ml to the CA, the seeded cells have shown strong attachment on CA/GO 30 µg and had the best biocompatibility compared with other concentrations. So, CA/GO 30 µg become our selected scaffold for this study). The scaffolds were analyzed using FTIR, XRD, and microCT to observe surface topography and morphology. SSC were cultured and divided in to 2 culture groups: (SSC + basic medium), and (SSC + CA/GO 30 µg scaffold). Basic medium was DMEM-F12 with KSR 10%, consisting of Bmp4 40 ng/ml and Retinoic acid 10-6 M. The stem cells related markers for differentiation of SSCs (*SYCP3* and *TEKT1*) were detected on all experimental groups by RT-qPCR and ICC.

Results: Incorporation of GO into CA matrix increased both crosslinking density as indicated by the reduction of crystalline peaks in the XRD patterns and polyelectrolyte ion complex as confirmed by the FTIR. MicroCT analyses indicate that the scaffold had a highly porous and interconnected pore structure with porosity of 81.56 %. The RT-qPCR results showed that the expression of *SYCP3* and *TEKT1* genes were higher after 14 days in the SSC + CA/GO group compared to control ($p < 0.05$). ICC assays results showed that the mean expression of *SYCP3* for SSCs cultured on the CA/GO 30 µg scaffold after 14 days was 49.37 ± 6.20 while Its mean expression for SSCs cultured on the basic medium after 14 days was 35.97 ± 4.70. The mean expression of *TEKT1* for SSCs cultured on the CA/GO 30 µg scaffold after 14 days was 73.12 ± 3.94 however this marker's mean expression for SSCs cultured on the basic medium after 14 days was 53.46 ± 3.09. So, the expression of *SYCP3* and *TEKT1* for (SSC + CA/GO 30µg scaffold) had significant increase than the other scaffold group ($p < 0.05$).

Conclusion: The most expression of differentiation markers was in CA/GO 30 µg group. This scaffold has biocompatibility and degradable properties and graphene oxide helps to strengthen the scaffold and thus improves cell culture. This scaffold will provide a more improved structural environment for increased differentiation of SSCs.

Key words: *Spermatogonial stem cells, Chitosan-Alginate Scaffold, Graphene oxide nanocomposite, Differentiation.*

P-36

Evaluation of antioxidant and anti-inflammatory effects of tannic acid on sperm survival and motility in sepsis-infected male rats

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Background: One of the problems caused by infectious diseases is the reduction of sperm count and motility. In this study, sepsis model was used to investigate the oxidative stress and inflammation in testicular and sperm structure. Since tannic acid has antioxidant and anti-inflammatory effects on various organs of the body, in this study, the effect of tannic acid on the above-mentioned indices as well as testicular and sperm function and structure were investigated.

Objective: The main aim of this study was to investigate the protective effect of tannic acid on short-term infertility due to oxidative and inflammatory conditions.

Materials and Methods: Twenty-four male Wistar rats in the weight range of 300-250 g were randomly divided into 3 groups of 8: 1) sham 2) sepsis 3) tannic acid. In the sham group, the animals were anesthetized and then underwent laparotomy, but sepsis induction was not performed in this group. In the sepsis group, the animals underwent anesthesia and laparotomy to induce sepsis, then 30-40% of the end of the cecum was double-tied with a double layer of silk suture. Two needle holes were then made in the closed cecum area with a needle number 25 to allow the infection to enter the abdominal cavity. In the tannic acid group, the animals received tannic acid at a dose of 20 mg/kg at 6, 12, and 24 hr after sepsis induction. Thirty hr after induction of sepsis, the animals were anesthetized and testis was fixed in 10% formalin for histological examinations. The end of the epididymis was used to examine sperm motility and survival.

Results: The percentage of motile sperm and the percentage of sperm survival decreased significantly in the sepsis group. The use of tannic acid significantly improved the inflammatory and oxidative status of testicular tissues as well as improving sperm parameters.

Conclusion: The results of this study showed that the reproductive system as well as the sex cells of male rats are strongly affected by the conditions created during sepsis. Tannic acid as an antioxidant and anti-inflammatory agent improves short-term infertility caused by infection.

Key words: *Short-term infertility, Sepsis, Oxidative stress, Inflammation, Tannic acid.*

P-37

The association between fatty acids and steroids gene expression in abdominal subcutaneous fat depot: A comparison between pregnant PCOS and non-PCOS pregnant women

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Background: It was reported that steroid-related gene

expressions in the adipose tissue (AT) of women differ between women affected with polycystic ovary syndrome (PCOS) (case) and non-PCOS (control). Although association between PCOS in mother and offspring's health is a crucial issue, there are few studies focusing on effectiveness of AT profiles on steroids genes expression in pregnant women suffering from PCOS.

Objective: Our objectives were to assess association between fatty acid (FA) and genes related to steroids metabolism expression in abdominal subcutaneous AT of 12 PCOS (case) vs. 32 non-PCOS (control) age- and BMI-matched pregnant women.

Materials and Methods: Twelve pregnant women with PCOS (case) and thirty two non-PCOS pregnant women (control) (age- and BMI-matched) undergoing cesarean section were enrolled for the present study. Expressions of fifteen genes related to steriodogenesis in abdominal subcutaneous AT were investigated using quantitative real-time PCR. Fatty acids profiles assessed by gas chromatography. Linear regression was performed to determine the association of FA and gene expression in subcutaneous AT.

Results: Age and BMI were similar among two groups at delivery day. Current study showed that omega-3 fatty acids had the highest association with steroids gene expression rate ($r = 0.500$; $p < 0.05$).

Conclusion: It seems that fatty acids, both direct and by metabolites, can play a role in many diseases through extensive signaling pathways, specifically in exacerbating PCOS, although pregnancy can double the role of nutrition in exacerbating these effects.

Key words: *Polycystic ovary syndrome, Subcutaneous adipose tissue, Sex steroid, Fatty acids.*

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P-38

Investigating the correlation between ubiquitination with motility, morphology, and DNA methylation in rat sperm

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Background: There are various techniques for treatment of male infertility, nowadays. At the beginning, evaluations are performed to determine the cause of infertility and the treatment. These include advanced molecular evaluations and assessment of sperm parameters. There is no study investigating the relationship between sperm parameters as an elementary index of male infertility, and deoxyribonucleic acid (DNA) methylation as an important epigenetic mechanism with rat sperm ubiquitination, so far.

Objective: The aim of this study was to evaluate the motility, morphology, DNA methylation, and ubiquitination in rat sperm and to determine the relationship between them.

Materials and Methods: First, 10 male mature rats were kept in experimental condition for 9 weeks (one cycle of spermatogenesis). After sacrificing, their semen samples were used to determine the sperm parameters and smear preparation. Through prepared smear and immunofluorescence assay, percentage of ubiquitinationed and methylated sperm were determined and finally, the correlation coefficient between them was calculated.

Results: There was no significant correlation between the ubiquitination and DNA methylation. However, there was an inverse and significant correlation between the percentage of ubiquitination and morphological abnormalities in spermatozoa ($p < 0.05$). The percentage of ubiquitinized sperm and sperm motility showed no significant correlation.

Conclusion: Ubiquitination, as one of the important molecular processes, prevents the participation of defective sperm in fertilization, and transmission of disorders to next generation. DNA methylation and ubiquitination affect sperm chromatin, but these two processes act in an independent manner.

Key words: *Sperm motility, DNA methylation, Infertility, Ubiquitination.*

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P-39

Protective effects of zinc on rat sperm chromatin integrity involvement: DNA methylation, DNA fragmentation and protamination after bleomycin etoposide and cis-platin treatment

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Background: Testicular cancer is the most common malignancy that threatens the male in their reproductive age. Combined treatment by bleomycin, etoposide, cis-platinum (BEP) is the most effective strategy for patients with testicular cancer, and these chemotherapeutic agents can increase the 5- years survival rate. BEP treatment has revealed negative side

effects on different germ cells, finally impacting reproductive function and fertility. In addition to cancerous cells, oxidative stress due to BEP treatment can destroy testicular germ cells and induce changes in chromatin integrity.

Objective: We decided to investigate recovery effect of zinc (Zn) on chemotherapy-induced complications in rat chromatin integrity and protamination.

Materials and Methods: The male rats (n = 40) were treated with BEP at appropriate dose levels of BEP (0.75, 7.5, and 1.5 mg/kg) for 9 week, with or without Zn; Sperm DNA methylation through immunofluorescence, DNA fragmentation and protamination were evaluated through acridine orange staining and Chromomycin A3 staining.

Results: The mean percentage of global DNA methylation sperm was significantly reduced as compared with the control group ($p < 0.001$). In BEP+ Zn group, the mean percentage of global DNA methylation increased compared to BEP group. In Zn group, the mean percentage of global DNA methylation had no significant difference compared with the control group. Following BEP treatment, the mean sperm count that represented DNA fragmentation was significantly increased ($p < 0.001$). In addition, the mean percentage of DNA fragmentation was reduced in the BEP + Zn group in comparison with the BEP group, but not as much as the control Group ($p < 0.001$). The mean percentage of DNA fragmentation in rats treated with Zn indicated no significant difference compared with the control group. Rats treated with BEP showed significantly increased protamine deficiency sperm as compared with the control group ($p < 0.001$). In the BEP + Zn group, recovery in protamination was observed compared with the BEP group, but there is still significant difference in comparison with the control group ($p < 0.05$). The significant difference was observed between Zn and control group ($p < 0.05$).

Conclusion: Our findings confirm the recovery effects of Zn on rat sperm chromatin integrity following BEP consumption. It is suggested that Zn be utilized as an antioxidant following chemotherapy.

Key words: *Spermatozoa, DNA methylation, Protamination.*

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P-40

Application of platelet-rich plasma increases in vitro proliferation of human spermatogonial stem cells in two-dimensional and three-dimensional culture systems

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Background: According to the American Cancer Society, the annual incidence rate of childhood tumors aged birth to 19 years old is 186.6 per 1 million adolescents. Regarding the improvement in life expectancy through the efficient medical procedures, side effects of treatment such as life quality and infertility are of grown importance. Spermatogonial stem cells (SSCs) are very sensitive to chemotherapy and radiotherapy, so male infertility is a great challenge for prepubertal cancer survivors. Cryoconservation of testicular cells before cancer treatment can preserve SSCs from treatment side effects. Different two-dimensional (2D) and three-dimensional (3D) culture systems of SSCs have been used in many species as a useful technique to in vitro spermatogenesis.

Objective: Since there is no available data on the proliferative effect of platelet-rich plasma (PRP) on SSCs, this research focused on the self-renewing of adult human SSCs in two-dimensional and three-dimensional culture systems of PRP.

Materials and Methods: Human testes samples taken from four brain-dead donors at 17, 21, 25, and 26 years old from November 2018 to September 2019. Approval from the family of each donor was acquired by the Organ Procurement Unit (OPU) of Imam Khomeini Hospital affiliated to Tehran University of Medical Science. Testicular cells cultivated in 2D pre-culture system, characterization of SSCs performed by RT-PCR and flow cytometry analysis. PRP prepared and dosimetry carried out to determine the optimized dose of PRP. After preparation of PRP scaffold, SSCs cultivated into three groups: Control, 2D culture by optimized dose of PRP and PRP scaffold. Finally, the diameter and number of colonies measured.

Results: After 2D pre-culture of testicular cells a significant increase in expression of OCT4, Vimentin, and VASA observed in comparison to after digestion ($p < 0.01$). Our results indicated that 16.2 % of all cells were positive for PLZF after enzymatic digestion, whereas after the 2D pre-culture significantly increased the purity of SSCs to 80.2%. After cultivation of SSCs in experimental groups, the number and diameter of colonies in the PRP-2D group increased significantly

($p < 0.01$) as compared to the control group. Interestingly, in the PRP- scaffold group only the mean number of colonies increased significantly ($p < 0.01$) related to the control group.

Conclusion: Our results suggested that PRP scaffold can reconstruct a suitable structure to the in vitro self-renewal and proliferation of human SSCs. The cytokines and growth factors obtained.

Key words: *Spermatogonial stem cell, Proliferation, Two dimensional culture system.*

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P-41
The hormone-sensitive lipase polymorphism (C-60G) is related with recurrent pregnancy loss in women with polycystic ovary syndrome

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Background: Lipid metabolism disruption is related to the development of polycystic ovary syndrome (PCOS) in women of reproductive age. Hormone-sensitive lipase (HSL) is an intracellular lipase that has a crucial role in normal lipid metabolism.

Objective: This study aimed to assess the frequency of C-60G polymorphism of HSL in healthy women and PCOS women, and its correlation with infertility and abortion in PCOS patients.

Materials and Methods: A total of 324 PCOS patients (including 199 infertile patients and 125 patients with a history of recurrent abortion) and 144 healthy controls enrolled in this study. Biochemical parameters were measured and the genotypes of C-60G polymorphism of the *HSL* gene were determined using PCR-restriction fragment length polymorphism techniques.

Results: There was no significant differences in the genotype and allele frequencies of C-60G polymorphism between PCOS, PCOS-infertile woman and non-PCOS subjects. However, a higher percentage of combined variants (CG+GG) and CG genotypes, as well as G allele was found in the PCOS-abortion group in comparison with non-PCOS women. The presence of the G allele conferred a 2.4-fold risk for abortion in women with PCOS (OR: 2.4, 95% CI [1.22-4.70], $p = 0.011$). Furthermore, a significant correlation between CG or GG genotype of HSL and the level of free testosterone was observed.

Conclusion: According to the obtained results, the C-60G polymorphism in the HSL promoter was associated with PCOS-related abortion, possibly through increasing the level of free testosterone. Therefore, the rs34845087 polymorphism may be considered as a promising prognostic biomarker for abortion in women with PCOS.

Key words: *Polycystic ovary syndrome, Hormone-sensitive lipase, Infertility, Abortion.*

P-42
Circulating level of plasma Complement C1q/tumor necrosis factor-related protein 15 in polycystic ovary syndrome

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Background: Poly-cystic ovarian syndrome (PCOS) is one of the frequent metabolic and endocrine disorder in women population that has a close relation with parameters of metabolic syndrome and obesity. Studies have shown perturbation of adipokines levels in PCOS patients. Complement C1q/tumor necrosis factor-related protein 15 (CTRP15) is a prologue of adiponectin that indicated a close relation with insulin, glucose and lipids metabolism.

Objective: In the present study we sought to evaluate the levels of this adipokines and its relation with cardiometabomic data.

Materials and Methods: This case-control study carried out on 120 PCOS patients and 60 controls. Serum levels of adiponectin and CTRP15 were determined using ELISA technique.

Results: Serum levels of CTRP15 elevated in patients with PCOS compared to controls while adiponectin decreased considerably. In addition, CTRP15 indicated a relation with BMI, insulin resistance and FSH levels.

Conclusion: Elevated levels of CTRP15 could be a compensatory response in patients with PCOS in response to obesity and insulin resistance, however further studies are needed to dissect the possible underlying mechanism.

Key words: *Polycystic ovary syndrome, Insulin resistance, CTRP15, Adiponectin.*

P-43
Comparing the effect of reduced graphene and graphene-L-arginine on sperm fertilizing ability

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Background: Sperm cell maturation occurs for fertility with longevity in the female reproductive system. Inducing and maintaining the ability of in vitro fertility of sperm is an important and effective factor in the success of pregnancy techniques and in vitro fertilization. So, various strategies such as addition of calcium or creatine phosphate, can be used for enhancement of the fertilizing capacity of sperm during in vitro fertilization. The change of chemical agents in sperm membrane can alter its fertility. Graphene, an allotrope of carbon, has interesting physical and chemical properties such as high electron mobility, high surface area, stiffness, strength and toughness. This nanostructure and their derivatives have been used in different fields of sciences, especially in biological and medical sciences.
Objective: The aim of this study was the comparison of the effect of reduced Graphene and Graphene-L-Arginine on sperm fertilizing ability.
Materials and Methods: In this study, we synthesized reduced and L-arginine-functionalized graphene by microwave method and characterized by transmission electron microscopy, scanning electron microscope, fourier-transform infrared spectroscopy, and raman spectroscopy. Acquired sperm samples from healthy volunteers were treated with different concentrations of reduced Graphene and Graphene-L-Arginine (1, 2, 3, 4, 5, 8, 10, 12, and 15 µg/ml).
Results: Results showed that water solubility of Graphene-Arginine was higher than reduced Graphene. This increase in solubility facilitates the use of functionalized graphene in chemical and physiological fluids. Between reduced Graphene and Graphene- L-Arginine, Graphene- L-Arginine had more significant effects on increase of sperm fertilizing ability in same concentrations. On the other hand, reduced Graphene has more toxicity than same concentrations. In concentrations of 1, 2, 3, 4, 5, 8, and 10 µg/ml of Graphene- L-Arginine and 1, 2, 3, and 4 µg/ml of reduced Graphene, significant increase of fertilizing ability for sperm was occurred. In concentrations of 12 and 15 µg/ml of Graphene- L-Arginine and 5, 8, 10, 12, and 15 µg/ml of reduced Graphene, cell death, reduction of sperm motility and viability as well as membrane lysis was significantly observed. The reason for the higher activity and effect of L-Arginine-functionalized graphene is the synergistic effect of graphene (cholesterol extraction and enhancement of fertilizing ability) and L-Arginine (enhancement of nitric oxide synthesis and prevention of membrane lipid peroxidation) on increase sperm fertility.
Conclusion: Based on this study, L-Arginine-functionalized graphene in low concentrations can be used in assisted reproductive technology for in vitro increase of sperm fertilizing ability.
Key words: *Assisted reproductive technology,Sperm fertilizing ability, Graphene, -Arginine, Nanostructure.*

P-44

Relationship between leptin and its polymorphism (-2548 G/A) and recurrent pregnancy loss in women with polycystic ovary syndrome

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders result in infertility and abortion in women. Leptin has a vital role in the regulation of body weight via interacting with its specific leptin receptor. Polymorphism of their related genes may play an important role in etiology and pathogenesis of PCOS related disorders.
Objective: This study investigate the relationship of leptin gene (*LEP -2548 G/A*) and its plasma level with the risk of infertility and recurrent pregnancy loss (RPL) in women with PCOS.
Materials and Methods: A total of 324 PCOS patients (including 199 infertile patients and 125 patients with a history of RPL) and 150 Non-PCOS enrolled in this study. Biochemical parameters were measured and the leptin gene (*LEP -2548 G/A*) was genotyped using PCR-restriction fragment length polymorphism (RFLP) techniques.
Results: There was a significant difference in GG genotype of leptin polymorphism in PCOS-infertile woman as compared to Non-PCOS subjects (p = 0.043, OR = 0.47, 95% CI = 0.22-0.97). Leptin level was significantly higher in PCOS-infertile (33.27 ± 8.45 ng/ml) and PCOS-RPL (36.47 ± 7.41 ng/ml) sub-groups compared to Non-PCOS group. Leptin level elevated the risk of PCOS (1.203, 95% CI [1.009-1.435]) as well as RPL related PCOS (1.267, 95% CI [1.054-1.522]) in females.
Conclusion: Our findings showed that high leptin level was associated with PCOS related disorders. Evidence suggests that high level of leptin increase the risk of RPL in PCOS women. However, more researches with large sample size is needed to find more leptin gene polymorphism in PCOS related disorders.
Key words: *Polycystic ovary syndrome, Leptin, Infertility, Recurrent pregnancy loss.*

P-45

In vitro mouse spermatogenesis on artificial testis engineered by 3D printing of extracellular matrix

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Background: Male infertility accounts for about 50% of all infertility cases, and 25 % of infertile men are azoospermic. Due to the very small number of spermatogonia stem cells (SSCs) in testicular tissue biopsy specimens, SSCs culture for infertile patients can be important.

Objective: The proliferation of SSCs on printed scaffold derived from the extracellular matrix (ECM) of testicular tissues evaluated.

Materials and Methods: Ram testicular tissue was decellularized using hypertonic solution -Triton X-100 for 30 min. The extracted ECM (5% ratio) was used as a bio-ink for the fabrication of artificial testes along with alginate and gelatin. Testicular cells were then isolated from the testes of 3-7 days old neonate mice after enzymatic digestion. The nature of SSCs was confirmed by flow cytometry and RT-PCR for specific markers Plzf, Id4, Gfrα1, and Prm1. Finally, cell viability evaluated using MTT test and testicular cell proliferation process on printed alginate-gelatin scaffolds (group I) and ECM-alginate-gelatin scaffolds (group II) using immunocytochemistry, flow cytometry, and real-time PCR techniques was assessed.

Results: The MTT test indicated that the cell viability on the composite scaffold was significantly higher than the hybrid scaffolds and control group ($p > 0.05$). The results of 2 wk of proliferation on the printed system showed that the expression of *Plzf, Id4, Gfrα1* gene using real-time PCR in group II was significantly higher than group I ($p > 0.05$). Flow cytometry analysis also showed that the number of Plzf-positive cells in group II was significantly higher than group I ($p > 0.05$). Immunocytochemistry results confirmed that Plzf, Id4, and Gfrα1 markers were expressed in both groups, but their expression in group II was significantly higher than group I ($p > 0.05$).

Conclusion: We concluded that the culture of testicular cells on scaffolds containing ECM increases the viability, colonization, and proliferation of SSCs and achieves a high number of cells for differentiation in vitro. Therefore, 3D printing using the ECM of the testis can be an ideal strategy for the regeneration of seminiferous tubules.

Key words: *Spermatogonia stem cells, Extracellular matrix, 3D printing, Proliferation.*

P-46
In vitro spermatogenesis in artificial testis

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Background: As a valuable resource for cell therapy, human spermatogonial stem cells (hSSCs) have raised hopes for the treatment of male infertility. Various 3D methods have been developed to produce cellular aggregates and mimic the organization and function of the testis. The rate of progression and breakthrough in vitro spermatogenesis is lower than that of SSC transplantation, but newer methods are also being developed.

Objective: Therefore, this paper discusses the promising methods of artificial testis development, which can be used for sperm production in vitro.

Materials and Methods: The relevant articles were searched in PubMed, Google Scholar, and ScienceDirect databases.

Results: The production of an artificial reproductive organ capable of supporting SSC differentiation will certainly be a major step forward in male infertility. The mammalian extracellular matrix (ECM) increases proliferation, migration, and/or differentiation of different stem cells and can facilitate the survival of hSSC in the culture medium. The use of testicular ECM for culture of germ cells has recently been reported. Given the importance of testicular ECM, seeding stem cells onto such decellularized scaffolds to create artificial tissues and organs can be promising for the restoration, preservation, or improvement of tissue/organ function in clinical therapy. ECM hydrogels as substrates for cell culture can also be appropriate for hSSC culture. Organoids can be formed from pluripotent stem cells or under the support of a scaffold (generally matrigel) and tissue-specific growth factors and morphogens. Recently, few studies have been conducted on the development of dynamic culture systems including bioreactor and microfluidic systems in testicular tissue that the tissue is exposed to a continuous, controlled flow of fresh media. 3D bio-printing is a novel way of developing organs or functional structures that allow cells and tissues to accumulate with great accuracy. Bioprinting can support gamete differentiation in a matrix-rich 3D environment.

Conclusion: Fabrication of biofunctional testis can be one of the new options for in vitro sperm production, maintaining fertility, or transplantation without the risk of cancer cell infestation to restitute spermatogenesis in vivo.

Key words: *Spermatogonial stem cell, Bioartificial testis, Differentiation.*

P-47

Effect of intrauterine injection of platelet-rich plasma and the number of injections in increasing endometrial thickness and pregnancy rate in patients with thin endometrium: A clinical trial, before and after

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Background: Adequate endometrial growth is a principal factor for implantation and pregnancy. Thin endometrium is associated with lower pregnancy rate in assisted reproductive technology.

Objective: In this study we assessedthe effectiveness of intrauterine injection of platelet-rich plasma (PRP) and number of injections in increasing endometrial thickness (ET) and pregnancy rate in patients with thin endometrium.

Materials and Methods: In this clinical trial, 26 women that referred to infertility center of Imam Khomeini Hospital, Sari, Iran, from September 2019 to January 2020 participated. They had history of frozen-thawed embryo transfer cycle failure due to thin endometrium. ET was determined on the tenth day of cycle, and if ET was (< 7 mm), intrauterine injection of PRP was done on day 11-12 and it was repeated on day 13-14 until ET reached an optimal pattern, then embryo transfer was performed ($p < 0.05$ were considered as statistically significant).

Results: Mean age of women was 34.96 ± 3.86 yr. Mean of ET pre-PRP was 5.64 ± 0.79 mm which significantly (n = 26), increased to 7.20 ± 1.27 mm post-first PRP (n = 26) and increased to 7.65 ± 1.29 mm post-second PRP (n = 13) ($p < 0.001$). Chemical and clinical pregnancy rate was 23.1% and 19.2% respectively. One patient had ectopic pregnancy and five patient had normal ongoing intrauterine pregnancy. Of these 5 people, one had twin pregnancy that miscarriage at 19 wk of gestational age, and the other 4 had live birth at term.

Conclusion: Our findings showed that the PRP injection can be used as a method to increase ET and improvement the results of in vitro fertilization. To achieve the optimal results, PRP as a safe and low risk method can be performed twice, between 24 till 72 hr in in vitro fertilization cycles.

Key words: *Platelet rich plasma, Endometrium, Fertilization in vitro, Pregnancy rate.*

P-48

Effect of fennel supplementation along with high-protein, low-carbohydrate weight-loss diet on insulin resistance and percentage of fat and muscle mass in overweight/obese women with polycystic ovary syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common reproductive disorder with prevalence of 5-10% in premenopausal women, which is identified with hyperandrogenism and ovarian dysfunction.

Objective: The aim of this study was to investigate the effects of fennel supplementation with energy-restricted diets on body fat and muscle percentage and insulin resistance in women with PCOS.

Materials and Methods: Sixty-four overweight/obese women with PCOS were randomly allocated to 4 groups for 12 wk as follows: (1) standard diet + fennel (SDF), (2) high-protein, low-carbohydrate diet supplemented with fennel (HPF), (3) standard diet + placebo (SDP), and (4) high-protein, low-carbohydrate diet + placebo (HPP).

Results: After 12 wk of intervention, there were significant changes in the percentage of body fat and muscle in all groups. Decreasing in fasting insulin was −4.12 micIU/ml ($p = 0.01$) for HPF and −4.5 micIU/ml ($p = 0.03$) for SDP groups. In addition, HOMA-IR significantly decreased in HPF ($p = 0.02$) and SDP ($p = 0.02$) groups.

Conclusion: Energy-restricted diets independent of dietary composition improved the body fat and muscle percentage and insulin resistance indices in women with PCOS. High-protein diet and fennel compared with standard diet and placebo had no significant effect on insulin resistance, body fat and muscle.

Key words: *High-protein diet, Standard diet, Fennel, Body fat percentage, Insulin resistance index.*

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P-49
Which sperm preparation technique separate the best quality sperm?

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Background: of all infertility cases, approximately 40-50% is due to male factor in fertility. One of the known causes of male infertility is associated with low sperm parameters and high level DNA fragmentation. Sperm preparation techniques in intracytoplasmic sperm injection procedures is used in order to obtain the best-quality sperm.
Objective: The present study was designed to compare Microfluidic, and Swim-up methods for sperm preparation and the effect of these methods on semen parameters and sperm DNA Integrity in Infertile men.
Materials and Methods: In this study, semen samples were collected from 25 infertile men. Each sample was divided into 2 groups, one part for preparing by Microfluidic method and the other one was prepared by swim up method. Then sperm count, viability, motility and morphology were assessed according to World Health Organization 2010. DNA damage were assessed by Sperm DNA Fragmentation assay.
Results: Sperm parameters including viability, motility, and morphology in the Microfluidic method were significantly improved and sperm DNA damage were significantly lower than the swim up method ($p < 0.05$).
Conclusion: Our results showed that Microfluidic method improved the sperm parameters and decreased sperm DNA damage, and it can be an effective way to improve sperm quality of infertile male compared to conventional preparation methods.
Key words: *Microfluidic, Swim up, DNA fragmentation.*

P-50
Prevalence of smoking in infertile men referred to the infertility ward of Ali ebn-e Abitaleb Hospital of Zahedan from 2017 to 2019

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Background: Infertility and its individual and social problems are one of the most important issues for couples. A significant problem with male infertility is that infertility is only detectable in 40% of cases and is not pathologically detectable in 60% of cases.
Objective: The aim of this study was to evaluate the frequency of smoking in male infertility referred to the infertility ward of Ali ebn-e Abitaleb Hospital of Zahedan from 2017 to 2019.
Materials and Methods: The present study was a cross-sectional study and included 200 infertile men with male factor referred to the infertility clinic of Ali ebn-e Abitaleb Hospital of Zahedan. The sampling method was easy or available and a questionnaire was used to collect information and SPSS software was used to analyze the data.
Results: The results showed that infertile patients with male factor that are non-smoking had the highest number with 174 (87%). Also, there was no significant relationship between smoking and sperm concentration in spermogram in infertile couples with male factor ($p = 0.293$). There was no significant relationship between smoking and sperm morphology in spermogram in infertile patients with male factor ($p = 0.130$). There was no significant relationship between smoking and sperm motility in spermogram in infertile patients with male factor ($p < 0.05$).
Conclusion: Although smoking as a risk factor can cause infertility, but in the present study, (the cross-sectional study), we were not able to show the cause-and-effect relationship between smoking and infertility.
Key words: *Smoking, Male infertility, Semen analysis.*

P-51
Evaluation of miRNAs involved in patients with endometriosis as diagnostic and therapeutic biomarkers using bioinformatics analysis

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Background: Endometriosis is one of the most common reproductive diseases in women. About 10% of women are pregnant, and about 50% of women between the ages of 30 and 50 and between 30 and 50% of women experience pelvic pain or infertility due to endometriosis. There are several treatments available for people with endometriosis, but facilitating and optimizing early detection of the disease can help manage its treatment. miRNAs are important regulatory molecules at the cellular level that control the expression of many genes due to their inhibitory effect. miRNAs can be found in human secretions. Therefore,

these regulatory molecules can be used as diagnostic biomarkers for endometriosis.

Objective: This study examined the ectopic and eutopic tissue data of patients with endometriosis and isolated the miRNAs in the extracellular matrix.

Materials and Methods: In this study, using bioinformatics analysis, we first isolated the appropriate data through the GEO database. We then uploaded the hypex gene to examine the signal path in the Enrichr database and the KEGG library. We loaded the genes involved in important pathways into the STRING database and measured their protein network. We then used the Targetscan database to obtain miRNAs.

Results: 700 low-expression genes were selected with LogFC < 2. These genes played a more prominent role in complement and coagulation cascades, cell adhesion molecules, TGF-beta, and Hedgehog signaling pathways. Of these pathways, 34 genes were involved in this pathways and extracellular matrix. After the examination, the hsa-miR-4800-3p, hsa-miR-4473, hsa-miR-614, hsa-miR-4671-3p, and hsa-miR-3659 miRNAs were identified more clearly.

Conclusion: Finally, this study showed that miRNAs, between ectopic and eutopic tissues, were significantly identified and could be found in the serum or plasma samples of patients with endometriosis.

Key words: *Endometriosis, Biomarkers, Bioinformatics analysis, Micro RNAs.*

P-52

"Protester Caregivers" semantic reconstruction of infertility outcomes in marital relationship of infertile women

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Background: Spouses, who experience infertility, are much more overwhelmed by failure, anxiety, stress, and unpleasant marital relationships than other spouses. Infertility often has negative effects on the relationships between spouses. Infertile women are more likely to suffer from these unpleasant marital relationships.

Objective: By using a qualitative grounded theory approach, 21 women were selected and studied using theoretical sampling. Theoretical sampling continues until data saturation occurs. These qualitative interviews were conducted between January 2019 and January 2020. Data were collected and analyzed using open and axial coding.

Materials and Methods: The findings of this study included 7 main categories and one core category called Protester Caregivers. Consequently, conceptual tables, paradigm model and theoretical schema were presented. Determining the results in general indicates the phenomenon of "Protester Caregivers" in the target community.

Results: The findings of this study included 7 main categories and one core category called Protester Caregivers. Consequently, conceptual tables, paradigm model and theoretical schema were presented. Determining the results in general indicates the phenomenon of "Protester Caregivers" in the target community.

Conclusion: Based on the traditional culture of the target community, infertile women struggle with the men who play the role of "caregivers" as well as consistently protest their infertility. This paradoxical phenomenon leads to the emergence of contradictory strategies and outcomes, and make infertile women to rethink about their choices.

Key words: *Infertile women, Marital relationship, Semantic reconstruction, Protester caregivers.*

P-53

Comparison of GnRH-agonist+ vaginal progesterone and vaginal progesterone effects on luteal phase support in frozen–thawed embryo transfer cycles: An RCT

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Background: As it seems that the progesterone alone isn't enough treatment for luteal phase support (LPS) specially in frozen embryo transfer (FET) cycles, so gonadotropin releasing hormone agonist (GnRH-a) was suggested as a adjuvant therapy with combination to progesterone for LPS.

Objective: This study aimed to evaluate the effects of the administration of a multiple doses of GnRH-a to routine LPS in FET cycles.

Materials and Methods: In this clinical trial study, 240 infertile women who were candidate for in vitro fertilization cycle were enrolled and divided into two groups (n = 120/each). Group 1 received 800 mg vaginal progesterone daily and group 2 received 0.1 mg dipherline in days 0, 3, and 6 of FET for LPS. Implantation rate, clinical pregnancy rate, ongoing pregnancy rate, and spontaneous abortion were checked and measured.

Results: Results showed that there was no significant difference between the mean age of women and also duration of infertility (p = 0.70, p = 0.60). There was no significant in term of implantation rate and rate of spontaneous abortion (p = 0.19, p = 0.31) respectively. In term of clinical pregnancy rate, significant difference were seen between groups (n = 37, 30.8% in group 1 and n = 57, 47.5% in group 2, p = 0.008). As a term of ongoing pregnancy rate (till 3 months after FET), significant difference between two groups were seen (p = 0.05).

Conclusion: The GnRH-a+cyclogest as opposed to cyclogest for LPS after FET cycles may be the superior choice.

Key words: *FET, ART, LPS, Cyclogest.*

P-54
Cytokines as biomarkers for embryo selection

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Background: Studies have shown that the morphological assessments to select the best embryo for transfer could not provide satisfactory outcomes. Therefore, many studies have been conducted to find predictive biomarkers that can distinguish embryos with high implantation potential.

Objective: In the current study, we comprehensively reviewed the possibility of using embryo-secreted cytokines as potential biomarkers for embryo selection in assisted reproductive technology.

Materials and Methods: The present review involved published research articles that have investigated cytokines in the embryo secretome. A search in Google Scholar and PubMed was performed with no limitation on the date of publication using a combination of the following search terms: "secretome", "culture media", and "cytokine (s)".

Results: It can be postulated that the embryo secretome can well reflect the embryo condition. Since the immune system has an indubitable role in implantation and also the immunological factors are involved in the embryo-endometrial crosstalk, the embryo-secreted cytokines can be used as potential biomarkers.

Conclusion: In conclusion, the following three points should take into consideration while using embryo-secreted factors as biomarkers: 1) The culture media should be evaluated at a certain stage of embryo development (e.g. cleavage and blastocyst), 2) The measurement method should be able to detect very small levels of factors, and 3) Changing in the concentration of several embryo-secreted factors in combination should be evaluated to propose an appropriate embryo selection method**.**

Key words: *Embryo, Cytokines, Implantation, Secretome, Culture media.*

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P-55
Effect of dietary polyunsaturated fatty acids on the status of uterine prostaglandins during the window of pre-implantation

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Background: The role of prostaglandins (PGs) in embryo implantation can be influenced by polyunsaturated fatty acids supplementation.

Objective: Therefore, the present study was conducted to investigate the effects of dietary omega-3 and -6 fatty acids on uterine PGs and their relevant receptors during the pre-implantation period in mice.

Materials and Methods: Twenty female mice were randomly divided into three groups and fed a standard pellet (control group), standard pellet +10% (w/w) fish oil, and +10% (w/w) soybean oil. The uterine levels of PGI2, PGD2, and PGF2α, the mRNA expression of PG I, D, and F synthesis enzymes (PGIS, PGDS, and PGFS, respectively), and protein expression of their receptors (PI, PD, and PF, respectively) were evaluated in uterine tissues of all treated groups at days 1-5 of pregnancy.

Results: Our results showed that the uterine levels of PGI2, PGD2, and PGF2α and expression of their synthesis enzymes were markedly high on the 5th day of pregnancy, while protein expression showed significant elevation only for PF and PI during this day ($p < 0.05$). Omega-6 significantly raised uterine levels of all three PGs on the fifth day of pregnancy compared to mice received omega-3 ($p < 0.05$). Furthermore, the omega-6 group showed higher expression of PGDS and PGFS than the omega-3-supplemented group on days 5 and 4 of pregnancy, respectively. In addition, we found positive correlations between the implantation rate and expression levels of PGIS, PGFS, IP, and FP and the PGI2 uterine levels.

Conclusion: Our study showed the positive effect of omega-6 PUFA supplementation on PGFS and PGDS expression together with the uterine levels of PGI2,

PGD2, and PGF2α which makes PGs status as a possible indicator of successful implantation.

Key words: *Embryo implantation, Pre-implantation window, Polyunsaturated fatty acids, Omega-3, Prostaglandins.*

P-56
Effects of bacteria on male fertility: Spermatogenesis and sperm function

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Background: Interestingly, bacteria can induce different damages on sperm cells such as DNA fragmentation, cell membrane peroxidation, and acrosome impairment. Such negative effects can be mediated by bacteria-secreted toxins and metabolites or by direct attachment of bacteria on the sperm cells and subsequent activation of signaling pathways related to oxidative stress, apoptosis, and inflammation.

Objective: In this study, we reviewed the impact of male urogenital bacteria on spermatogenesis and sperm functions as well as the underlying mechanisms by which the bacteria can damage sperm.

Materials and Methods: The present review involved all published research articles that have investigated the effect of bacteria on spermatogenesis and sperm function. Combinations of the following terms were searched in Google Scholar, PubMed, and Science Direct databases with no limitation on the date of publication: "sperm", "spermatozoa" "spermatogenesis", "bacteria", and "bacterium".

Results: Interestingly, bacterial contamination of semen, which ordinarily originates from the urinary tract or by the partner through sexual intercourse, can induce DNA fragmentation, cell membrane peroxidation, acrosome impairment, vacuolization, and mitochondrial damage in sperm cells. Bacteria can exert these effects by toxins or by direct attachment to the sperm cells and subsequent activation of signaling pathways related to oxidative stress, apoptosis, and inflammation. These bacteria-induced changes in the sperm can impair semen parameters including concentration, motility, morphology, viability, and fertilization capacity, and subsequently cause infertility.

Conclusion: Given the significant destructive effect of some bacteria on sperm cells, the type of bacterial contamination in the patient's genital tract should be diagnosed and its potential negative effects on male fertility, and the underlying mechanisms should be taken into account in the treatment of bacteria-induced subfertile men. Furthermore, future studies are recommended to investigate possible therapeutic strategies to inhibit bacteria-induced sperm damage based on the type of bacterium and its potential damages on sperm cells.

Key words: *Bacteria, Sperm, Fertility, DNA fragmentation.*

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P-57
Effect of one-way blunt testis on sperm parameters in acute and chronic periods after injury in mice

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Background: So far, the effects of blunt trauma on sperm parameters and reproductive capacity have not been firmly established and diverse reports have been presented.

Objective: The aim of this study was to investigate the effect of unilateral blunt testis on sperm parameters in acute and chronic periods after injury in mice.

Materials and Methods: In this study, 40 adult male NMRI mice with a weight of 35-30 gram were selected randomly and divided into 3 groups: control, sham (Mice in this group were only surgically treated) and experimental (Mice were surgically treated in this group and the blunt was hit by their left testes). Sampling was performed in two acute (48 hours after surgery) and chronic (1 month and 2 months after surgery), after anesthesia the tail of epididymis was separated and placed in Ham's F10 solution. Then sperm samples were examined microscopically in terms of motility, number

(with 40x lens), viability (eosin stain color and hypoosmotic swelling).

Results: In the case of rapid sperm motility, the control group with acute experimental, one and two month chronic, acute sham group with sham one month, acute and chronic experimental one and two months, and sham group two months with acute experimental groups and sham one and two months were significant. Regarding the sperm viability, the control group with one month acute and chronic sham, the acute sham group with one month sham groups, an acute and chronic one month experimental, and a one month chronic group with acute and chronic two month experimental groups were significant. Regarding sperm count, a one month chronic group with control groups, acute sham, one to two months sham and an acute experimental with one month chronic group was significant.

Conclusion: The testicular blunt has affected sperm parameters. In other words, the mean number of sperm (ml /ml) and the percentage of sperm survival and motility (progressive, non-progressive, and inactivity) were significant between the control and sham groups and the experimental group, which was not effective on fertility.

Key words: *Blunt, Testis, Sperm parameters, Mice.*

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P-58

The effects of morphine abuse on sperm parameters, chromatin integrity and apoptosis in men consuming morphine

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Background: Morphine is one of the major psychoactive chemicals in opium that can increase the production of free radicals and thus can negatively affect spermatogenesis.

Objective: The purpose of this survey was to demonstrate the effect of morphine consumption on sperm parameters, DNA integrity and apoptosis in men taking morphine.

Materials and Methods: In this case-control study, 30 man abusing morphine (cases) and 30 healthy men (controls) were compared for sperm parameters (count, motility and morphology) and sperm chromatin quality, with aniline blue, toluidine blue and Chromomycin A3 stainings. The participants were matched for age, weight, amount and duration of cigarette smoking.

Results: In men with morphine dependency, sperm progressive and total motility ($p = 0.038$ and $p < 0.001$ respectively) showed significant decreasing compared to control group. Regard to morphine abusing, although morphine can decrease the sperm chromatin condensation and increases the rate of sperm apoptosis, but these differences were not statistically significant.

Conclusion: According to our result morphine dependence can reduce male fertility by affecting sperm parameters and also it may affect sperm chromatin/DNA integrity.

Key words: *Morphine, Sperm parameters, Chromatin, Human.*

P-59

Comparison of the Betatrophin level and its association with metabolic and inflammatory parameters in PCOS and non-PCOS infertile women condidated for IUI

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Background: Betatrophin may be associated with metabolic diseases.

Objective: To investigate the betatrophin level and its association with metabolic and inflammatory parameters in infertile women with polycystic ovary syndrome (PCOS) and other infertile women during the intrauterine insemination cycle.

Materials and Methods: This case control study was conducted on 90 infertile women (45 PCOS and 45 non-PCOS) chosen by convenience sampling method, in Tehran. Participants were interviewed to obtain age, body mass index, reproductive history. Fasting brachial venous blood samples were obtained on the 3rd day of the menstrual cycle in order to measure the betatrophin, fasting blood sugar, insulin, luteinizing hormone, follicle-stimulating hormone, low-density lipoprotein cholesterol, estradiol, and high-sensitivity C-reactive

protein. To analyze the data, SPSS 25 software and statistical tests such as independent Student's *t* test, chi-square test, and multiple linear regression were used.
Results: The results showed that the level of betatrophin in women with PCOS was significantly higher than in the control group ($p = 0.05$). Based on multiple linear regression analyses, the effects of metabolic and inflammatory parameters on betatrophin were not significant ($p > 0.05$). The results showed no significant difference between groups in folliculogenesis ($p = 0.57$).
Conclusion: According to the results, betatrophin levels were higher in PCOS infertile women than in infertile non-PCOS women. Indirectly, one might argue that there is a potential association between an increase in betatrophin and a possible increase in the incidence of PCOS syndrome. Further studies with a larger sample size are needed to investigate the role of betatrophin in insulin resistance, lipid metabolism, and its effects on infertility treatment outcomes.
Key words: *Betatrophin protein, Human, Infertility, Polycystic ovarian syndrome, Iran.*

P-60
Investigation and comparison of the effect of TGF-β3, kartogenin and Avocado/Soybean unsaponifiables on the in-vitro and in-vivo chondrogenesis of human adipose derived stem cells on fibrin scaffold

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Background: There are lack of suitable therapeutic approaches to cartilage defect.
Objective: This study was designed to determine the effect of TGF-β3, avocado/soybean (ASU) and Kartogenin (KGN) on chondrogenic differentiation in human adipose-derived stem cells (hADSCs) on fibrin scaffold.
Materials and Methods: hADSCs seeded in fibrin scaffold and cultured in chondrogenic media. These cells divided in to 4 groups (control, TGF-β3, ASU and KGN). Cell viability was estimated by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, or MTT assay, differentiated cells evaluated by histological and immunohistochemical techniques. Expression genes [sex determining region Y-box 9 (SOX9), Aggrecan (AGG), type II collagen (Coll II) and type X collagen (Coll X)] assessed by real-time PCR. For study on animal model, differentiated cells in fibrin scaffolds were subcutaneously transplanted in rats. Histological and immunohistochemistry was done in animal model.
Results: The results of the real-time PCR indicated that *SOX9*, *AGG* and *Col II* genes expression in TGF-β3, KGN and ASU groups were significantly higher ($p < 0.01$) compared to the control group, *Col X* gene expression only in TGF-β3 group was significantly higher ($p < 0.01$) compared to the control group. The glycosaminoglycan (GAG) deposition was higher in TGF-β3, KGN and ASU groups compared to the control group. The immunohistological analysis showed the distribution of collagen type X in the extracellular matrix in fibrin scaffold TGF-β3 group was significantly higher in control, KGN and ASU groups, ($p < 0.001$).
Conclusion: ASU, and in particular KGN was suitable for successful chondrogenic differentiation of hADSCs and a suppressor of the consequent hypertrophy.
Key words: *TGFβ3, Avocado/Soybean, Kartogenin, Human adipose-derived stem cells.*

P-61
An overview of application of stem cells as a resource for male infertility treatment

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Background: Male infertility due to decreased semen quality is a growing global problem.
Objective: Commonly used strategies for treating infertility include medication, intrauterine insemination, and in vitro fertilization. In recent years, mesenchymal stem cells have created new opportunities to treat a variety of disorders, including infertility and new expectations for managing reproductive disabilities. Stem cells are undifferentiated cells that are able to regenerate and proliferate and are also able to produce specialized cells under appropriate conditions. They are present in all stages of the fetus, embryo and adult and can multiply in different cells.
Materials and Methods: We searched the articles published in English from 1985-2020 using the keywords nanoparticles, male infertility, imaging, and toxicity in Scopus and PubMed databases. While many questions remain about stem cells, stem cells have undoubtedly opened up new avenues for infertility treatment.
Results: In summary, most studies have shown that mesenchymal stem cells can be used as a viable option for the treatment of azoospermia in men. However, there is a need for further evaluation of the effectiveness of these cells in treating infertility.
Conclusion: In this review, we discuss and summarize different stem cell approaches to the treatment of male infertility to provide updates on stem cell therapy research.
Key words: *Stem cells, Male infertility, Treatment, Transplantation.*

P-62

Investigation the effect of avocado soybean unsaponifiables and icariin on the chondrogenesis of human adipose derived stem cells on poly (Lactic-Co-Glycolic) acid/fibrin hybrid scaffold

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Background: Avocado soybean unsaponifiables (ASU) and icariin (ICA) components are described to have a chondroprotective.
Objective: The aim of this study was to investigate the effect of ASU and ICA on the chondrogenesis of human adipose derived stem cells (hADSCs) on poly (lactic-co-glycolic) acid (PLGA)/fibrin hybrid scaffold.
Materials and Methods: hADSCs seeded in PLGA/fibrin scaffold and cultured in chondrogenic media. These cells divided into 5 groups (control, TGFβ-3, ASU, ICA and ASU/ICA). After 14 days, the viability of cells in all groups were calculated by MTT. The gene expression of chondrogenic was quantified by real time PCR. Protein expression levels were evaluated by Western blotting.
Results: The cell viability in ASU/ICA group significantly increased in comparison with the TGF-β3 group. Genes expression levels of type II collagen (Col II) and SOX9 significantly increased in all groups in comparison with the control group. Aggrecan (*AGG*) gene significantly increased in TGF-β3, ASU and ASU/ICA groups in comparison with the control group. Type X collagen (*Col X*) gene significantly increased in TGF-β in comparison with the all groups. Genes expression levels of type X collagen (Col I) significantly increased in TGF-β3 group in comparison with the ASU/ICA group. Protein levels of Col II significantly increased in all groups in comparison with the control group. Protein levels Col X significantly decreased in the groups of ASU, ICA and ASU/ICA in comparison with TGF-β3.
Conclusion: Using ASU, ICA and particularly synergist form can induce chondrogenesis in hADSCs in PLGA/Fibrin composite scaffold.
Key words: *Human adipose-derived stem cells, Avocado/Soybean, Icariin.*

P-63

Association of soluble leptin receptor level and its polymorphism (rs1137101) with infertility and abortion in Iranian women with polycystic ovary syndrome

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Background: Leptin is an adipocyte-derived adipokine that plays a crucial role in metabolic and reproductive functions via interacting with its specific leptin receptor (LEPR). A form of LEPR binds to leptin in the circulation and modulates its level in plasma. It has been indicated that the level LEPR and also rs1137101 polymorphisms of the *LEPR* gene are associated with metabolic disorders.
Objective: This study was to investigate the levels of the soluble LEPR, and also the frequency of rs1137101 polymorphism in subjects with polycystic ovary syndrome (PCOS) and those without PCOS.
Materials and Methods: A total of 324 PCOS patients (including 199 infertile patients and 125 patients with a history of recurrent pregnancy loss) and 150 non-PCOS were included in this study. Biochemical parameters and plasma level of soluble LEPR were measured and the genotype of rs1137101 polymorphism was determined using PCR-restriction fragment length polymorphism techniques.
Results: There was a significantly lower level of LEPR in PCOS (58.13 ± 24.3 ng/ml), PCOS-infertile (58.74 ± 24.04 ng/ml), and PCOS-abortion (57.62 ± 24.67 ng/ml) compared to the non-PCOS group (72.95 ± 22.95 ng/ml). Our data also shown that there was significant differences in allelic (G) and genotypic (GG) frequencies for the LEPR rs1137101 polymorphism in PCOS women when compared with the non-PCOS subjects (p = 0.033, OR = 0.67, 95% CI = 0.46-0.96 and p = 0.02, OR = 0.39, 95% CI = 0.18-0.86, respectively). The analysis of LEPR rs1137101 polymorphism gene revealed significant differences in GG genotype and G allele in PCOS-infertile women as compared to non-PCOS subjects.
Conclusion: According to the results, the levels of soluble LEPR were associated with PCOS, and rs1137101 polymorphism was correlated to PCOS-related infertility. Thus, this polymorphism may be considered as a prognostic biomarker of infertility in PCOS women.
Key words: *Polycystic ovary syndrome, Leptin receptor, Polymorphism, Infertility.*

P-64

Polymorphism of hormone-sensitive lipase C-60G in obese and non-obese polycystic ovary syndrome patients

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder in women of reproductive age. Since most PCOS patients are obese, abnormal lipid metabolism has an essential role in the pathological development of PCOS. Hormone-sensitive lipase (HSL) is an intracellular lipase that has a crucial role in normal lipid metabolism.
Objective: This study aimed to assess the frequency of C-60G polymorphism of HSL in healthy women and PCOS women.

Materials and Methods: 324 women with PCOS and 144 healthy controls were enrolled in this study. All subjects were further divided into Non-PCOS and body mass index (BMI) ≥ 25 (n = 72), PCOS and BMI ≥ 25 (n = 197), Non-PCOS and BMI < 25 (n = 67) and PCOS and BMI < 25 (n = 117) subgroups. Biochemical parameters were measured and the genotypes of C-60G polymorphism of the *HSL* gene were determined using PCR-restriction fragment length polymorphism techniques.
Results: Age, BMI, Insulin, HOMA-IR, FT, and follicle-stimulating hormone levels were significantly different between subgroups. Our results have shown that there was no significant difference between fasting blood sugar, triglyceride, serum total cholesterol, luteinizing hormone low-density lipoprotein, and high-density lipoproteins levels in cases and controls. The genotypic and allelic frequencies of HSL showed no significant differences between PCOS – with BMI ≥ 25 and/or BMI < 25- and non-PCOS subjects.
Conclusion: According to the obtained results, the C-60G polymorphism in the HSL promoter was not associated with PCOS and BMI.
Key words: *Polycystic ovary syndrome, Hormone-sensitive lipase, Polymorphism.*

P-65
The protective effects of omega3 on ubiquitination and protamination of rat sperm after bleomycin, etoposide, and cisplatin treatment

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Background: Generation of oxidative stress during chemotherapy leads to imbalance between oxidant and antioxidant in different organs, that consequencely, increase the risk of male infertility. Bleomycin, etoposide, cisplatin (BEP) are popular agents for testicular cancer treatment. Chemotoxic effects of BEP on reproductive organ including: weight loss, significant decrease in sperm concentration and motility. Also mentioned abnormal chromatin condensation induced by BEP can influence histone and reduced translation of protamine1. Ubiquitin is a small chaperone protein that ubiquitinated Histone2A protein during chromatin remodeling. Histone modification plays critical role for regulating nucleosome stability in order to control gene transcription and DNA repairment. During spermiogenesis, 95% of somatic histones replaced with protamine that results chromatin condensation. Every abnormalities in chromatin remodeling can lead to infertility. Omega3 (polyunsaturated fatty acids) has antioxidant, antiapoptotic and anti-inflammatory properties. Also, omega3 inhibits generation of reactive oxygen species that protects against oxidative damage and lipid peroxidation in testis.
Objective: The purpose of this study is evaluation the protective effect of omega3 on rat sperm protamination and ubiquitination after treatment with BEP drugs**.**
Materials and Methods: In this present study, 40 male rats were divided into four groups: Control, BEP, omega3 and BEP+omega3. Sperm protamination and ubiquitination were assessed using chromomycin A3 and immunofluorescence staining respectively.
Results: The mean percentage of ubiquitinated sperm in BEP group was significantly increased relative to control group ($p < 0.001$). But, the mean percentage of sperm protamination significantly decreased in BEP group relative to control group ($p < 0.001$). Rats in BEP+omega3 group showed a significantly decreased in the mean percentage of sperm ubiquitination as compared to BEP group ($p < 0.05$) while, sperm protamination increased significantly relative to BEP group ($p < 0.001$). Administration of omega3 after chemotherapy showed an improvement in sperm ubiquitination and protamination.
Conclusion: Our data indicated that omega3 after chemotherapy may be beneficial for chromatin remodeling during spermatogenesis following BEP treatment.
Key words: *Chemotherapy, Ubiquitination, Protamination, Rat sperm.*

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P-66
The role of the *SYCE1* gene in the male and female fertility: A literature review

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Background: Infertility is described as the inability to become pregnant after at least a year of unprotected intercourse. It affects approximately 10-15% of couples worldwide and can involve males and females. There are different risk factors for infertility. Genetic factors are one of the most important among those and some of them affect synaptonemal complex (SC). It is a protein set that facilitates synapsis formation and crossing over during meiotic division in males (spermatogenesis) and females (oogenesis).
Objective: Here, we try to summarize the roles of SC especially, the synaptonemal complex central element-1 (SYCE1) in fertility.
Materials and Methods: We used the search term TITLE-ABS-KEY (SYCE1) from article names,

keywords, and abstracts to search the article in PubMed. In addition, we screened all references to related papers for extra research.

Results: The SC comprises several compartments: lateral elements (LEs), which are located on both sides of homologous chromosome axes, and a central region containing a central element (CE), and transverse filaments (TFs). The protein components of the mammalian SC include synaptonemal complex protein-2 (SYCP2), synaptonemal complex protein-3 (SYCP3) in the LEs, synaptonemal complex central element protein-1 (SYCE1), synaptonemal complex central element protein-2-testis-expressed protein-12 (SYCE2-TEX12), synaptonemal complex central element protein-3(SYCE3), and Six6 opposite strand transcript 1 (SIX6OS1) in the CE and synaptonemal complex protein-1(SYCP1) in the TFs. All components have been associated with meiosis division, so mutations in those can be associated with abnormalities of gametogenesis. Mutant mice have been associated with infertility for all SC proteins, except for female mutants in Sycp2 and Sycp3. Functional studies revealed that the male *Syce1*-knockout mice had excessive primary spermatocytes with maturation arrest, and the females had small ovaries with almost complete lack of follicles. In humans, the function of the *SYCE1* gene also appears to be as important as in mice. To date, homozygous mutations of *SYCE1* affecting infertility have been reported in three families worldwide. In 2014, a nonsense homozygous mutation (c.613C>T; p.Gln205*) in two sisters affected with primary ovarian insufficiency (POI) was reported. Thereafter, in 2015 and recently in our study in 2020 by whole exome sequencing (WES), two multi affected families were described with non-obstructive azoospermia (NOA), who carried splice-site mutations c.197-2A>G and c.375-2A>G, respectively. Whereas, the C-terminal of the SYCE1 protein is essential for interaction with SYCE3 and thereby assembly of SC, all three aforementioned mutations produce the truncated proteins without C-terminal of the normal protein. It can be possible that nonsense mediated decay (NMD) causes no expression of the mutated mRNA.

Conclusion: To date, all three reported families with mutations in *SYCE1* have originated from the Middle East; Israeli-Arab, Iranian-Jewish, and Iranian families. Therefore, screening of SYCE1 mutations among the large cohorts of infertile males and females in Iran and other Middle East countries is recommended.

Key words: *Synaptonemal complex, SYCE1, Infertility, Iranian population.*

P-67

Evaluation the remote organ functions in polycystic ovary syndrome-induced by estradiol valerat in rats

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Background: Polycystic ovarian syndrome (PCOS), Metabolic and heterogeneous disorder, prevalence in women between 5-10%. PCOS presents itself by a numerous clinical manifestation that may affect remote organs such as brain, liver and kidney as well as ovaries.

Objective: The main aim of this study is to investigate the effects of PCOS on remote organs (brain, liver, and kidney).

Materials and Methods: Twelve female rats were randomly assigned to 2 experimental groups: 1) Sham and 2) PCOS. In the Sham group PCOS induction was not performed. In the PCOS group animals received 4 mg/kg estradiol valerate in 0/2 mg sesame oil as a single dose intra muscular. After 31 days, animals were anesthetized and blood, liver, and brain tissues were collected for evaluation of kidney function markers, Urea and plasma creatinine, and liver enzymes, Alanine transaminase and Aspartate transaminase, and measurement of oxidative stress (Malondialdehyde and superoxide dismutase) in liver and brain.

Results: PCOS altered kidney function and liver enzymes significantly as well as the oxidative stress markers in liver and brain as malondialdehyde levels increased and superoxide dismutase activity decreased.

Conclusion: The current study showed that PCOS may affect other organs like kidney, liver, and brain via oxidative stress. Thus notice to remote organs in PCOS is as important as reproductive organs.

Key words: *PCOS, Oxidative stress, Remote organs, Brain, Liver.*

P-68

Evaluation the effects of polycystic ovarian syndrome-induced by estradiol valerat on ovaries function, oxidative stress and histological changes in female rats

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Background: Polycystic ovarian syndrome (PCOS) is the most prevalent endocrine disorder of women of reproductive age usually accompanied by polycystic ovarian morphology, hyperandrogenism and anovulation or oligomenorrhea.

Objective: The aim of this study is to clarify tissue changes and oxidative stress status in PCOS rats.

Materials and Methods: The PCOS rat model was developed by the once intra muscular injection of Estradiol valerat. Twelve wistar female rats were randomly assigned to two control and PCOS groups. Control group was without any manipulation; PCOS group was administered with Estradiol valerat (4 mg/kg) dissolved in sesame oil (0.2cc); Ovary functional parameters (folliculogenesis, corpora lutea, stage of follicles), SOD activity and MDA levels were measured in ovary samples.

Results: Significant changes were observed in ovary functional parameters, ovarian SOD activity and MDA levels in compared to control group.
Conclusion: This study showed that due to oxidative stress in ovary, the growth of follicles in the preantral stage, folliculogenesis and the number of corpora lutea were changed in PCOS. Therefore, in PCOS the chance of fertility may reduce.
Key words: *Estradiol valerat, Polycystic ovarian syndrome, Oxidative stress.*

P-69
Inhibition effect of gamma-aminobutyric acid ergic system on oxidative stress in the dorsal hippocampus in an experimental model of polycystic ovary syndrome induced by morphine

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Background: Because of noticeable occurrence of endocrine disorders in women such as polycystic ovary syndrome, researchers have conducted extensive experimental studies to detail the mechanism of the disease by using the animal model that simulates this type of complication in the model.
Objective: We used the baclofen as a GABAB receptor agonist to reduce oxidative stress induced of polycystic ovary syndrome (PCOS) in active brain regions such as the dorsal hippocampus.
Materials and Methods: For this experiment, 48 female Wistar rats (in the diestrous phase) were randomly divided into seven groups: control (saline), morphine (5 mg/kg), baclofen alone (at doses of 10, 20, and 30 mg/kg), and pre-injection of baclofen doses to the morphine. 24 hr after the experiment, the animals' brains were removed and the hippocampus was isolated on ice for histological and oxidative stress studies. The results were analyzed by analysis of variance (ANOVA) with an error coefficient of 0.05.
Results: In this morphine-induced experimental model of PCOS, the level of dorsal hippocampustissue stress was significant compared to the control group, but in the groups pretreated with baclofen, stress in the dorsal hippocampus decreased.
Conclusion: Reproductive difficulties such as PCOS cause oxidative stress in the active brain areas such as the dorsal hippocampus. The use of baclofen as an agonist of the gamma-aminobutyric acid ergic system shows a protective effect in this complication. Therefore, the gamma-aminobutyric acid ergic system may involve in stress inhibition circuits in this area.
Key words: *Polycystic ovary syndrome, Morphine, Baclofen, Oxidative stress, Dorsal hippocampus.*

P-70
The stereological evaluation of testis structure on protective effect of quercetin against lead acetate toxicity

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Background: Exposure to environmental pollutants tightly impacts on male fertility sometimes are irretrievable.
Objective: In the present study, we studied the toxic effects of lead acetate (Pb) on testicular structure, and the possible effect of quercetin on qualifying these effects.
Materials and Methods: Experimental groups, including the Pb, quercetin (QE), (Pb + QE), and control mice, were treated at least one spermatogenic cycle. The fixed testes were dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections were prepared using Cavalieri method in a series of equal parallel planes (5 and 20-µm thickness).Then the samples were evaluated by stereological methods.
Results: Testicular weight, both absolute and relative, was higher in Pb-exposed mice in comparison with the control and Pb-quercetin groups. The increase in the size of testis was related to the lumen and connective tissue in this group. Lead acetate induced different patterns in testicular cell number; as spermatogonia, spermatocyte, and Sertoli cells number did not affect in lead acetate exposed group, while the total number of round spermatids and long spermatids significantly reduced.
Conclusion: In conclusion, Pb administration adversely impacted on the cellular organization and activation of the apoptotic pathways in the testis; on the other hand, quercetin co-administration with lead partially ameliorated these adverse effects.
Key words: *Antioxidant, Heavy metal, Male fertility.*

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P-71
Evaluation of H2S level changes and oxidative stress in the ovary in the polycystic ovary syndrome in rat model

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Background: Polycystic ovary syndrome (PCOS) is one of the common endocrine and metabolic disorders and occurs in reproductive-aged women. Recent findings have shown that hydrogen sulfide, as one of the gaseous transmitters, is involved in the process of egg maturation and follicogenesis.
Objective: In this study, we investigated changes in H2S levels and their relationship with changes in oxidative stress index levels in this disease.
Materials and Methods: Twelve female rats are randomly selected and divided into 2 groups of 6: 1) control 2) PCOS. In order to induce the polycystic ovary, we dissolve 4 mg of estradiol valerate in 0.2 ml of sesame oil , then inject it intramuscularly in a single dose. Ovarian tissue samples were taken after 21 days to measure the level of oxidative stress indices and determine the level of H2S.
Results: In this study, there were observed that after induction of PCOS, the level of H2S and SOD activity in ovarian tissue reduced and the MDA concentration increased compared with the control group.
Conclusion: This study showed that there is a relationship between H2S level and polycystic ovarian syndrome. Measurement of this parameter may be considered as a reliable diagnostic test for patients with PCOS.
Key words: *H2S, Ovary, Oxidative stress.*

P-72
Protective effect of Sophora pachycarpa root extract on testicular histopathology and sex hormones level in acid in carbon tetrachloride-intoxicated in male rats

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Background: Carbon tetrachloride (CCl4) is an industrial solvent that causes liver, kidneys, lungs, testicular and brain damage as well as in blood diseases by generating free radicals. Alterations in the spermatogenic cycle and degeneration in seminiferous tubules has been induced with CCl4 in rat. Previous studies on the chemical composition of Sophora pachycarpa (S.pachycarpa) have shown the presence of antioxidant compounds such as flavonoids.
Objective: The purpose of this study was to investigate the protective effects of S.pachycarpa roots extracts on testicular histopathology and serum level of sex hormones in carbon tetrachloride-intoxicated in male rats.
Materials and Methods: Thirty six male wistar rats (195-200 g) were selected and randomly divided into 6 groups (n = 6): pre-treatment groups I, II, III received S.pachycarpa extract at doses 50 mg/kg/day, 100 mg/kg/day and 250 mg/kg/day by gavage for 21 days prior to intraperitoneal injection of CCl4 500 µl/kg on 21st day, control group, CCl4 group received 500 µl/kg CCl4 on the 21st day, post-treatment group received extract at doses 100 mg/kg/day for 10 day at 12 h after CCl4 250 µl/kg injection. At the end of the treatment, blood was collected by cardiac puncture from all of the animals and serum levels of Follicle Stimulating Hormone, Luteinizing Hormone and Testosterone were assessed, also the testis tissues were harvested for histological examination.
Results: Serum levels of testosterone and follicle stimulating hormone were significantly increased in serum of pre-treatment group III and serum level of luteinizing hormone in serum of pre-treatment group III compared to CCl4 was significantly increased ($p < 0.05$). treatment of S.pachycarpa extract (250 mg/kg) showed noticeable improvement in histopathalogical changes induced by CCl4 in testis sections.
Conclusion: From the results it is suggested that S.pachycarpa extract can partly ameliorate toxic effects of CCl4 in male reproductive system, possibly through antioxidant effects of its bioactive compounds.
Key words: *Sophora pachycarpa, Carbon tetrachloride, Testis, Sex hormones, Male rat.*

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- *Banan Khojasteh SM, Javanmard khameneh R, Houresfand M, Dehghan G, Heidari R, Iranshahi M. Investigation in protective effects of Sophora pachycarpa extracts on serum level of sex hormones, urea and uric acid in carbon tetrachloride-intoxicated in male rats. J Med Plants 2016; 15(60): 94-100.*

P-73
Insulin resistance defects in male fertility

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Background: Insulin resistance (IR) in men with unexplained infertility can be a possible cause of hypogonadism and idiopathic oligozoospermia along with other metabolic abnormalities.
Objective: In this study we investigate IR and following that inflammation effects in male infertility.
Materials and Methods: 45 men with IR deficiency (case group) and 30 men without IR deficiency (control group) were enrolled in this study. Body mass index, testicular volume, semen samples, serum hormone/lipid profiles and high sensitive C-reactive protein (hsCRP) were compared in two groups.
Results: Both case and control groups have shown no significant differences in terms of age, testicular volume, serum hormone, and lipid profiles and body mass index. Nevertheless, HOMA-IR was associated with hsCRP levels ($r = 0.92$, $p < 0.0001$).
Conclusion: Lifestyle management is an essential aspect of IR on men's health and fertility which include, nutrition therapy, physical activity, smoking cessation. It is assumed that male infertility pathophysiology discovery should be effective in therapeutic interventions.
Key words: *Infertility, Dyslipidemias, Insulin resistance, C-reactive protein.*

P-74
Bacteriospermia and its association with seminal fluid parameters and infertility

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Background: *Chlamydia trachomatis* (*C. trachomatis*), *Ureaplasma urealyticum*, *Ureaplasma parvum* (*U. parvum*), *Mycoplasma hominis* (*M. hominis*) and *Mycoplasma genitalium* (*M. genitalium*) are among the most prevalent sexually transmitted bacteria. The impact of these bacteria on semen quality and their role in male infertility remains a controversial issue.
Objective: This study was conducted to determine the prevalence of bacteriospermia in infertile and fertile men and evaluate the correlation between the presence of these bacteria with infertility and semen quality.
Materials and Methods: In this cross-sectional study, 100 infertile and 100 fertile men attending to the research and clinical centers for ifertility in Kerman, Iran were enrolled from July to December 2019. Semen analysis was performed using the methods outlined by the World Health Organization. Polymerase chain reaction was used for detection of *C. trachomatis*, *Ureaplasma urealyticum*, *U. parvum*, *M. hominis*, and *M. genitalium*. Then the correlation between the presence of mentioned bacteria with infertility and the semen quality was evaluated.
Results: There was a significant difference in the presence of *M. genitalium* and *C. trachomatis* between infertile and fertile men ($p = 0.003$). The mean values of volume, progressive motility, non- progressive motility, sperm concentration, total progressive motility and viability were significantly lower in infertile men than in fertile ones ($p < 0.05$). Statistically significant correlations were observed between the presence of *M. genitalium* and progressive sperm motility ($p = 0.04$), the presence of *M. hominis* and semen volume ($p = 0.03$), contamination with *U. parvum* and the normal form of sperm ($p = 0.02$) and finally between the presence of *C. trachomatis* and the progressive motility of sperm as well as sperm viability ($p = 0.03$). Logistic regression analysis showed that the presence of *M. genitalium* (OR = 8.06, $p = 0.007$) and *C. trachomatis* (OR = 16, $p = 0.016$) was significantly associated with infertility in men.
Conclusion: According to these results, clinician should consider *C. trachomatis* and *M. genitalium* in men with decreased sperm progressive motility and viability during the infertility assessment.
Key words: *Chlamydia trachomatis, Ureaplasma, Mycoplasma hominis, Mycoplasma genitalium, Infertility, Sperm quality.*

P-75
Development and characterization of a novel PEGylated liposome-encapsulated Ceratonia siliqua to improved sperm parameters

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Background: Ceratonia silique is found to have antioxidative properties that may inactivate free radicals and minimize the oxidative stress inside the testis cells in infertile male. Also, it is reported as a protective pharmacological agent against several diseases. In order to increase the drug stability, bioavailability, and to deliver the drug in the targeted tissue, it can be incorporated in a nano-sized vesicle of liposome formulations.
Objective: The aim of this study was to optimize the PEGylated liposome-containing carob to enhance the therapeutic response.
Materials and Methods: Polyethylene glycol (Lipoid PE 18:0/18:0-PEG2000, DSPE-mPEG 2000) and 1, 2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) were purchased from Lipoid GmbH (Ludwigshafen, Germany). Cholesterol and all chemicals, solvents, and salts were bought from Sigma Chemical Company in St. Louis: USA. In this study, we synthesized liposome

formulations containing cholesterol: DPPC: DSPE-PEG2000 at a molar ratio of 70:30:5 and thin-film evaporation method was used for the preparation of stealth controlled- release liposomal the carob. Outcome parameters were mean size of the vesicle, zeta potential, entrapment efficiency and in vitro drug release.

Results: The formulation of liposomal carob demonstrated that the optimum nano-vesicle size with an average diameter of below 100 nm. The vesicles have a suitable negative charge that are stable without aggregation at 4°C. The entrapment efficiency (EE%) for carob was above 80%. Moreover, the release profile of carob during 72 h was slow and continuous with an initial rapid release period followed by a slower release phase.

Conclusion: In conclusion, we successfully developed a stealth liposomal carob with a high potential for systemic delivery. The sustained-release properties of liposomal carob as a successful lipid-based nano-carriers that improves in vivo stability of the drug and leads to pharmaceutical benefits increase and loss of the drugs followed by the high-dose drug.

Key words: *Liposome, Ceratonia silique, Infertility, Sperm.*

P-76
Effects of plasma rich in growth factors treatment in neonate mouse testicular organ culture

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Background: One of the major causes of infertility is the destruction of germ cells in children caused by chemotherapy and radiotherapy. Therefore, sustaining fertility is very critical with respect to patients under such treatments.

Objective: Therefore, developing a proper culture system in order to sustain fertility by inducing in-vitro spermatogenesis in mouse testicular germ cells is of high priority. In the present study, due to the effectiveness of plasma rich in growth factors (PRGF), it was used as a serum in the culture medium of testicular tissue of neonatal mice and then evaluated correspondingly.

Materials and Methods: In this study, mouse testicular tissue fragments were cultured on agar by gas-liquid interphase method lasting for a duration of two wk. Also, PRGF was prepared from a platelet bag and three groups with concentrations of 5, 10 and 20% were selected to find the best-optimized dosage of PRGF in culture medium. Moreover, KSR was used as a serum for the control group. Finally, the obtained samples were examined morphologically and morphometrically which then were analyzed by SPSS software using ANOVA and Tukey tests.

Results: Morphological and morphometric findings revealed that the diameter of the seminiferous tubules of testicular tissues in the culture medium with 5% the PRGF was larger than that of other concentrations ($p < 0.05$). The tissue structure of the tubules was better preserved at this concentration and the number of spermatogonium cells in each tubule was more than that of other groups ($p < 0.05$). Besides, at this concentration, the volume of testicular tissues was shown to be higher than other concentrations. In the control group, i.e. KSR, the diameter of the tubules was larger than what we obtained in the case of the PRGF group with a concentration of 5% and the structure of the tubules was better preserved ($p < 0.05$). Similarly, the number of spermatogonium cells were higher than the PRGF group with a concentration of 5% ($p < 0.05$). Also, the tissue volume was higher than the PRGF group with a concentration of 5%.

Conclusion: Concentration of 5% PRGF can better preserve seminiferous tubules and their cells and tissue volume. However, KSR can better maintain the structure of tubules and their cells and tissue volume compared to 5% PRGF.

Key words: *Testicular organ culture, PRGF, Spermatogenesis.*

P-77
Trehalose attenuates detrimental effects of freeze-drying on human sperm parameters

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Background: Freeze-drying is one of the sperm preservation methods leading to preserve sperm genetic material for a long period.

Objective: Our main goal was to evaluate the effect of trehalose in freeze-drying method on sperm motility, viability, morphology, acrosome, and DNA integrity compared with standard protocol without trehalose.

Materials and Methods: Twenty-five normozoospermic samples were included in this prospective study. Direct swim-up was used for sperm preparation. Experiment was performed on freeze-dried samples containing trehalose (0.2 M) and the results were compared to that of without trehalose. The sperm parameters including count, motility, morphology, viability, acrosome reaction, DNA denaturation, and DNA fragmentation were evaluated before and after freeze-drying in both groups.

Results: The spermatozoa were totally immotile after freeze-drying in both groups. Sperm viability, acrosome reaction, and non-denatured sperm DNA were significantly higher in the trehalose group in comparison with that of without trehalose group. Non-fragmented sperm DNA showed an increasing trend in the trehalose group compared to the without trehalose group. While freeze-drying significantly reduced normal morphology, the addition of trehalose did not affect this parameter.

Conclusion: The results of this study showed that trehalose can attenuate the detrimental effects of freeze-drying on human sperm parameters.

Key words: *Lyophilisation, Cryodesiccation, Sperm.*

P-78
Effects of plasma-rich in growth factors on cryopreservation of human sperm

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Background: Sperm cryopreservation has been used worldwide in infertility clinics. During freezing process, sperm cell is exposed to oxidative stress that results in damage to membrane integrity, sperm viability, sperm motility and DNA structure. In order to protect sperm cell, many strategies have been proposed.

Objective: In this study, we evaluated the effect of plasma rich in growth factors (PRGF) on sperm parameters during sperm freeze-thaw process.

Materials and Methods: Twenty normozoospermic semen samples were included in this study. All semen samples were processed with direct swim-up. All sperm parameters including sperm motility, viability, acrosome reaction, DNA denaturation and fragmentation and chromatin packaging were evaluated before freezing. After that each sample was divided in two groups: control and 1% PRGF. We used rapid freezing technique to freeze all samples. After thawing all sperm parameters were evaluated again and compared to before freezing.

Results: After thawing, all sperm parameters were significantly decreased compared to before freezing. PRGF supplementation was able to improve all sperm parameters compared to control group. Therefore supplementation of freeze-thaw medium with 1% PRGF could significantly improve all sperm parameters including sperm motility, sperm, normal morphology, acrosome integrity, DNA denaturation and fragmentation and chromatin packaging.

Conclusion: According to this study, we conclude that supplementation of freeze-thaw medium with 1% PRGF could significantly improve all sperm parameters including sperm motility, viability, normal morphology, acrosome integrity, DNA denaturation and fragmentation and chromatin packaging.

Key words: *PRGF, Platelet, Growth factor.*

P-79
Evaluating the role of alginate on human sperm cryopreservation

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Background: Sperm freezing is currently a widely used method to preserve sperm fertility in infertility clinics. During this process, sperm cells undergo considerable damage, including membrane, acrosome area, DNA integrity. Consequently, several ways have been developed to protect sperm cells against freezing damage, one of which is encapsulating sperm cells using alginate.

Objective: We aimed to evaluate the impact of alginate on human sperm motility, viability, morphology, acrosome reaction, and DNA integrity during freeze-thawing process.

Materials and Methods: Twenty-five human normozoospermic samples were included in this study. The sperm parameters were examined before and after direct swim-up. Eventually, the samples were divided into two groups of containing 1% alginate and the control group lacking alginate. The samples were then frozen by rapid freezing, and after melting, the sperm parameters were examined in terms of number, motility, morphology, viability, acrosome reaction, and DNA denaturation and fragmentation.

Results: All the measured parameters were significantly reduced following freezing, compared to their measurement before freezing. Motility was shown to be noticeably lower in the alginate group, while viability, morphology and DNA fragmentation was not significantly different between alginate and control group. Acrosomal integrity and DNA denaturation were significantly increased in the alginate group in comparison with the control.

Conclusion: Based on the obtained results, alginate might be capable of inhibiting premature acrosome reaction as well as preserving DNA against denaturation caused by rapid freezing.

Key words: *Sperm DNA, Alginate, Rapid freezing.*

P-80
Evaluation of the *ALX1* and *PDHX* genes expression in endometriotic tissues of women with endometriosis in comparison with the normal endometrium

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Background: Endometriosis (EMs) is a benign, estrogen-dependent disease and the leading cause of infertility in women of reproductive age. This disease is

characterized by the presence of endometrial glandular tissue and stroma outside the uterus. EMs is considered as a multifactor disease affected by genetic, hormonal and environmental factors. Among genetic factors, aristaless-like homeobox1 (*ALX1*) and pyruvate dehydrogenase protein X (*PDHX*) genes are considered in this study. Studies show that upregulation of the *ALX1* gene cause cell proliferation, migration, and invasion in cancer cells. *PDHX* is involved in cellular metabolism and acts as a tumor suppressor gene while maintaining normal homeostasis. Till now, the specific roles of the *ALX1* and *PDHX* in EMs remain unclear.

Objective: In this study, we investigated the expression of the *ALX1* and *PDHX* in endometriotic tissues of women with EMs in comparison to control endometrial samples.

Materials and Methods: In this case control study, ten eutopic and ectopic endometrium tissues (EMs group) as well as ten normal endometrium (as a control group) were collected. Ectopic biopsies were obtained using diagnostic laparoscopy, while the endometrial control samples and eutopic samples were collected via pipelle. RNA extraction and cDNA synthesis were done then the expression of *ALX1* and *PDHX* genes evaluated by quantitative real-time polymerase chain reaction, using designed primers for the candidate gene. Data analysis performed using One-way ANOVA analysis (SPSS software) considered the significant level of $p < 0.05$.

Results: Results showed a significant decrease in the expression levels of the *ALX1* in eutopic endometrial samples from patients compared to normal endometrium ($p = 0.007$). Although the expression of *ALX1* increased in ectopic endometrium tissues of women with endometriosis compared with eutopic endometrium tissues, this increase was not statistically significant ($p > 0.05$). However, *PDHX* mRNA expression in both eutopic and ectopic groups was significantly reduced than in the control group ($p = 0.017$ and $p = 0.021$, respectively), although the *PDHX* mRNA decrease was not statistically significant between the endometriosis group ($p > 0.05$).

Conclusion: It is suggested that deregulation of *ALX1* and *PDHX* genes could be involved in the pathogenesis of endometriosis.

Key words: *Endometriosis, ALX1, PDHX, Gene expression.*

P-81

Cannabinoid receptor type-1 (*CB1*) and its correlation with *CB1* gene polymorphism-1359G/A in ectopic pregnancy compared to the control group

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Background: Ectopic pregnancy (EP) is one of the most important causes of maternal mortality. Novel information could help to identify the signaling pathway and mechanism of fetal transfer disruption and its potential use in the diagnosis and prevention of this disorder.

Objective: This study aimed to evaluate the immunohistochemical expression of the cannabinoid receptor type 1 (CB1) and its association with *CB1 -1359G/A* gene polymorphism (rs1049353) in the fallopian tubes in EP compared to controls.

Materials and Methods: In this case control study, 100 women with EP (cases) and 100 women that underwent abdominal surgery due to the hysterectomy or uterine tubal ligation (healthy controls) were included. Genotyping of CB1-1359G/A polymorphism, tissue expression of CB1 at the protein and mRNA levels were studied using restriction fragment polymorphism, immunohistochemical (IHC) method, and quantitative real-time polymerase chain reaction (qRT-PCR) analysis.

Results: Genotyping showed that in EP, the frequency of AA, AA+AG genotypes, and A allele was significantly higher than healthy control subjects ($p = 0.001$). Also, patients with EP had significantly increased IHC expression of CB-1 compared to the control samples ($p = 0.016$). Patients with AA and AG genotypes had a significantly higher IHC expression of CB-1 compared to the GG genotype. Quantitative real-time PCR analysis showed that patients with EP had significantly increased expression of CB-1 compared to the control samples ($p < 0.001$). Patients with AA and AG genotypes had higher significant mRNA expression of CB-1 compared to the GG genotype.

Conclusion: Based on molecular and cellular analysis of CB1, the frequency of an allele and expression of CB1 were higher in patients with ectopic tubal pregnancy. Identifying the causes of the EP is essential to find effective methods of prevention and treatment of EP. CB1 is likely to be effective in creating innate immunity in humans and can affect the process of EP in the fallopian tube. CB1 is also a pathological valuable factor in identifying the pathway of inflammation during ectopic implantation. However, it is not possible to claim that a single SNP could be the only cause of the EP. In other words, EP is a polygenic disease, but the possible effects of these genetic changes in EP remain unknown. More investigations are required to introduce a risk prediction tool for susceptibility to EP.

Key words: *Cannabinoid receptor type-1, Ectopic pregnancy, Polymorphism, Immunohistochemistry.*

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P-82
Protective effects of curcumin on sperm and stereological parameters in testes of formaldehyde-exposed NMRI mice: An experimental study

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Background: Formaldehyde (FA) exposure is one of the important causes of cellular injury and oxidative damage in testis, and leads to infertility.
Objective: This study aimed was to assess the protective effects of curcumin on sperm and stereological parameters in testes from formaldehyde-exposed NMRI mice.
Materials and Methods: At 6-8 weeks of age, 24 adult male NMRI mice weighing 30-35 g were separated into four groups (n = 6) based on the treatment they received: Group I (control) no treatment, group II received FA (10 mg/kg), group III received FA (10 mg/kg) and curcumin (100 mg/kg), and group IV (Solvent) received dimethyl sulfoxide (DMSO) (0.2 ml/day). Materials were administered intraperitoneally for 35 days. After excision, epididymis tissues were placed in 1-ml aliquots of Ham's F10 medium at 37°C for 20 min and were then used in analyses of sperm parameters. Testes were fixed and stained with Hematoxylin and Eosin (H & E) for investigations of stereological indices. We also determined lipid peroxidation levels using malondialdehyde (MDA) assays.
Results: Mean sperm parameters (count, motility, viability, and morphology) differed significantly between groups II and III ($p \leq 0.001$). Stereological indices, including Leydig and spermatogonia cell numbers and surface-to-volume ratios of seminiferous tubules were significantly higher in group III than in group II ($p \leq 0.001$). Finally, MDA levels in group III were significantly lower than in group II ($p = 0.001$).
Conclusion: Our data showed that the curcumin, as an antioxidant, reduced FA-induced damage in sperm parameters and stereological indices in mice testis.
Key words: *Formaldehyde, Curcumin, Testis, Mice.*

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P-83
The effect of different concentrations of cerium oxide during pregnancy on ovarian follicle development in neonatal mice

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Background: Cerium is one of the rare chemical elements of earth and belongs to the Lanthanides group. The most commonly used commercial compounds of cerium are cerium oxide and are widely used in human life.
Objective: In this study, we investigated the effect of different doses of Cerium (IV) oxide (CeO_2) during pregnancy on neonatal mice ovaries, as well as its effect on blood biochemical parameters**.**
Materials and Methods: Thirty pregnant NMRI mice were divided into five groups: Control and 4 groups treated with CeO_2 (10, 25, 80, 250 mg/kg.bw i.p) at the Gestational day 7 and Gestational day 14. Postpartum, the ovarian histological of neonatal (2 and 6 day-olds), as well as blood serum of neonates at 15-dpp were analyzed.
Results: Count ovarian primordial follicles in neonates at 2 dpp showed a significant decrease in the groups treated with 80 and 250 mg/kg.bw doses of CeO_2, and ovarian primordial and primary follicles in neonates at 6-dpp at 250 mg/kg.bw doses of CeO_2 into control there was a significant decrease ($p < 0.05$). There was no significant difference in serum levels of Malondialdehyde and Total antioxidant capacity between the experimental and control groups.
Conclusion: Our results suggest the effects of CeO_2 on the ovarian tissue of neonatal mice during pregnancy may depend on the dose.
Key words: *Cerium oxide, Malondialdehyde, Neonate, Ovarian.*

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P-84

The effect of cerium oxide during pregnancy on the development of the testicular tissue of newborn NMRI mice

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Background: Cerium (IV) oxide (CeO_2) is widely used as a catalyst in all aspects of human life and human beings are exposed to these materials.

Objective: The purpose of this experimental study was to investigate the effect of CeO_2 during pregnancy on alterations in the testis tissue and blood biochemical parameters in newborn mice.

Materials and Methods: Pregnant NMRI mice were divided randomly into five groups (n = 6 for each group) including one control group and 4 treatment groups. Injection of CeO_2 solution was administered intraperitoneally at the doses of 10, 25, 80, and 250 mg/kg.bw, respectively, on Gestational day 7 and Gestational day 14. At the end of treatment period, the testicular histological and biochemical parameters of 2- and 6-day-old newborns were analyzed, as well as the biochemical parameters in serum samples of 15- day-old newborns.

Results: The number of spermatogonia, Sertoli, and Leydig cells in the testis of the 2-day-old newborn and spermatogonia and Leydig cells in the testis of the 6-day-old newborns in the 250 mg/kg.bw CeO_2 treatment group was significantly reduced compared with the control group ($p < 0.05$). Testis malondialdehyde concentration (MDA) of the 2- and 6-day-old newborns in the treated group receiving 250 mg/kg.bw of CeO_2 was significantly higher than the control group ($p < 0.001$). There was no significant difference between serum MDA and total antioxidant capacity levels between the treated groups with different doses of CeO_2 compared with the control group.

Conclusion: Therefore, CeO_2 given to dams during pregnancy may affect the testicular tissue and blood biochemical parameters in neonates and may be dose-dependent.

Key words: *Cerium oxide, Pregnant mice, Newborn, Testicular tissue.*

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P-85

Association of *MMP2* polymorphism with premature reduction of ovarian reserve

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Background: Premature ovarian insufficiency (POI) is one of the infertility factors, which affects about 1-3% of women under 40 years of age. There is an increasing list of genetic causes, but POI is mostly idiopathic. Several Studies show that Matrix Metallopeptidase 2 *(MMP2)* gene polymorphisms are effective in reducing ovarian reserve earlier than scheduled.

Objective: This study aim to investigate the association of *MMP2* gene polymorphism (rs243865) with POI in Iranian women undergoing IVF treatment referred to Yazd Infertility Center.

Materials and Methods: One hundred women under the age of 35 with anti-mullerian hormone under 1.7 ng/ml referred to the recurrent abortion clinic in Yazd Reproductive Sciences Institute, and 100 women with normal ovarian reserve with the same age entered as cases and controls in this study. After obtaining the consent to participate in this study, blood samples were taken in an EDTA tube and genomic DNA was extracted by DNA Extraction Kit (JAPA). Genetic variability of *MMP2* was tested using the tetra ARMS-PCR method. A few cases and controls were sequenced for confirmation of the desired SNP.

Results: Genotypes frequency of TT, TC and CC for *MMP2* polymorphism among women with premature ovarian insufficiency was 4%, 36%, and 60%, while in the control group these values were 2%, 58%, and 40% respectively. The diffrences of the frequency of these genotypes between cases and controls were significant. The frequency of the T and C alleles was 8% and 92% in the case and 12% and 88% in control groups respectively, which the differences between them were significant.

Conclusion: According to the results, *MMP2* rs243865 polymorphism may increase the susceptibility to premature reduction of female ovarian reserve.

Key words: *Premature ovarian insufficiency, Matrix metalloproteinase 2, Low anti-mullerian hormone.*

P-86

The effects of glycerophospholipid micelles on the thawed rooster semen

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Background: Today, with the expansion of commercial poultry farms, artificial insemination is used as an efficient method to improve livestock and poultry reproduction programs. For this reason, techniques such as sperm cryopreservation make reproductive programs more efficient. However, due to the abundance of unsaturated fatty acids in plasma membrane, sperm is sensitive to the freezing-thawing process, and sperm cryopreservation increases peroxidation of membrane lipids, decreases membrane integrity, and consequently decreases sperm fertility.
Objective: Membrane lipid replacement with oxidized membrane lipids would restore cellular membrane, and improves its stability. The aim of this study was to investigate the effects of glycerophospholipid micelles on the cryo-survival of thawed rooster semen.
Materials and Methods: Semen samples were collected from six 29-wk Ross broiler breeder roosters, twice a wk, then mixed and divided into five equal parts. The samples were diluted with the Beltsville extender containing different concentrations of glycerophospholipid micelles (GPL) according to the following groups: 0% (GPL-0), 0.1% (GPL-0.1), 0.5% (GPL-0.5), 1% (GPL-1), and 1.5 % (GPL-1.5), then diluted semen was gradually cooled to 4 °C during 3 h and stored in liquid nitrogen. The optimum concentration of GPL was determined based on the quality parameters of thawed sperm such as total motility, progressive motility, viability, apoptosis rate, malondialdehyde level, membrane integrity, and mitochondrial membrane potential.
Results: Exposure of sperm to GPL-1 significantly increased total, progressive motility, average path velocity (VAP), straight linear velocity (VSL), and curvilinear velocity (VCL), and the percentage of viability and membrane integrity were significantly higher in the GPL-1, and GPL-1.5 groups compared to the other groups ($p < 0.05$). Moreover, the lowest rate of apoptosis and lipid peroxidation were observed significantly in GPL-1 and GPL-1.5 groups in comparison to the frozen control group. Mitochondrial activity of thawed sperm was not affected by GPL ($p > 0.05$).
Conclusion: Our findings indicated that membrane lipid replacement with GPL micelles (1%-1.5%) could substitute damaged lipids in membrane and protect sperm cells against cryoinjury.
Key words: *Cryopreservation, Rooster semen, Glycerophospholipid.*

P-87
Engineering biomimetic Polycaprolactone /Gelatin based nanostructure, promise for human spermatogonial stem cells culture

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Background: Improvement of the culture system and increasing the proliferation of spermatogonia stem cells (SSCs) in vitro is essential as a treatment for infertility before autologous transplantation.
Objective: In this study, the proliferation of human SSCs on the electrospun polycaprolactone-gelatin nanocomposite was evaluated.
Materials and Methods: For this purpose, nanofiber porous scaffolds were prepared using the electrospinning method and their structure was confirmed using scanning electron microscope. After swelling and biodegradability tests, human SSCs were cultured on scaffolds, and survival status evaluated using the MTT assay, and their proliferation during two weeks of culture was assessed using immunocytochemistry, flow cytometry, and Real-time polymerase chain reaction techniques compared with the control group.
Results: Scanning electron microscope images showed the presence of fibers with suitable diameter and arrangement and sufficient porosity in nanocomposite scaffolds and showed good biocompatibility and biodegradability. Results showed a significant increase in the number of SSCs in the cultured group on scaffold compared with the control group ($p < 0.05$). The results showed that the expression of promyelocytic leukemia zinc finger *(Plzf)*, *Integrin a6* and, *β1* gene using Real-time polymerase chain reaction in nanofiber scaffolds was significantly higher than the control group ($p > 0.05$). Flow cytometry analysis also showed that the number of Plzf-positive cells in nanofiber porous scaffolds was significantly higher than the control group ($p > 0.05$). Immunocytochemistry findings also confirmed the presence of human spermatogonia stem cell colonies.
Conclusion: In general, it seems that the designed nanocomposite scaffold provides a suitable capacity for self-renewal of human SSCs that can have a good application potential for use in research and reconstructive medicine in the field of male infertility.
Key words: *Spermatogonia stem cells, Polycaprolactone, Gelatin, Electrospinning, Proliferation.*

P-88
The effect of progesterone treatment in women with recurrent pregnancy loss

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Background: Despite advances in research and technology, recurrent abortion remains a clinical and emotional problem for patients and physicians. Recurrent pregnancy loss (RPL), which affects about one percent of fertile couples, is defined by the American Reproductive Medicine Association as two or more failed pregnancies that have been confirmed by ultrasound or histopathology. The three main causes of widespread abortion that are widely accepted include parental chromosomal abnormalities, antiphospholipid antibody syndrome, and structural disorders of the uterus.
Objective: The aim of this study was to evaluate the effect of progesterone treatment in women with recurrent miscarriage.
Materials and Methods: We searched SID, Google Scholar, PubMed, and UpToDate database covering the period of 2000-2021. The search terms habitual abortion, pregnancy loss, treatment, progesterone and recurrent miscarriage were used.
Results: In the reviews, 36 articles were found, and finally 12 articles were selected for review. All studies have examined the effect of progesterone therapy in women with RPL. Some studies do not suggest supplemental progesterone therapy once a pregnancy has been stablished because treatment does not appear to increase the live birth rate. However, some studies have shown a notable improvement in pregnancy outcome after progesterone supplementation in women suffering from RPL. High-quality data on management of RPL are limited; accordingly, therapist suggestions are largely based on clinical experience and data from observational studies.
Conclusion: Since most studies on this topic are statistically insufficient, more research is required on the effectiveness of progesterone therapy in affected women.
Key words: *Pregnancy loss, Treatment, Progesterone, Recurrent miscarriage.*

P-89
A review of factors affecting recurrent pregnancy loss

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Background: Abortion is used to describe a pregnancy that fails to progress, resulting in death and expulsion of the embryo or fetus before gestational age less than 20 week or weight less than 500 gr .Recurrent pregnancy loss is defined as three or more times abortion.
Objective: The aim this study is getting to know risk factors for recurrent pregnancy loss.
Materials and Methods: A comprehensive search in online English databases including PubMed, Google Scholar, Scopus, and Persian databases including: Magiran, Civilica and SID, were conducted to find eligible studies published between 2010 and 2021 in Persian or English. The articles including counseling and treatment were removed. Finally 25 eligible studies were included in our review.
Results: Various risk factors were observed including the followings: age > 40. Family history, Infection: genital tuberculosis, Chlamydia, Trachomatis, Syphilis and cytomegalovirus. Lifestyle factors: obesity, high daily caffeine intake, alcohol consumption, smoking, use of nonsteroidal anti-inflammatory drug, high physical or psychical stress during work, and too much high impact physical exercise. Anatomic defect: chronic endometritis, intrauterine adhesions, myomas, polyps, surgical trauma, congenital uterine abnormality, müllerian ducts including septate, bicornuate, unicornuate, didelphic, and arcuate uteri) Genetic: chromosomal abnormality, displacement robertsonian, HLA, dermatologicall marker, homozygous mutation, p.Leu127Trpfs, in CAPS, and XPO5 (2257082 rs) polymorphism. Decrease serum malondialdehyde concentrations Endocrine factors: Thyroid disorders, hyper prolactinomia, poly cystic ovarian syndrome, hyper insulinemia, hyper androgenemia, and luteal phase deficiency. Immunological factors: anti phospholipid syndrome, antinuclear antibody positivity, protein C and S and anti thrombin 3 deficiencymale factors: Sperm DNA fragmentation, increase in abnormal sperm parameters, nuclear chromatin decondensation, sperm aneuploidy, and chromosomal abnormality.
Conclusion: There are several factors that affect recurrent pregnancy loss, identifying and controlling the causes of recurrent pregnancy loss helps its prevention and treatment.
Key words: *Recurrent abortion, Recurrent early pregnancy loss, Miscarriage, Recurrent.*

P-90
Association of miR-122a expression with high level of oxidative stress in grade III varicocele

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Background: Varicocele is one of the main leading causes of male fertility, and present in ~10% of adult men. In some severe cases, it can lead to dysfunctional spermatogenesis due to unbalanced increase of reactive

oxygen species (ROS) in testis. However, current evidence indicates that ROS-induced oxidative stress is the central mediator contributing to infertility in men with varicocele. Although the harmful effects of ROS on sperm DNA, proteins, and lipids are well documented, its impact on the expression of miRNAs in spermatozoa has not been fully understood. Recent evidences have revealed that specific miRNA contributes to the modulation of oxidative stress and their irregular results due to the impairment in spermatogenesis of infertile males.

Objective: We evaluated the expression pattern or miR-122a as a key factor in germ cell development of spermatozoa of men in three different groups; grade III varicocele patients with normal (VN) and abnormal (VA) spermogram and fertile control (FC) men with proven fertility.

Materials and Methods: In this study, the semen samples were obtained from patients with normal (VN, n = 15), abnormal (VA, n = 15) spermogram and fertile controls (FC, n = 15) in each group. Semen was separated by a density-gradient centrifugation (DGC) to gathered spermatozoa for subsequent RNA extraction. The real-time PCR was performed to analyze the expression of the miR-122a throughout three groups.

Results: Our results showed that the expression levels of miR-122a ($p < 0.001$) were significantly decreased in patients with grade III varicocele with normal (VN) and abnormal (VA) spermogram in comparison with the fertile control (FC) group. A significant reduction in miR-122a expression was also detected in patients with grade III varicocele with normal (VN) compared with patients with grade III varicocele with abnormal (VA) spermogram ($p < 0.046$). Moreover, increased levels of oxidative stress were determined in semen samples of varicocele patients compared with the fertile control ($p < 0.0001$).

Conclusion: In conclusion, our results demonstrated a significant decrease in the level of oxidative stress-related miRNAs in severe varicocele patients, particularly those with defective spermatogenesis. We hypothesized that miRNAs play crucial roles in response to oxidative stress that is induced in spermatozoa of men with varicocele.

Key words: *Grade III Varicocele, Male infertility, Oxidative stress, miRNAs, Reactive oxygen species.*

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P-91

Microfluidic sperm selection improved the sperm quality and ICSI clinical outcomes: A pilot study

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Background: An appropriate sperm preparation technique isolates and collects high quality spermatozoa for intracytoplasmic sperm injection.

Objective: The aim was to assess sperm quality parameters, DNA integrity, embryo development and clinical outcomes using a practical and accessible microfluidic sperm sorting (MSS) technique.

Materials and Methods: 95 ICSI cases performed by spermatozoa was prepared with our designed MSS (group 1) or Direct Swim Up (DSU) methods (group 2). Both sperm quality parameters and DNA fragmentation index were compared between the groups. DNA fragmentation was assessed using Sperm Chromatin dispersion test and fine morphology was assessed using Motile Sperm Organelle Morphology Examination. Also, embryo development and clinical outcomes were compared between the groups.

Results: In the MSS treatment group, progressive motility and the fraction of Class I morphology of spermatozoa were significantly higher compared to DSU control group ($p < 0.01$ and $p < 0.001$, respectively). Moreover, the rates of DNA fragmentation and immotile spermatozoa were significantly lower in MSS group compared to DSU group ($p < 0.001$). Also, higher rates of high quality embryo formation ($p < 0.001$), implantation ($p = 0.04$) and pregnancy ($p = 0.05$) were achieved in in MSS compared to DSU group.

Conclusion: The designed MSS technique was a noninvasive, disposable, easy to use, and inexpensive method for the separation of high quality spermatozoa. Both laboratory parameters and clinical outcomes were improved with the application of MSS for sperm preparation in intracytoplasmic sperm injection procedure.

Key words: *Intracytoplasmic sperm injection, Embryonic development, Sperm sorting.*

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P-92

The effects of soy milk enriched with *lactobacillus casei* on the sexual hormone in ovariectomized rats

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Background: Loss of ovarian function causes estrogen deficiency, followed by menopause and uterine atrophy**.**
Objective: The aim of the present study was to investigate the effects of soy milk Enriched with *Lactobacillus casei* on the sexual hormone, as hormonal replacement therapy in ovariectomized (OVX) rats.
Materials and Methods: Fifty female Sprague Dawley rats were randomly assigned into 5 sets: control, sham-operated, OVX, OVX + soy milk, and OVX + soy milk + *Lactobacillus casei* groups. The soy milk and *Lactobacillus casei* were fed to OVX groups at the concentration of (1×10^9 CFU/ml/day) for 4 wk. Finally, the rate of serum estradiol and progesterone was measured using Elisa reader and the data were analyzed using the SPSS statistical software (V. 23) and Tukey test.
Results: The results showed a significant decrease in serum estradiol, progesterone, in the OVX group compared to the control and sham groups ($p < 0.05$). On the other hand, all the treated groups significantly increased the serum estradiol, progesterone, compared to the OVX group ($p < 0.001$).
Conclusion: The results indicated that soy milk Enriched with *Lactobacillus casei* ameliorate the changes arising from ovariectomy on the sexual hormone as hormonal replacement therapy when ovarian hormones are absent.
Key words: *Soy milk, Lactobacillus casei, Ovariectomy, Rat.*

P-93
The effect of Alpha-lipoic acid on oxidative stress in mouse transplanted ovarian tissue

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Background: Although ovarian tissue transplantation is used to preserve fertility in young women undergoing chemotherapy, but due to the occurrence of ischemia-reperfusion injury and oxidative stress, ovarian function is disturbed and large amounts of follicles are lost. Alpha-lipoic acid (ALA) is a strong free radical scavenger used in the prevention of oxidative stress and cellular damage.
Objective: We aimed to investigate the effect of ALA on the serum level of malondialdehyde (MDA) and total antioxidant capacity (TAC) following mouse ovarian tissue transplantation.
Materials and Methods: 24 mice were randomly divided into: control, autograft (ovarian tissue transplanted to the gluteus superficialis muscle) and autograft + ALA (100 mg/kg intraperitoneal injections of ALA 30minutes prior to transplantation). Serum concentrations of MDA and TAC were measured 7 days after ovary transplantation. Data were analyzed using one-way ANOVA and Tuckey's test and the means were considered significantly different at p-value < 0.05.
Results: The MDA level in the autograft group significantly increased in compare to the control, while it showed a significant decrease in the autograft + ALA group compared to the autograft group ($p < 0.05$). Moreover, the serum level of TAC decreased significantly in the autograft group compared to the control counterpart, whereas it increased significantly in the autograft + ALA group compared to the autograft group ($p < 0.05$).
Conclusion: ALA can reduce oxidative stress and cell damages following ovarian tissue transplantation which can improve the function of the grafted ovary.
Key words: *Ovarian tissue transplantation, Alpha-lipoic acid, Oxidative stress.*

P-94
Alpha-lipoic acid reduces inflammation in the mouse ovarian tissue following transplantation

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Background: Ovarian tissue transplantation can restore fertility in cancerous patients. Ischemia-reperfusion injury and inflammation are major restrictions of ovarian tissue transplantation. Alpha-lipoic acid (ALA), as a potent anti-inflammatory and antioxidant agent, is capable of scavenging free radicals.
Objective: We aimed to evaluate the effect of ALA on the serum level of inflammatory factors such as interlukin6, 10 (IL6,10) and Tumor necrosis factor-α (TNF-α) following mouse ovarian tissue transplantation.
Materials and Methods: 24 Mice were divided into: control, autograft + saline (whole ovarian tissue transplanted in the gluteus superficialis muscle, receiving intraperitoneal injections of saline), autograft + ALA (receiving 100 mg/kg" intraperitoneal injections of ALA, 30 minutes before transplantation). 7 days after ovary transplantation, serum concentrations of IL-6, IL-10 and TNF-α were assayed. Data were analyzed using one-way ANOVA and Tuckey's test and the means were considered significantly different at p-value < 0.05.
Results: Serum concentrations of TNF-α and IL-6 in the autograft group increased significantly compared to the control, while it showed a significant reduction in the autograft + ALA group compared to the autograft group. Moreover, the serum level of IL-10 was significantly lower in the autograft group when compared to the control counterpart. Whereas it showed a significant increase in the autograft + ALA group compared to the autograft group ($p < 0.05$).

Conclusion: ALA can decrease inflammation through its antioxidant effects, therefore can prevent ischemia-reperfusion induced damages and improve the function of the grafted ovary.

Key words: *Ovarian tissue transplantation, Alpha-lipoic acid, Ischemia-reperfusion, Inflammation, Free radicals.*

P-95

The effects of L-carnitine, repaglinide, and mesenchymal stem cell-conditioned medium on in vitro maturation and early embryo development of oocytes derived from normal and endometriosis NMRI mouse

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Background: Endometriosis is an estrogen-dependent chronic inflammatory disorder that adversely affects women in their reproductive age, inducing infertility and pelvic pain. Indeed, oocytes retrieved from endometriosis women are more likely to fail in vitro maturation (IVM), exhibit altered morphology, and lower cytoplasmic mitochondrial content. More importantly, this condition is responsible for 30% of female infertility, and between 30 and 50% of women with endometriosis experience difficulties in becoming pregnant. L-carnitine (LC) is a lysine derivative with anti-oxidative properties that clears hydrogen peroxide and products of lipid peroxidation. Repaglinide (RG) is an anti-diabetic drug that increases intracellular Ca^2+concentration through opening the cells' calcium channels, resulting in insulin release from pancreatic β cells. Mesenchymal stem cell-conditioned media (MSC-CM) contain various growth factors, cytokines, bioactive factors, and tissue regenerative elements generated by mesenchymal stem cells, which can enhance IVM and subsequent embryonic development.

Objective: The current study was aimed to explore the comparative effects of mesenchymal stem MSC-CM, RG, and LC on IVM, in vitro fertilization (IVF), embryo development and formation, as well as on total blastocyst cell numbers.

Materials and Methods: Immature oocytes were collected from two groups of normal and endometriosis induced female NMRI mice ovaries (6-8 weeks old). Oocytes cultured in IVM medium supplemented with 0.0 (control group), 1µM RG, 0.3 and 0.6 mg/ml LC, and 25% and 50% MSC-CM. After 24h of oocyte incubation, the IVM rate was evaluated. Subsequently, MII oocytes were put in the IVF and culture medium then early embryo cleavage was evaluated for 1 to 5 days.

Results: Endometriosis caused a devastating impact upon ovarian histopathology, oocyte maturation, fertilization, early embryo development, and oxidant/antioxidant status ($p < 0.05$). Conversely, in both normal and endometriosis induced mice, different concentrations of RG, LC, and MSC-CM, especially 50% MSC-CM, significantly improved IVM, IVF, and embryo formation rates compared to control groups ($p < 0.05$). Strikingly, better improvement in alleviating EMS-induced injuries was seen in the MSC-CM groups in comparison with other groups.

Conclusion: By way of conclusion, supplementation of IVM medium with RG, LC, and MSC-CM improved oocyte developmental parameters such as maturation, fertilization, and embryo cleavage rates. Our findings indicated that 50% of MSC-CM was the most efficient supplement to reverse endometriosis-evoked deleterious impacts. Indeed, MSC-CM not only does it possesses anti-oxidative properties, but also it contains growth factors, bioactive factors, cytokines and performs anti-inflammatory actions. Present findings have potential applications for improving the clinical trials of humans suffering from endometriosis-related sub/infertility.

Key words: *Endometriosis, In vitro fertilization, Mesenchymal stem cell-conditioned medium, Repaglinide, L-carnitine.*

P-96

The effect of probiotic *Bifidobacterium longum* on testis tissue and testosterone hormone in alloxan-diabetic rats

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Background: Diabetes is a metabolic endocrine disorder that has a major impact on male reproductive system damages. Probiotics are beneficial microorganisms that could have preventive and therapeutic effects in various metabolic disorders beyond gastrointestinal health.

Objective: The present study carried out to investigate the effectiveness of Probiotic *Bifidobacterium longum* on the amelioration of some diabetes complications in the testis tissue in diabetic rats.

Materials and Methods: In this study, 30 male rats were randomly divided into three groups including; control, diabetic (induced with Alloxan 120 mg/kg), and diabetic+*Bifidobacterium longum*. Alloxan was administered intraperitoneally, while the rats in group diabetic+*Bifidobacterium longum* was fed orally by gavage with 1 mL (1×10^9 CFU/ml/day) of probiotics for 48 days. After dissection, fasting blood glucose, oxidative stress markers, and the amount of tumor necrosis factor-alpha as an inflammatory cytokine were estimated. The rats' testes were quickly removed and put

in 10% formalin for further stereological analysis. All data are expressed as mean ± SEM. Statistical analysis was performed using One-way ANOVA followed by Tukey's post hoc tests using SPSS10 (v23) analytic software.

Results: The results showed that malondialdehyde, fasting blood glucose, and tumor necrosis factor-alpha levels decreased, but the level of serum testosterone also anti-oxidant enzymes including superoxide dismutases, and glutathione peroxidase increased significantly in the diabetic group receiving *Bifidobacterium longum* compared to the diabetic control group ($p < 0.05$). The evaluation of testis tissue indicated that diabetic rats treated with *Bifidobacterium longum* significantly increased the number of spermatogonia, spermatocyte, spermatids, a spermatozoid, Leydig cells, and restoration of testis architecture compared to the diabetic group($p < 0.05$).

Conclusion: The results of the present study indicated that the *Bifidobacterium longum* decreased some diabetes complications in the testis tissue. More specifically, our results confirming the protective effects of *Bifidobacterium longum* through repairing the stereological damages induced by Alloxan. Therefore it might be a good candidate for treatment purposes.

Key words: *Testis, Bifidobacterium longum, Diabetic, Rat.*

P-97

Improving effects of Trifolium pratense extract on histopathological changes in the testis of diabetic rats

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Background: Diabetes is the most common endocrine disorders. It has adverse effects on male reproductive function. There are many natural agents available to treat and control diabetes *Trifolium pratens*e L. (*T. pratense*) has traditionally been used for their anti-diabetic and antioxidant effects.

Objective: The purpose of the current study was to examine the effect of hydroalcoholic extract of *T. pratense* on testicular tissue changes in diabetic rats.

Materials and Methods: In this study, 42 male Wistar rats (200 ± 10 g) were divided into groups: control, diabetic, 100 and 200 mg/kg doses of *T. pratense* extract and diabetic treated groups by 100 and 200 mg/kg extract. Rats were treated for 21 days. Diabetes was induced by intraperitoneal injection of streptozotocin at a dose 55 mg/kg. The immunohistochemical expression of bcl-2 and p53 were assessed. Testicular tissue changes were also examined.

Results: The expression of p53 and bcl-2 were increased by diabetes, but it was decreased significantly ($p < 0.001$) in diabetic treated rats with *T. pratense* extract. Furthermore, the extract reduced the testicular tissue destruction induced by diabetes.

Conclusion: *T. pratense* extract improved the undesired side-effects of diabetes on reproductive indices by improved diabetes-induced impairment in testis.

Key words: *Trifolium pratense, Sperm, Testis.*

P-98

The relationship between *FOXR2* gene expression profile and epithelial ovarian cancer

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Background: Several factors have been evaluated for their competency as applied biomarkers regarding diagnosis and therapy of ovarian cancer as one of the most causes of death due to gynecologic malignancies. However, some Fox-factors have been shown to modulate cancer progression primarily by their impacts on the proliferation of the cells, the expression and potential function of *FOXR2* (Forkhead Box R2), newly identified as a probable oncogene in a few human cancers, remains undecided in ovarian cancer.

Objective: The aim was to evaluate the *FOXR2* and some epithelial-mesenchymal transition (EMT) related genes expression profiles in epithelial ovarian cancer (EOC) tissues and their healthy samples as well as an ovarian cancer cell line (SKOV-3).

Materials and Methods: In this observational study, twenty epithelial ovarian adenocarcinoma (case group) and their marginal samples (controls), obtained from 20 patients with EOC, besides SKOV-3, were investigated for the relative gene expression levels of *FOXR2*, *CDH1* (encoding E-cadherin), and *FN1* (fibronectin-1) in two groups using qualitative real-time PCR (qRT-PCR) technique.

Results: The findings demonstrated a significant up-regulation of *FOXR2* and *FN1* despite the *CDH1* down-regulation in case samples compared to controls ($p < 0.05$). There was a significant correlation between *FOXR2* gene expression profile and EMT-related markers in high-grade tumors. Furthermore, the biomarker index of 0.772 was obtained for *FOXR2* gene expression levels.

Conclusion: The findings indicated that the expression levels of *FOXR2* have a significant association with ovarian cancer as far as it can be used as a diagnostic and therapeutic molecular biomarker in ovarian cancer.

Key words: *FOXR2, Gene expression, Ovarian cancer, Epithelial-Mesenchymal transition.*

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P-99

In vitro differentiation of human induced pluripotent stem cells to primordial gem cell-like cells with RA and BMP4 induction

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Background: Since there is still no cure for men with non-obstructive azoospermia infertility to have a biological child, in vitro production of male germ cells from human induced pluripotent stem cells (hiPSCs) is very important because these cells, in addition to having all the characteristics of embryonic stem cells (ESCs), can be produced from patients somatic cells and there is no risk rejection in transplantation to the patient. Researchers have significant results in the differentiation of mouse iPSCs (miPSCs) into primordial germ like cells (PGCLCs) and have shown that human iPSCs (hiPSCs) can also differentiate into PGCLCs; however, the efficiency of PGCLC induction from hiPSCs is < 5%.

Objective: The aim of this study was establish the protocol for in vitro differentiation of embryiod bodies (EBs) derived from hiPSCs into PGCLCs with bone morphogenic protein 4 (BMP4) and retinoic acid.

Materials and Methods: EBs derived from hiPSCs were divided into 3 groups, the first group for 2 days, the second group for 4 days and the third group for 6 days treatment with differentiation culture medium containing 100 ng/m BMP4 and 1 mM RA. The expression of germ cell specific genes and pluripotent genes on the second, fourth and sixth days were evaluated by molecular study. After determining which group had the highest gene expression immunocytochemistry (ICC) was performed for that group.

Results: The result of molecular study showed that in 3 groups, the expression of *PGC* specific genes, *DDX4*, *STRA8* and *DAZL* were increased significantly ($p < 0.05$) and expression of pluripotent genes that including NANOG and OCT4 were decrease significantly ($p < 0.05$), and among the 3 groups, the second group, which was treated for 4 days, expressed higher expression of *PGC* specific genes. ICC images showed that severe expression of PGC specific proteins including STRA8, VASA and c-KIT were detected in this group.

Conclusion: The results of this study showed that the treatment of EBs derived from hiPSCs with differentiating inducers, BMP4 and RA causes the differentiation of these cells and differentiated cells expressed the germ cell markers at the gene and protein levels.

Key words: *Human induced pluripotent stem cells, Primordial germ cells, Differentiation, BMP4.*

P-100

The role of STK31-hsa-circ-0133980, LRWD1-hsa-circ-0003327 in the spermatogenesis and sperm quality from semen samples with different factors of male infertility

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Background: Male infertility is responsible for approximately 50% of infertility. It has been reported that some circularRNAs can act as biomarkers in the diagnosis and treatment of various types of infertility and in fact, may play an important role in regulation of gene expression and spermatogenesis.

Objective: Evaluation of the expression of STK31-hsa-circ-0133980, LRWD1-hsa-circ-0003327 in the normal semen samples (fertile men) and their expression changes in different groups of infertile men.

Materials and Methods: For this study, semen samples were collected from infertile men with different factors of male infertility and fertile men (control group) from IVF center. Part of each sample was used to analyze the expression of STK31-hsa-circ-0133980, LRWD1-hsa-circ-0003327 as well as the expression of genes related with sperm apoptosis (e.g., BAX, BCL2, and Caspase3) using Real Time-qPCR. Also, another part of the same semen samples was used to evaluate sperm quality such as evaluation of chromatin condensation (aniline blue staining), chromatin integrity (toluidine blue staining), and sperm membrane integrity (eosin-nigrosine staining).

Results: It was observed that gene expression in different groups of infertile men was different compared to the control group. Based on the results, it was observed that the morphology, motility and concentration of sperm in the oligozoospermia, teratozoospermia, oligoasthenoteratozoospermia groups were lower than normal compared to the control group.

Conclusion: According to the results, there is a relationship among changes in the STK31-hsa-circ-0133980, LRWD1-hsa-circ-0003327 expression, male fertility potential, sperm quality and spermatogenesis. So that this relationship may indicate the potential of male fertility. Therefore, the analysis of these biomarkers can help to better understand the fertility potential of men and assisted reproductive techniques (ART) outcomes.

Key words: *CircularRNA, Spermatogenesis, Male infertility, Sperm, Semen quality.*

P-101
Association between mir-335-5p and mir-424-5p microRNA expression and semen quality of men with different infertility factors

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Background: Approximately 20-30% of infertility cases are due to male infertility. MicroRNAs are small non-coding single stranded RNA molecules (22 nucleotides). Todays, microRNAs have been used as noninvasive diagnostic biomarkers in male infertility.
Objective: The aim of this study is the evaluation of expression level of mir-424-5p and mir-335-5p innormozoospermia and their alterationsin different groups of infertile men.
Materials and Methods: In this study, semen samples of couples referred to in vitro fertilisation center with male infertility factors were used. The samples were divided into different groups of asthenozoospermia, azoospermia, normozoospermia, oligozoospermia, teratozoospermia and oligoasthenoteratozoospermia. Part of the semen samples wereconsideredfor evaluation of *mir-424-5p* and *mir-335-5p microRNA* expression and *bcl2, caspase3* and *bax*genes using Real Time-quantitative polymerase chain reaction. Also, a part of the same semen samples were used to assay sperm quality, including evaluation of sperm chromatin integrity, chromatin condensation and evaluation of sperm viability.
Results: The percentage of sperm viability and normal chromatin integrity in oligozoospermia men and the percentage of normal chromatin integrity in asthenozoospermia groups increase slightly. Instead, oligoasthenoteratozoospermia grouphas dramatic decrease in normal chromatin condensation (about 35%) and normal chromatin integrity (about 30%) compared to normal group (normozoospermia). Beside these exceptions other infertility factors shows modest decline than control. In this study, also changes in mir-335-5p and mir-424-5p microRNA expression was detected, and it association with decline spermquality found.
Conclusion: In general, there is a correlation between sperm qualityand the expression of semen biomarkers. So that, they can demonstrate the type of male infertility. Moreover, the different microRNA expression of normal and abnormal semen samples may enable the direct diagnosis of semen abnormalities.

Key words: *Male infertility, Spermatogenesis, miRNA, Non-invasive, Spermatozoa.*

P-102
Critical appraisal of published studies on the causes of male infertility in Iran

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Background: In order to provide appropriate treatment policies and strategies for male infertility in each region, it is necessary to know the affecting factors. However, to the clinical application of the result of the various studies in this field, the accuracy of the results must be ensured. Critical appraisal is an essential skill in performing evidence-based practice to integrate the best evidence in clinical care.
Objective: The aim of this study was a critical appraisal of the published studies on the factors affecting male infertility in Iran.
Materials and Methods: Male infertility-related articles in Iran were extracted from PubMed, Google Scholar, Scopus, SID databases with no time limitation, then were evaluated for the inclusion and exclusion criteria. Finally, 7 articles in the English language and 7 articles in the Persian language were appraised. For this purpose, the JBI checklist consisting of 9 different sections was used. The minimum and maximum points that could be obtained for each study ranged from 0 to 9. The overall quality of the articles was classified into three areas: good, average, and poor.
Results: Evaluated articles were indexed in online databases between the years of 2008 and 2018 as follows: 50% in PubMed (7 articles), 57/1% in Scopus (8 articles), 71/4% in Google Scholar (10 articles), and 21/4% in SID (3 articles). The results revealed that 11 articles (78/6%) had good quality, and 3 articles (21/4%) had average quality. Totally, 6 articles were in compliance with all sections of the JBI checklist. Only in 50% of the articles, a suitable sampling method was used.
Conclusion: The quality of published article reports on male infertility factors in Iran were average and good.

To improve the quality of articles as much as possible, it is suggested that researchers apply standard instructions in writing articles. Also, in order to development the simplest and most complete checklist, it is suggested that with different checklists evaluated similar studies, so we will be enable to appraised articles in a short time, critically.

Key words: *Epidemiology, Factor, Male infertility.*

P-103
The most important causes of secondary infertility in women: A narrative review

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Background: Infertility is a relatively common health problem that has a growing trend and has many consequences. Secondary infertility is the inability of the mother to become pregnant again or to continue the pregnancy following the birth of one or more children so that the birth of the previous children does not involve any assisted reproductive technology or fertility drugs.

Objective: The present study was designed to determine the most important causes of secondary infertility.

Materials and Methods: The present study was a brief review study designed in 2021. PubMed/Medline and Google Scholar databases were used to search for similar studies and extract content. Selected keywords for the search included “Infertility”, “secondary infertility”, “women”, “risk factor” and “causes”. The articles were retrieved using advanced search and using AND and OR operators. The two researchers examined the extracted articles and included Latin and Persian articles on the most important causes of secondary infertility in women. Summaries of articles published in congresses and conferences were excluded from the study. Also, articles that did not have a full text were excluded. Initially, about 69 articles were obtained, and after applying the exclusion criteria and reporting the results, 10 articles were finally evaluated.

Results: According to various studies, one of the main causes of secondary infertility is infections that lead to fallopian tube involvement in women. Obstruction of the fallopian tubes caused by a genital infection and the use of traditional medicines in the last postpartum period can also be causes if secondary infertility. Other factors such as age and age of marriage (which has a clear effect on increasing the chances of infertility), place of residence, race, overweight and the presence of some socio-economic factors are effective in secondary infertility.

Conclusion: As mentioned, infections play an important role in cases of secondary infertility, so it is possible to prevent secondary infertility to a large extent by observing personal hygiene during intercourse, pregnancy, during childbirth and after childbirth. Periodic checkups are also helpful for early detection of infection.

Key words: *Secondary infertility, Pregnancy, Causes, Infection.*

P-104
TNP1 as a biomarker in fertilization failure patients following ICSI

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Background: Fertilization failure has been majority attributed to the sperm's inability to stimulate oocyte activation with incidence of 1-3% following ICSI cycles. Phospholipase C zeta (PLCZ1) is considered to be main candidates in sperm for the induction of oocyte activation during fertilization. Transition nuclear protein 1(TNP1) is taking responsibility for condensed structure of the sperm nuclear chromatin. Dysregulation of this gene may results in fertilization failure.

Objective: The aim of present study was to assess the expression *PLCZ1* and *TNP1* genes in patients with failed fertilization undergoing ICSI cycles in comparison with healthy fertile men. In addition, the relationship between expression of these genes and DNA fragmentation was evaluated.

Materials and Methods: In this experimental study, semen samples of 10 fertilization failure men and 15 healthy fertile men were collected. Expression of PLCZ1 and TNP1 were assessed by Real-time PCR. DNA fragmentation of sperm were assessed by SCD method.

Results: Expression of both *PLCZ1* and *TNP1* genes were significantly reduced in fertilization failure patients undergoing ICSI cycles compared to healthy fertile men ($p < 0.01$). Expression of the genes was not correlated with DNA fragmentation of sperm.

Conclusion: The result of our study indicated TNP1 and PLCZ1 may provide useful markers for the fertilization capacity of sperm in fertilization failure patients undergoing ICSI cycles.

Key words: *Fertilization failure, PLCZ1, TNP1.*

P-105
Identification of *Hub* genes and key pathways involved in ovarian carcinomas by integrated bioinformatics analysis

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Background: Ovarian carcinoma is one of the most aggressive cancers among women. Although the main mechanism of this cancer is not clear, several studies have revealed the fundamental role of genetic abnormalities in the pathogenesis of the disease.
Objective: This study aimed to investigate the potential genes and pathways in susceptibility to ovarian carcinomas for prognosis and developing therapeutic strategies.
Materials and Methods: The microarray data of gene expression profile for GSE66957 including 57 ovarian carcinomas and 12 ovarian normal samples based on Rosetta/Merck Human RSTA Custom Affymetrix 2.0 microarray platform was downloaded from Gene Expression Omnibus database. To identify differentially expressed genes (DEGs), GEO2R tool based on R language was applied. Then, we used Database for Anno-tation, Visualization, and Integrated Discovery online tools to perform gene ontology and kyoto encyclopedia of genes and genomes (KEGG) analyses for DEGs ($p < 0.05$ as a significant level). In the next step, the STRING database and cytoscape software were employed to construct protein-protein interaction network. This network was used to predict hub genes in the development of ovarian carcinomas considering maximal clique centrality algorithm.
Results: Our study revealed that up-regulated DEGs were enriched in the membrane (cellular component), protein binding (molecular function), regulation of the biological process, and cell adhesion molecules (KEGG pathway). Down-regulated DEGs were also enriched in the postsynaptic membrane (cellular component), sequence-specific DNA binding (molecular function), positive regulation of cell proliferation (biological process), and Neuroactive ligand-receptor interaction (KEGG pathway). In addition, PPI network analysis revealed 10 hub genes including *CDH1*, *EPCAM*, *CLDN4*, *CLDN7*, *CLDN3*, *KRT8*, *KRT5*, *CD24*, *ESRP1*, and *RAB25*, which were enriched in the lateral plasma membrane (cellular component), structural molecule activity (molecular function), cell-cell adhesion (biological process), and cell adhesion molecules (CAMs) (KEGG pathway).
Conclusion: Our bioinformatics study suggested that these ten genes might play fundamental roles in the progression of ovarian carcinomas, which pave the way for further functional studies to identify biomarkers and future treatments for the disease.
Key words: *Ovarian cancer, Microarray, Gene expression, Bioinformatics.*

P-106
Isolation and culture of germline stem cells from adult mouse ovary

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Background: It has been generally accepted for more than half a century that in most mammalian species, oocytes cannot renew themselves in postnatal or adult life and that the number of oocytes is already fixed in fetal or neonatal ovaries. This assumption, however, has been challenged in 2004 by the finding that mouse ovaries possess rare female germline stem cells (GSCs). However, the existence of these cells in postnatal mammalian ovaries still remains a controversial issue among reproductive biologists and stem cell researchers. The study of these cells depends on the validation of a reliable strategy for the purification of these cells from postnatal ovary tissue. In this regard, we used a differential plating approach to isolate and enrich female GSCs (fGSCs) from mouse ovaries and subsequent in vitro culture of cells.
Objective: Certainly, any potential approach to reform the oocytes in vitro would be of great improvement because any stem cell-based strategies for ovarian regeneration and oocyte production have been proposed as future clinical therapies for treating infertility especially in women facing the risk of Premature ovarian failure after exposure to gonadotoxic chemotherapy and radiation for cancer therapy.
Materials and Methods: Ovarian tissues of adult mouse were collected and dissociated by enzymatic and mechanical methods then subjected to differential plating culture system. Non-attached cells were collected and cultured on the mouse embryonic fibroblasts coated plate. Gene and protein expression analysis were done to characterize the fGSCs. Expression of some of the oocyte markers detected in the spontaneous differentiated cells oocyte like cells.
Results: The fGSCs successfully were enriched and cultured by differential plating approach. fGSCs were proliferated in vitro and expressed germ cell-specific markers (Vasa, Dazl, Blimp1, Fragilis, Stella, Oct4). The cells were cultured for more than four months and maintain the expression of germ cell-specific markers over these times. In vitro cultured fGSCs produced oocyte like cells spontaneously which expressed oocyte-relevant genes and proteins.
Conclusion: Our results demonstrates that differential plating allow reproducible isolation of fGSCs that are able to proliferate in vitro over time. This source of fGSCs can serve as suitable material for studying mechanisms underlying female germ cell development and function.
Key words: *Female germ line stem cells, Oocyte like cells, Premature ovarian failure.*

P-107

Prosopis farcta improves histopathological disorders and reduces oxidative stress of testicular tissue in diabetic rats

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Background: Prosopis farcta (PF) has antioxidant effects and might be effective in avoiding damaging effects of diabetes on the testicular tissue.
Objective: This study purposes to examine the effect of PF on oxidative stress and the structure of testis in diabetic rats.
Materials and Methods: 32 male adult Wistar rats were randomly divided into control, PF, diabetic and diabetic/PF. Streptozotocin was administered intraperitoneally to induce diabetes mellitus in rats. PF group and PF-treated diabetic group received intraperitoneally 300 mg/kg extract of PF for 30 days. At the end of the study, the rats were weighed and dissected. Then, oxidative stress and histopathology of testis were examined.
Results: The level of malondialdehyde in diabetic rats treated with PF decreased in compared with diabetic group ($p = 0.001$), although PF extract increased the level of superoxide dismutase in the diabetic group (373.9 ± 16.6) ($p < 0.001$). Moreover, PF decreased testicular damage caused by diabetes mellitus.
Conclusion: Hydroalcoholic extract of PF improves testicular tissue structure in diabetic rats via decreasing oxidative stress.
Key words: *Testis, Rat, Prosopis farcta.*

P-108

Evaluation of *PGRMC1* gene expression in premature reduction of ovarian reserve in women under 35 years of age in Yazd, Iran

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Background: Premature reduction of ovarian reserve is a heterogeneous condition that it may occure through mutations in genes involved in the normal growth of the ovaries and follicles. Among these genes, it is recognized that progesterone receptor membrane component-1 (*PGRMC1*) gene, as a reproductive gene, is associated with premature reduction of ovarian reserve.
Objective: The aim of this study is evaluation of *PGRMC1* gene expression in women under the age of 35 yr with premature depletion who have referred to Yazd Infertility Center.
Materials and Methods: 25 women under the age of 35 with anti molerian hormone AMH > 2 referred to the recurrent abortion clinicn in Yazd Reproductive Sciences Institute and 25 women with normal ovarian reserve with the same age entered as cases and controls in this study. After obtaining the consent to participant in this study, their blood samples were taken in an EDTA tube and their genomic DNA was extracted by DNA Extraction Kit (FAVORGEN). The expression of *PGRMC1* gene was tested using RT-PCR method.
Results: Our results indicated that the *PGRMC1* gene expression in premature reduction of ovarian reserve samples (positive group) increased relative to the negative group. Also, the area under the curve of PGRMC1 expression of all study populations showed discriminatory power of *PGRMC1* gene expression between the positive and control groups. Besides that, the receiver operating characteristic curve analysis showed the *PGRMC1* gene expression has a sensitivity of 82%, a specificity of 83%, and a cutoff level of 1.76.
Conclusion: This study points to a significant association between *PGRMC1* gene expression and decreased AMH levels in patients with premature reduction of ovarian reserve suggesting that quantitative expression of *PGRMC1* gene in samples may be a valuable biomarker for predicting premature decline in ovarian reserve in young women.
Key words: *Premature reduction of ovarian reserve, PGRMC1 gene, RT-PCR.*

P-109

Epigenetic changes of immune cells in polycystic ovary syndrome

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Background: Polycystic ovarian syndrome is one of the most prevalent causes of female infertility, with a complex etiology. Studies on human tissues have demonstrated a higher expression of inflammation markers among the patients. High level of antithyroglobulin antibodies is also reported among some patients. In recent years studies have reflected that the epigenetic alterations in immune system may be involved in pathogenesis of this syndrome.
Objective: To have a better view on epigenetic changes of immune system among polycystic ovarian syndrome patients a narrative review study was performed.
Materials and Methods: In this study, 98 papers that were indexed by PubMed before September 2020 were analyzed. The literature review was based on the following keywords: autoimmune diseases, cytokines, inflammation, epigenetic, polycystic ovary syndrome, immune system, hyperandrogenism, genomic imprinting, epidrug, CpG islands and DNA methylation.

Results: Studies have indicated that epimutations in immune cells are involved in the pathology of metabolic disorders among these patients. On the other hand, high level of some microRNAs including, miRNA-27b, miRNA-21, miRNA-155 and miRNA-103 were reported among obese patients who had hyperandrogenism. Furthermore, several reports have also confirmed significant hypomethylation of DNA in immune cells, including monocytes, B-lymphocytes, T-cytotoxic and T-helper cells.
Conclusion: In recent years, various epigenetic alterations have been identified among immune cells. However, still further studies are needed to find more epimutations. Comprehensive studies should investigate the exact effect of these changes on the pathology of the disorder in order to find epidrugs or environmental factors that can modify these alterations.
Key words: *Inflammation, Polycystic ovary syndrome, Immune system, Epigenomics.*

P-110
A review of counseling in the pregnancy with abnormal fetuses

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Background: This review examines the cases of improving the therapeutic skills of therapists and areas of counseling, and the important cases that need midwives' services when there is the diagnosis of an abnormal fetus which requires attention.
Objective: Finding the best counseling methods for healthcare providers.
Materials and Methods: In this review study, a search conducted by using the keywords congenital anomalies, psychological counseling and prenatal counseling in PubMed, Science Direct, Clinical Key, and Google scholar search engine. After screening, the complete data of 20 articles were included in this review article.
Results: The results of the studies showed that counseling in pregnancy with abnormal fetuses includes medical and psychological counseling. In medical counseling, full knowledge of the types of tests and their interpretation is important, and prenatal screening training programs for health care providers should be revised based on their educational needs. In psychological counseling, to meet the needs of a changing population of clients, midwives in the context of the wider health care system need more accurate knowledge of religious beliefs and religious and cultural contexts of their clients in order to take the best approach for relevant care. The diagnosis of a congenital anomaly during transmission from parents adds to the accumulation of stress-related events that may increase the risk of developing psychological symptoms in the early stages after diagnosis.
Conclusion: Considering the different cultures of different countries of the world, midwifery counseling skills play an important part in the diagnostic and therapeutic process. Therefore, creating extraordinary educational programs on university education is needed for midwives.
Key words: *Congenital anomalies, Psychological counseling, Prenatal counseling.*

P-111
An overview of the psychological effects of contraceptive methods on women

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Background: The psychological effects of using any method of contraception are not hidden from anyone but in different methods, they have different effects.
Objective: We aim to investigate the different psychological effects of common contraceptive methods in women.
Materials and Methods: A search conducted by key words "contraception", "psychological effects", and "women" in PubMed, Science Direct, Scopus, Clinical Key data bases. Finally, data retrieved from 12 articles were collected and analyzed.
Results: The positive and negative psychological effects were slightly different in consumers. The effect of oral contraceptive and intrauterine device and sterilization on sex life compared to condoms was reported to be positive and in menstrual experiences, oral contraceptive consumers reported higher satisfaction than other methods, in particular, intrauterine device. The regret in using sterilization was higher than in other methods. Psychopathological disorders and psychological disorders developed while using these methods should be differentiated. Negative psychological effects of women using contraceptive methods are often due to their mental background to a mother's role and fertility and the conflict that exists in these methods with their mental image. Also, cooperation and understanding of spouses on the acceptance of these methods and their positive or negative impact has been reported to be very effective.
Conclusion: Before providing any method of contraception, it is recommended to provide comprehensive counseling on each method and follow up with women while consuming to reduce these symptoms and improving their effectiveness.
Key words: *Contraception, Psychological effects, Women.*

P-112

The effectiveness of group counseling based on acceptance and commitment therapy on body image and self-esteem in patients with polycystic ovary syndrome: An RCT

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in reproductive age women which is known with irregular menstruation, hirsutism, alopecia, obesity, infertility, and acne. These symptoms cause a negative effect on the satisfaction of body image, self-esteem, and quality of life in these patients. Recent studies emphasize the need to consider the psychological problems in these women and also the need for appropriate interventions.

Objective: The aim of this study was to determine the effectiveness of group counseling based on acceptance and commitment therapy (ACT) on body image and self-esteem in patients with PCOS.

Materials and Methods: This randomized controlled trial was performed on 52 women with PCOS who met the inclusion criteria (aged 18-45 yr, Iranian resident in Yazd, and diagnosed with PCOS according to Rotterdam criteria and endocrinologist diagnosis). Then, these women were randomly allocated to intervention and control groups (n = 26/each) using the table of random numbers. Group counseling based on the acceptance and commitment therapy was held in eight sessions of 90 min once a week for the intervention group. The demographic questionnaire, Littleton development of the body image concern inventory and Rosenberg self-esteem scale were completed in both groups before, immediately after, and one month after the intervention. Data were analyzed using the SPSS software (version 16.0). Normality of data was analyzed using the Kolmogorov-Smirnov test. Due to the normal distribution, parametric statistical tests (Independent *t* test and repeated measure test) were used to analyze the data. $p < 0.05$ was considered as significant.

Results: The results showed that there was no significant difference in the mean of body image concern scores between the intervention and control groups before intervention, but this difference was significant between the studied groups in two stages of after the intervention and follow-up. In an intra-group comparison of intervention group participants, the results indicated a significant decrease ($p = 0.001$) in the body image concern scores in all three stages of intervention. The mean score of self-esteem before the intervention was not significantly different between the groups. But after the intervention, the mean changes significantly differed between the two groups, and one month after the intervention, there was no significant difference between the two groups. In an intra-group comparison of intervention group, the mean scores of self-esteem in three stages of the intervention was significantly ($p \leq 0.001$) changed.

Conclusion: Based on the findings of this study, use of cognitive-behavioral therapies in health care centers is recommended as a complementary method.

Key words: *Acceptance and commitment therapy, Body image, Self-esteem, Polycystic ovary syndrome, Cognitive behavior therapies.*

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P-113

Study of the relationship between sperm DNA fragmentation and classical parameters with consideration of various diseases

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Background: Sperm chromatin integrity has a major role in the normal embryo's development and pregnancy outcome. Nonetheless, lots of variable factors such as lifestyle, history of surgery and disease can destruction sperm DNA. Therefore evaluation of sperm DNA fragmentation (SDF) could be a good predictor of ART outcome and improvement of healthy pregnancy rate.

Objective: The present study was performed to evaluate the effect of various diseases (Diabetes Mellitus and Mumps) and varicocele and hernia surgery on SDF and sperm classic parameters in a big sample size of infertile couples who underwent ART treatment.

Materials and Methods: The demographic data of 1,191 records of men who were referred to Royan Institute, for SDF test from July 2018 to March 2020 were investigated. Following liquefaction, semen

quality parameters including semen volume, sperm concentration, count, motility, morphology were tested according to guidelines of the WHO, 2010. The sperm concentration and total motility including progressive motility plus non-progressive motility were measured by CASA. The sperm morphology was studied by Papanicolaou staining. The sperm chromatin structure assay (SCSA) test was applied for measuring sperm DNA fragmentation. Data were analyzed using the two-tailed Student's *t* test for independent data.

Results: Within the studied group, 295 men (25%) had varicocele surgery. The results demonstrated this group had higher mean of total DFI (25.77 ± 14.38%) than non-varicocele surgical group (22.70 ± 13.75%) and there is a significant correlation between men who had varicocele surgery and total DFI (p = 0.002) and also all sperm classic parameters including (count, motility, progressive motility, morphology (p = 0.000). Patients who had mumps in childhood had higher total DFI (26.07 ± 1.25%) than patient who had not (23.47 ± 0.44) and the difference was significant (p = 0.042). Also mumps group had lower motility (49.46 ± 0.01%) and lower normal morphology (1.77 ± 0.09%) rather than non- mumps group (54.91 ± 0.78%; p = 0.010), (2.05 ± 0.04%; p = 0.012), respectively. Moreover, 36 men (3%) had hernia surgery and the result demonstrates no significant correlation between hernia surgery and total DFI and sperm classic parameters. Men were divided into two groups based on whether they had diabetes or not (regardless of type I or type II diabetes). Although the mean of SDF in diabetics men was numerically higher than non-diabetics, the difference wasn't significant (p = 0.454).

Conclusion: These results indicate differences in SDF and semen parameters between men who suffering from mumps compare to healthy subjects which warrants further studies. Furthermore, in men who had varicocele surgery SDF was elevated.

Key words: *Sperm DNA fragmentation, Mumps, Varicocele.*

P-114

Evaluating the effect of ovarian stimulation and exogenous progesterone on CD31-positive cell density, VEGF protein, and miR-17-5p expression of endometrium immediately before implantation

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Background: MicroRNAs (miRNAs) form a special class of RNAs regulating endometrial functions like cell proliferation, differentiation, angiogenesis, and blastocyst implantation. In addition to providing suitable conditions for embryo development, angiogenesis is a prerequisite to natural pregnancy. The family of vascular endothelial growth factor (VEGF) and its receptors are the main physiological and pathological angiogenesis regulators in the endometrium. In the past, research has demonstrated alteration of angiogenesis and subsequent endometrial receptivity in the stimulated and luteal phase support cycles, when compared with natural cycles.

Objective: The objective of this study is to investigate the effect of ovarian stimulation and exogenous progesterone on the density of CD31-positive cell (Endothelial cell), VEGF protein, and miR-17-5p expression in the mouse endometrium immediately before implantation.

Materials and Methods: The endometrial CD31-positive cell density was determined by immunohistochemistry (IHC) staining, the level of VEGF protein by IHC and western blot analysis, and finally the miR-17-5p expression was determined by the real-time PCR method.

Results: The density of endothelial cell, VEGF protein, and miR-17-5p expression increased in all of the experimental mice when compared to the control group, with the maximum increase having been seen in the group that had received progesterone after ovarian stimulation.

Conclusion: This research indicates that ovarian stimulation and exogenous progesterone lead to an increase in the number of endothelial cells by upregulating the VEGF protein. Moreover, except for miR-17-5p, other microRNAs and molecules are presumably involved in angiogenic pathways, thereby requiring more studies.

Key words: *VEGF, miR-17-5P, Progesterone, Endometrium, CD31- positive cell.*

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P-115

The evaluation of retinoic acid and estrogen on mouse induced pluripotent stem cells differentiation into female germ cells

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Background: Using stem cells is possible for treatment of patients with genetics or induced abnormalities and diseases such as non-obstructive azoospermia. Today, more attention has been paid to self-induced induced pluripotent stem cells (iPSCs).

Objective: The aim of this study was to investigate the role of estrogen with retinoic acid on the differentiation of mouse iPSCs towards female germ cells.

Materials and Methods: In this study, mouse embryonic fibroblast cells were extracted as feeding cells and inactivated. The target groups such as *Stra8*, *Stella*, *Ddx4* and *GDF9* were adjusted for estrogen with retinoic acid at intervals of days 0, 4, and 7. Expression of these genes was performed by Real Time PCR technique.
Results: In this study, the expression of genes such as Stra8, Stella, Ddx4 and GDF9 was evaluated. Real-time data showed that the expression of these genes increased in estrogen group on day 4 of embryoid bodies culture, while the differences were not significant on other days.
Conclusion: It is very difficult to control the differentiation of mouse induced pluripotent stem cells (miPSCs) and the role of estrogen was carefully investigated in vitro in this study. Evidence suggests that female germ cells can differentiate from miPSCs in vitro. Treatment of cells with estrogen showed a greater effect on the differentiation process on day 4.
Key words: *Differentiation, Mouse induced pluripotent stem cells, Female germ cells, Estrogen, Retinoic Acid.*

P-116
Natural cycle versus modified natural cycle for endometrial preparation in women undergoing frozen-thawed embryo transfer: A randomized clinical trial

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Background: There are three main types of cycle regimens using for endometrial preparation: natural cycle, ovulation induction cycle, and artificial cycle. The natural cycle can be used only in normoovulatory women. Regarding the advantages and disadvantages of each method, various studies have been performed, but the optimal frozen-thawed embryo transfer (FET) cycle strategy in terms of the in vitro fertilization outcomes (clinical pregnancy rate, ongoing pregnancy rate, and live-birth rate) is still debated.
Objective: To compare the natural cycle versus modified natural cycle for endometrial preparation in women undergoing FET.
Materials and Methods: In this randomized clinical trial, a total of 110 infertile women undergoing FET, at Arash Women's Hospital, were included and randomly allocated into two groups: the true natural FET (tNFET) cycle with spontaneous luteinizing hormone surge and the modified natural FET (mNFET) who received 5000 IU hCG injection 36 hr before ET. The outcome measures were: patients' characteristics, implantation rate, chemical and clinical pregnancy, ongoing pregnancy and abortion rate.
Results: There were no differences in patients' baseline characteristics between implantation rate in the two groups. There was no difference in terms of the chemical pregnancy, clinical pregnancy, and abortion rate, while the implantation rate was significantly higher in the mNFET group (29.2 % versus 17.6 %; p = 0.036).
Conclusion: Our results demonstrated that both types of natural cycles are similar in terms of pregnancy outcomes, while the modified cycle may be associated with a higher implantation rate.
Key words: *Embryo transfer, Embryo Implantation, Human chorionic gonadotropin.*

P-117
Protective effect of vitamin E on sperm parameters, chromatin quality and DNA fragmentation in BALB/C mice treated with different doses of ethanol

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Background: Excessive consumption of alcohol induces increasing in oxidative stress production and can lead to detrimental effects in the male reproductive system.
Objective: The aim of the present study was to evaluate possible protective effects of co-administration of vitamin E, as a well-known antioxidant, on detrimental changes of sperm quality in mice intaken ethanol.
Materials and Methods: In this experimental study, 54 BALB/c mice were categorized into 9 groups (n = 6 /each). Group 1: The Control group received a basal diet. Groups 2, 5: Gavaged with 10% and 20% (V/V) ethanol (99% v/v, Merk, Germany) daily, respectively. Groups 3, 4: Gavaged with 10% (V/V) ethanol and injected with Vitamin E (Osveh Co., Iran) 100, 200 mg/kg intraperitoneally, respectively. Groups 6, 7: Gavaged with 20% (V/V) ethanol and injected with Vitamin E 100, 200 mg/kg intraperitoneally, respectively. Groups 8, 9: Received Vitamin E 100, 200 mg/kg intraperitoneally, respectively. The control group received basal diet and experimental groups including (alcohol 10% & 20%, alcohol 10% vit.E 100 & 200 mg, alcohol 20%/ vit.E 100 & 200 mg and vit.E 100 & 200 mg). After 35 days, the epididymis was dissected for analyzing sperm parameters include sperm motility, morphology, and viability. Sperm chromatin was assessed with Aniline Blue and Toluidine Blue staining. TUNEL assay was performed to evaluate the extent of DNA damage in spermatozoa.
Results: The results demonstrated a statistically significant reduction in motility rate (p = 0.04), normal morphology rate (p < 0.0001 and p < 0.0001,

respectively), viability rate ($p < 0.0001$ and $p < 0.0001$, respectively), increase abnormal DNA structure and packaging Toluidine Blue staining ($p = 0.01$) and DNA damage (TUNEL) ($p = 0.04$) in ethanol consumer groups compared to the control. In addition, the findings showed a significant increase in the above-mentioned parameters in ethanol and vitamin E consumer group compared to the counterpart ethanol consumer groups. However, the extent of protamine deficiency Aniline Blue was not different in any experimental group compared to the control. The ethanol group received 20% of the most damage among the groups. The group receiving vitamin E 100 mg/kg and the group receiving ethanol 10% with vitamin 200 mg/kg gained the highest benefit among the groups.

Conclusion: Result showed that sperm forward progressive motility, normal morphology rate and viability decreased significantly in ethanol (10% and 20%) treated groups when compared with the control group also the rates of spermatozoa with abnormal DNA structure and DNA fragmentation increased significantly in the ethanol intake group than the control group. While, co-treatment with vit. E could prevent some of these adverse effects. Our findings in the current study revealed that co-administration of vitamin E and ethanol can protect destructive changes in DNA structure and damage.

Key words: *Ethanol, Sperm parameters, Vitamin E.*

P-118
Association study of *TNF-α*-238G/A, *GSTT1* and *GSTM1* polymorphisms with the risk of recurrent pregnancy loss in Iranian women

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Background: Recurrent pregnancy loss (RPL) affects <5% of couples and shows an increasingly elevated frequency in industrial regions. The reactive oxygen species and cellular oxidative stress are involved in inflammation development. Tumor necrosis factor-alpha (TNF-α), one of the pro-inflammatory cytokines and Glutathione S-transferases are enzymes involved in oxidative stress handling. Polymorphisms of genes encoding mentioned molecules may potentially influence the risk and the outcome in RPL disease.

Objective: In this study, we studied the occurrence of genetic polymorphism in TNF-α, GSTM1, and GSTT1 genes with RPL in an Iranian population as a case control study.

Materials and Methods: In this study, we enrolled 85 women with RPL and 85 normal women (age and ethnically matched healthy controls with successful reproductive history). DNA was extracted from whole blood samples. The GSTM1 and GSTT1 null phenotype was identified by multiplex PCR and TNF-α-238G/A polymorphism was determined using the PCR-RFLP technique.

Results: The results of our study showed significant differences in genotypic frequencies of *TNF-α*-238G/A between case and control groups ($p = 0.0001$), but our results provided no evidence of a relationship between the *GSTM1* ($p = 0.635$) and *GSTT1* ($p = 0.493$) genes polymorphism and susceptibility to RPL in the studied population.

Conclusion: The *TNF-α*-238G/A variant is a possible genetic risk factor for RPL in our population, and this polymorphism can be used as a relevant marker to identify women at risk of developing endometriosis.

Key words: *Recurrent pregnancy loss, GSTT1 gene, GSTM1 gene, TNF-α gene, Polymorphism.*

P-119
Evaluation of sperm parameters and DNA integrity following different incubation times in PVP media

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Background: Polyvinylpyrrolidone (PVP) is a chemical material used in intracytoplasmic sperm injection program.

Objective: The aim of this study was to investigate the ideal time that sperm can be safely incubated in PVP with less structure and DNA damage.

Materials and Methods: Thirty-one oligoasthenoteratospermia samples were used. Sperm samples were prepared by discontinuous density-gradients method and incubated in 10% PVP at different time intervals (0, 5, 10, 15, 20, and 30 min). The effect of PVP was assessed on sperm DNA fragmentation and viability via sperm chromatin dispersion assay and Eosin-nigrosin staining.

Results: Data showed there was a significant increase in sperm DNA fragmentation after 10 min (36.76 ± 7.99, $p < 0.001$), 15 min (37.81 ± 8.11, $p < 0.0001$), 20 min (38.62 ± 8.00, $p < 0.0001$), and 30 min (40.05 ± 7.69, $p < 0.0001$) compared to 0 min. The viability rate also significantly reduced after 10 min (57.71 ± 10.85, $p = 0.04$), 15 min (55.81 ± 10.87, $p < 0.0001$), 20 min (53.19 ± 11.44, $p < 0.0001$), and 30 min (50.24 ± 11.81, $p < 0.0001$) compared to 0 min.

Conclusion: As a result, sperm samples could be incubated with PVP for 10 min with less DNA damage. While, prolonged incubation may significantly damage the sperm DNA integrity and viability.

Key words: *Polyvinylpyrrolidone, Oligoasthenoteratospermia, Sperm, DNA fragmentation, SCD test, Viability rate.*

P-120
The effect of different O-antigen serogroups of Escherichia coli on infertility in semen samples of fertile and infertile men

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Background: Male genital tract infections have been associated with infertility. Among the types of bacteria, Escherichia coli (*E. coli*) has drawn increasing attentions. However, its role in male infertility remains undefined.
Objective: This investigation aimed to characterize and compare the distributions of O-antigen serogroups of E. coli in the semen samples of fertile and infertile men.
Materials and Methods: In this case control study, semen samples were collected from 575 fertile and 1725 infertile men. The *E. coli-positive* samples were evaluated in term of concentration, morphology, viability and motility parameters according to World Health Organization 2010 guidelines. Finally, different serogroups of E. coli were identified by multiplex polymerase chain reaction targeting the O-antigen variations of the bacterium.
Results: The prevalence of *E. coli* among fertile men was significantly higher than infertile men ($p < 0.0001$). The sperm morphology, viability and motility, in the *E. coli-positive* fertile group was significantly higher than in the E. coli-positive infertile group ($p < 0.0001$). The E. coli O6 was the most prevalent serogroup found in both groups. However, there was no significant difference in frequencies of different serogroups of *E. coil* between two groups ($p = 0.55$).
Conclusion: Despite the higher prevalence of *E. coli* among fertile men, the E. coli have more detrimental effect on semen parameters in infertile men. There was no association between the types of E. coli serogroups between two groups.
Key words: *Male infertility, Semen, Escherichia coli, Serogrouping.*

P-121
Investigation of association between rs5030772 polymorphism of *FasL* gene with premature ovarian failure

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Background: Premature ovarian failure (POF), the pathogenesis might be indiscernible for a long time, is related with problems in fertility. In 74-90% of cases, POF is idiopathic but it could be familial (4-33%) or sporadic. The main recognized causes of POF are as follows: Autoimmune ovarian damage, genetic aberrations and environmental factors (i.e. viruses, toxins, etc). Genetic mechanisms of POF are decreased gene dosage as well as non-specific chromosome effect that disrupts meiosis, reducing the pool of primordial follicles along with increasing atresia because of apoptosis or impaired maturation of follicle. Fas ligand (FasL) is a mediator of apoptosis that plays a role in the differentiation of cells and the development of embryo. Polymorphism of FasL gene could be involved in the disease process.
Objective: Considering the importance of apoptosis in homeostasis of normal tissues as well as in disease conditions, we evaluated the impact of a common FasL polymorphism and its relationship with POF.
Materials and Methods: In this case-control study, the polymorphisms of FASLIVS2nt_124A/G were analyzed in Iranian women suffering from POF and healthy controls. Isolation of DNA was done by salting out method and genotype analysis was performed for all the subjects using PCR-RFLP restriction fragment length-polymerase chain reaction) method.
Results: Statistical analysis revealed no differences in codominant or other models of genotype nor allele frequencies between cases and controls ($p > 0.05$).
Conclusion: It appears that FasL INV2nt_124A/G rs5030722 SNPs (Single nucleotide polymorphisms) have no difference between POF patients and healthy subjects.
Key words: *FASL, Polymorphism, POF.*

P-122
Prevalence of cytomegalovirus in semen of male partners of infertile couples and the virus impact on sperm parameters

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Background: Genital tract infection is one of the causes of male infertility. Several studies have shown a role for human cytomegalovirus (CMV) in this context. In the present study, the prevalence of CMV in a population of male partners of infertile couples was estimated and the impact of CMV on sperm parameters was determined.
Objective: In the present study, the prevalence of CMV in a population of male partners of infertile couples was estimated and the impact of CMV on sperm parameters was determined.
Materials and Methods: In this cross sectional study, CMV DNA and virus copy number were ex-amined in the semen of 150 participants including 80 with normal semen analysis (SA) and 70 with abnormal SA, by quantitative Real-Time PCR. Sperm parameters were compared between CMV positive and negative groups. Comparisons with p-values under 0.05 were considered significant. Logistic regression was performed to control the effect of some variables with $p < 0.25$ on sperm parameters.
Results: CMV DNA was detected in the semen of 28 (18.6%) individuals. 21 men (30%) with abnormal SA and 7 (8.8%) with normal SA were positive for CMV DNA ($p = 0.001$). The mean virus copy number was 883.1 ± 4662.01 for the men with ab-normal SA and 2525.7 ± 12680.9 for those with normal SA ($p = 0.001$). Sperm count was (32.1 ± 23.5) $x10^6$ in CMV positive and (44.2 ± 24.1) $x10^6$ in CMV negative groups ($p = 0.022$). Normal sperm morphology was 2.73 ± 2.83% and 5.99 ± 5.44% in CMV positive and negative groups, respectively ($p < 0.001$). After controlling some variables, the sperm morphology remains the only statistically significant sperm pa-rameter that was reduced by CMV.
Conclusion: The higher CMV prevalence in the semen of males with abnormal SA compared to normal SA and significant reduction of sperm morphology in the pres-ence of CMV, are in favor of the negative impact of CMV on male fertility.
Key words: *Cytomegalovirus, Male infertility, Polymerase chain reaction.*

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P-123
Y chromosome-AZFc atypical partial microdeletions in Iranian severe oligozoospermia men

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Background: Azoospermia Factor c (AZFc) region contains important gene families (including DAZ, CDY1) for male spermatogenesis. Meiotic homologous recombination within amplicons is the etiologic cause for various partial microdeletions as well as the changes in copy number of mentioned genes. The association of AZFc partial microdeletions with impaired spermatogenesis and infertility is still debated.
Objective: The aim of this study was to investigate the nature and frequency of partial microdeletions (gr/gr, b2/b3 and b1/b3) in AZFc region.
Materials and Methods: Total DNA extracted from the peripheral blood of 200 oligozoospermic male (sperm count < 5 mil./mL) as patients and 200 fertile males as controls was used to detect partial microdeletions by multiplex PCR and six sequence-tagged sites (STS) markers. Also PCR-RFLP technique was used to detect deletion of gene copies.
Results: Other than 18/400 (4.5%) cases of typical partial AZFc microdeletions (gr/gr, b2/b3 and b1/b3), we detected two cases of deletion pattern of sY1191 and sY1161 (1%), 1 case of sY1201 (0.5%) and one case of sY1258 (0.5%) in the oligozoospermia group. The mechanism of the rearrangement occurring within the Y chromosome of these individuals is complex and unknown.
Conclusion: Similar to some other studies, we have shown that in addition to known AZFc partial microdeletions, other atypical types may also been detected in patients with spermatogenic defects. Although, these findings are extremely rare, very small in size, and may not have much effect on spermatogenesis, there is a risk of vertical transmission and even expansion in the size of deletions in male offspring leading to a boost of infertility rate in the mentioned generation. Therefore, genetic counselling and informed decision making (PGT as an option) will be necessary for such couples before ART. Hence, testing for AZFc partial deletion may be suggested in oligozoospermia cases with idiopathic reason.
Key words: *Male infertility, Y Chromosome, Oligospermia.*

P-124
Effects of lorazepam on uterus and sex hormones in Balb/C adult female mouse

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Background: Lorazepam is one of the drugs used in the treatment of depression, especially use worldwide among young people. The complications of antidepressant usage such as lorazepam, which is readily available, are due to temporary or permanent infertility.
Objective: In the present study, we investigated the effect of lorazepam on the uterus and sexual hormones of Balb/C adult female mice.
Materials and Methods: Lorazepam (Abidi Company, Iran) was injected intraperitoneally with a fixed dose of 2 mg/kg/bw for Balb/C mature female mice (n = 75). Mice were divided into five groups (n = 15/ each) including control (without injection), sham (physiological serum injection), and Lorazepam injection in at three different times of 5, 10, and 15 days. Next, estradiol and progesterone hormones were measured by Enzyme Linked Fluorescence Assay after serum preparation. Then, uterine samples were prepared and stained with eosin and hematoxylin for histological evaluation. Data were analyzed by SPSS software version 22 and Duncan statistical test and ANOVA method.
Results: Animal weight, the number of primary, secondary, growing, and graph follicles, corpus luteum, the number of open secretory glands and progesterone were significantly decreased with increasing the duration of drug injection in the experimental groups compared to the control group, respectively. But, a significant increase was observed in the number of destructive follicles, folded zona pellucida, the number of close secretory glands, oviduct diameter and estradiol compared to control group ($p < 0.001$). In evaluations related to fertility reversal in experimental groups due to increasing the duration of drug injection, a significant increase in fertility time and a significant decrease in the number of embryos was observed ($p < 0.05$ and $p < 0.001$, respectively). In uterine tissue observations, an increase in uterine diameter (endometrium, myometrium and perimetrium) was evident, but this increase was not statistically significant.
Conclusion: The use of lorazepam for a long time can have damaging effects on the female reproductive system and its use should be informed under medical supervision, especially in young girls.
Key words: *Lorazepam, Uterus, Estradiol, Progesterone, Mice.*

P-125
Epidemiology of infertility in Iran: A systematic review

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Background: Infertility refers to not being pregnant after 12 months of unprotected sex. In the world, 15% of reproductive-aged couples are affected by infertility. Childbearing is a social value for married women in developing countries such as Iran. Therefore, couples who suffer from infertility are prone to depression, anxiety and poor quality of life.
Objective: The aim of this review was to investigate the epidemiology of infertility in Iran.
Materials and Methods: This systematic review was based on preferred reporting items for systematic reviews and meta-analysis (PRISMA). A comprehensive search was conducted to find potentially relevant articles in PubMed, Scopus, Web of Science, Iranmedex, Irandoc, and SID using infertility, epidemiology, prevalence, and Iran keywords. Then, the titles and abstracts of the articles were reviewed and articles dealing with different aspects of the epidemiology of infertility were included in the review.
Results: Based on previous studies, in 2015, the overall prevalence of lifetime primary infertility among couples was 17.3%. In addition, the secondary infertility rate was 4.9%. According to the results of previous studies, the prevalence of infertility in Iran is increasing. The presence of a history of turbo-ovarian surgery, salpingitis, ectopic pregnancy, varicocele, and cryptorchidism were positive predictors of infertility. Also, the old age in women, high BMI, active smoking, and higher educational level, higher age at marriage, long-term health problems, and a partner who smoked had a significant association with infertility. The race had no effect on infertility.
Conclusion: In this study, the results of the literature review showed an increase in the prevalence of infertility in the Iranian couple. Infertility seems to be on the rise as couples change their lifestyle and risk factors increase. Therefore, depression and other infertility-related problems among people with infertility need more attention and care.
Key words: *Infertility, Prevalence, Risk factors, Iran.*

P-126
Factor V Leiden 1691G>A mutation and recurrent pregnancy loss risk: Evidence from meta-analysis and meta-regression analysis

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Background: Although numerous case-control studies have attempted to determine the association between factor V Leiden (FVL) 1691G>A mutation and susceptibility to recurrent pregnancy loss (RPL), there have been confliction among the results of various ethnic groups.
Objective: To address this limitation, here we implemented meta-analysis to provide with consistent conclusion of the association between FVL 1691G>A mutation and RPL risk.

Materials and Methods: After a systematic literature search, pooled odds ratio (OR) and their corresponding 95% confidence interval (CI) were used to evaluate the strength of the association.
Results: In this meta-analysis, 61 studies, containing 10255 cases and 9269 controls, were included in quantitative analysis. Overall population analysis revealed a significant positive association in the dominant (OR = 2.11, 95% CI = 1.81-2.47, p < 0.001, FEM), over-dominant (OR = 1.88, 95% CI = 1.61-2.19, p < 0.001, FEM), allelic (OR = 2.08, 95% CI = 1.81-2.40, p < 0.001, REM), and heterozygote (OR = 1.97, 95% CI = 1.68-2.30, p < 0.001, FEM) models. Moreover, a significant association of dominant (OR = 2.97, 95% CI = 1.93-4.57, p < 0.001, FEM), over-dominant (OR = 2.58, 95% CI = 1.65-4.02, p < 0.001, FEM), allelic (OR = 2.82, 95% CI = 1.31-6.04, p < 0.001, REM), and heterozygote (OR = 2.67, 95% CI = 1.71-4.18, p < 0.001, FEM) models was found in the Iranian population. The subgroup analysis, indicated strong significant association in Asian, European, and Africa population, but not in Americans.
Conclusion: The FVL 1691G > A mutation and the risk of RPL confers a genetic contributing factor in increasing the risk of RPL, particularly in Iranians, except for Americans.
Key words: *Recurrent pregnancy loss, Factor V Leiden, 1691G>A mutation, Meta-analysis.*

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P-127
MDM2 gene rs2239745 polymorphism and breast cancer risk: A systematic review and meta-analysis

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Background: Several case-control studies have previously assessed the association of mouse double minute 2 homolog (*MDM2*) gene rs2239745 polymorphism and the risk of breast cancer (BC) that has resulted in incongruous conclusions.
Objective: In order to clarify the conflicting outcomes obtained from different individual association studies, here we performed the most updated meta-analysis of rs2279745 polymorphism and risk of BC.
Materials and Methods: A comprehensive systematic search of literature, including Web of Science, Scopus, and PubMed/Medline was carried out prior to August 2020, and the pooled odds ratio (OR) and their corresponding 95% confidence interval (CI) were calculated to determine the overall association power in the pooled population.
Results: Literature search led to retrieving of 31 case-control studies, containing 14638 cases and 17767 non-cancer controls. The pooled analysis indicated that the dominant model (OR = 1.05, 95% CI = 1 - 1.11, p = 0.03), recessive model (OR = 1.16, 95% CI = 1.01 - 2.32, p = 0.03), allelic model (OR = 1.06, 95% CI = 1.02 - 1.10, p < 0.001), GG vs. TT model (OR = 1.23, 95% CI = 1.06 - 1.43, p < 0.001), and GT vs. TT model (OR = 1.05, 95% CI = 1 - 1.11, p < 0.001) was significantly associated with increased risk of BC. This polymorphism was also associated with increased risk of BC in Caucasians (dominant, allelic, and homozygote models) and Asians (allelic, homozygote, and heterozygote models).
Conclusion: The current meta-analysis suggests that *MDM2* gene rs2239745 polymorphism is a predisposing genetic factor in BC, particularly in Caucasians and Asians.
Key words: *Breast cancer, MDM2, Polymorphism, Meta-analysis.*

P-128
Determining an optimal cut-off value for follicle-stimulating hormone to predict microsurgical testicular sperm extraction outcome in patients with non-obstructive azoospermia

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Background: Determining the success of sperm retrieval for infertile men before using assisted reproductive technologies can decrease the costs. Using endocrine markers, as an inexpensive and non-invasive method is considered to be a clinically suitable marker in assessment of infertile men.
Objective: To determine the optimal cut-off value for follicle stimulating hormone (FSH) to predict the outcome of microsurgical testicular sperm extraction (micro-TESE) in patients with nonobstructive azoospermia (NOA).
Materials and Methods: We included a total number of 180 patients with NOA. The serum level of FSH was determined and all the subjects underwent micro-TESE. We determined the optimal cut-off value for FSH and

assessed whether the test could be effectively used as a successful predictor of sperm retrieval by calculating the receiver operating characteristic area under the curve.

Results: Overall, we included a total number of 171 patients with mean age of 34.3 ± 8.6 yr. The micro-TESE was considered to be successful in 79 (43.8%) while it failed in 92 (56.2%) patients. We found that the mean level of serum FSH was significantly higher in group those with failed micro-TEST compared to successful group (P < 0.001). The cut-off value for FSH was calculated to be 14.6 mIU/mL to predictive the outcome of micro-TESE with a sensitivity of 83.5% [73.5%-90.9%] and a specificity of 80.3% [69.5%-88.5%]. At this value, the other parameters were calculated to be PPV, 81.5%; NPV, 82.4; LR+, 4.23; and LR-, 0.21.

Conclusion: The results of the current study indicate that FSH plasma levels above 14.6 mIU/mL can be considered to be the failure predictor of the micro-TESE in NOA patients.

Key words: *Follicle-stimulating hormone, Non-obstructive azoospermia, Microsurgical testicular sperm extraction.*

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P-129

Design of a new coating agent based on graphene oxide and antimicrobial/spermicidal peptide (Sarcotoxin Pd) for condom coating: new strategy for prevention of unplanned pregnancy and sexually transmitted infections

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Background: Sarcotoxin Pd has been introduced as potent contraceptive agents in the prevention of unplanned pregnancy and sexually transmitted infections (STIs). The limitation for application of these peptides is low stability in various environments (with different pH and temperatures). Nanotechnology can be used to design new biocompatible and biodegradable system for enhancement of peptide stability, slow release, targeted delivery, maintenance of peptide's structure and function, and so on.

Objective: The aim of this study was to design of a new coating agent based on graphene oxide and antimicrobial/spermicidal peptide, Sarcotoxin Pd, for condom coating as new strategy for prevention of unplanned pregnancy and sexually transmitted infections.

Materials and Methods: Microwave method was used for synthesis of functionalized graphene oxide (GO) with antimicrobial/spermicidal peptide, Sarcotoxin Pd. Characterization was done by FTIR, TEM, and SEM microscope. Antimicrobial and spermicidal activity as well as peptides stability on functionalized GO were evaluated in comparison with naked peptides.

Results: The results approved that Sarcotoxin Pd-functionalized GO (GO-Pd) had broad-spectrum antimicrobial activities against examined pathogens, especially vaginal infections such as Candida Vulvovaginitis. This antimicrobial activity was more than pristine peptides. GO-Pd had also the higher inhibitory activity on motility and viability of sperm than pristine peptides. Evaluation of stability showed that in all examined conditions, GP-Pd had high stability and activity. But, naked peptides had low stability and activity after incubation in acidic pH and high temperatures (more than 38°C). In all tests, there was the highest significant difference between GO-Pd with naked peptides.

Conclusion: This study showed that GO-Pd had higher stability, antimicrobial activity and spermicidal activity in comparison with naked peptides. On the other hand, due to high surface-area-to-volume ratio, these synthesized nanocariers could carry a large amount of peptides on their own surfaces and easily stabilized on surface of condom for the prevention of unplanned pregnancy and especially, prevention of STIs.

Key words: *Graphene oxide, Sarcotoxin Pd, Spermicidal, Vaginal infections, STIs.*

P-130

High titers of phosphatidylserine/prothrombin antibody in non-APS RIF patients

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Background: Immunological causes of recurrent implantation failure (RIF) are of controversial aspects of implantation; mainly because antiphospholipid antibodies (aPL) are an important cause of recurrent pregnancy loss. This is why they may play a role in RIF. Recently, strong association between anti phosphatidylserine/prothrombin antibodies, as an extra-criteria aPL, and the clinical manifestations of

antiphospholipid syndrome (APS), one of the reasons for RIF, is highlighted. So, the emerging role of aPL on implantation and pregnancy complications led us to investigate these anti-bodies in RIF patients.

Objective: High titers of phosphatidylserine/prothrombin antibody in non-APS RIF patients.

Materials and Methods: For this respect a pilot study was designed to compare titers of IgG/IgM antibodies against cardiolipin, B2GPI, phosphatidyl ethanolamin and anti-phosphatidylserine/prothrombin of RIF and healthy women. After obtaining of written constant, blood samples were collected from 30 RIF women referred to Royan Institute through ART procedure and 10 volunteer healthy women with at least one child (control group) and detection of aPLs was conducted using Enzyme-Linked Immunosorbent Assay. Also lupus anti-coagulant test was performed on all samples.

Results: The results of anti-coagulant test as well as anti cardiolipin antibody and B2GPI antibody tests showed that none of the women had the APS. The level of other tested antibodies in RIF women was not significantly different from the control group although some of aPLs (antibodies against cardiolipin, B2GPI and phosphatidyl ethanolamine) had higher levels in RIF women vs. control. Surprisingly titer of aPS/PT antibody in RIF women was significantly higher than healthy women.

Conclusion: Although the test results showed that RIF women do not have APS but altered levels of their aPL titers are not negligible specially anti phosphatidylserine/prothrombin antibodies. It may be better to investigate non-criteria factors in the category of immunological reasons for implantation failure in non-APS RIF women and take a deeper look at the issue.

Key words: *Embryo Implantation, Antiphospholipid Antibodies, Enzyme-Linked Immunosorbent Assay.*

P-131

Improvement of sperm parameters and chromatin quality in asthenospermic men by oral co-administration of pentoxifylline and anti-oxidants

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Background: Oxidative stress affects male fertility by defecting spermatozoa. Asthenozoospermia is referred to reduced or complete sperm motility. Reactive oxygen species is one of the major reasons of higher sperm DNA fragmentation. Sperms with high DNA fragmentation are higher in asthenozoospermia, teratozoospermia and oligozoospermia. Also, sperm DNA fragmentation level is higher in men with sperm motility defects. The imbalance between the production of Reactive oxygen species and physiological status leads to damage which is known as oxidative stress. So, antioxidants supplements and Pentoxifylline (PTX) probably improve sperm quality by reducing oxidative damage.

Objective: The present retrospective study aimed to investigate the possible effect of oral co-administration of PTX + folic acid (FA) + vitamin E (Vit E) on sperm parameters, apoptosis, and sperm chromatin in asthenospermic men.

Materials and Methods: Semen samples of 30 infertile asthenospermic men, who referred to Yazd Reproductive Sciences Institute ere collected. Sperm parameters (count, motility, morphology, and viability), apoptosis, and DNA and chromatin quality were evaluated before and three months after consumption of PTX + FA + Vit E. DNA integrity and chromatin quality were assessed by Aniline blue (AB), Toluidine blue (TB), and Chromomycin A3 (CMA3) staining. Also, apoptosis was assessed by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Sperm morphology was assessed by Papanicolaou staining.

Results: Our results showed that after co-administration of PTX and antioxidants, sperm motility and morphology increased significantly ($p < 0.0001$). Semen volume and sperm count were also increased, but not significantly ($p < 0.05$). Following the intervention, AB, TB, and CMA3 staining showed that the number of sperms with good chromatin quality and DNA integrity was increased, although it was not significant ($p < 0.05$). The mean ± S.D. of chromatin condensation, which were measured by AB, TB, and CMA3 before taking the drugs were 37.48 ± 8.84, 47.10 ± 15.43. and 37.72 ± 9.52, respectively and after taking the drug were 34.51 ± 7.27, 44.51 ± 14.99, and 35.68 ± 9.37, respectively. Also, the mean ± S.D. of sperm apoptosis by TUNEL test before and after taking the drug was 14.89 ±3.49, and 14.06 ± 3.74, respectively.

Conclusion: Based on these data, the cocktail of PTX + FA + Vit. E significantly increased the normal motility and morphology of sperm in asthenospermic men. But still more studyies with larger sample size is needed.

Key words: *Pentoxifylline, Folic acid, Vitamin E, Sperm chromatin, Asthenospermia.*

P-132
Stabilization and immobilization of decellularized amniotic membrane as scaffold using a simple plastic ring composed of polyethylene terephthalate

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Background: Recently, the amniotic membrane (AM) has attracted the attention of researchers as an significant potential source for scaffolding material. The AM is the innermost layer of the placenta and made up of a basement membrane which contains blood vessels and epithelial cells. Its structure and biological nature make it a good candidate for use as a scaffold in tissue engineering studies.
Objective: The study was aimed to resolve the problem of using AM as scaffold in culture condition, i.e. floatation on the surface of culture medium. For stabilization and immobilization of AM scaffold on the culture plate, we used the ring used for holding the plastic bottle cap of mineral water composed of Polyethylene Terephthalate (PET).
Materials and Methods: The PET ring was firstly cleaned by 70% ethanol and then autoclaved at 121°C and 1.5 bar. To decellularized AM, it was agitatedly incubated by 0.25% trypsin-EDTA on shaker for 30 min at 100 rpm, three times. Finally, AM was cut up in 3 cm pieces and used for as scaffold to culture amniotic fluid-derived mesenchymal stem cells (AF-MSCs), characterized in previous studies. The cells were cultured in DMEM supplemented with 10% FBS, 1% Pen-Strep and 0.5% fungizone, during 21 days.
Results: No contamination and toxic effect was observed during a 3 wk monitoring of cultures contained the PET ring-immobilized AM scaffold compared to the cells only cultured on vessels. Decellularization and attachment of the cells to the AM was confirmed with scanning electron microscope (SEM).
Conclusion: The results of this study showed that the use of PET rings does not have a significant negative effect on cell growth and their ability to attach to the AM scaffold. It seems that these simple plastic rings can be used in studies of scaffolds that have difficulty of floating in culture medium.
Key words: *Amniotic membrane, Scaffold, Decellularized, Stabilization.*

P-133
Effect of chromium supplementation on metabolic biomarkers in women with polycystic ovary syndrome: A systematic review

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Background: Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder in females at the age of fertility and is the main cause of infertility. PCOS affects 11-18% of females' population in the world and 6-8% of females at the age of fertility globally. According to the ESHRE guideline 2018, lifestyle modification (diet and physical activity) is the first treatment line of PCOS.
Objective: The purpose of the present study was to determine the effects of chromium supplementation on metabolic biomarkers in women with PCOS.
Materials and Methods: PubMed, Scopus, and Google Scholar databases were searched for literature published between September 2005 and December 2020. The applied Mesh terms were "chromium picolinate," "polycystic ovary syndrome," and "polycystic ovary syndrome treatment". The collected data contained 10 clinical trials, of which 10 were reviewed systematically. All studies were randomized placebo-controlled trials in women with PCOS that investigated the efficacy of chromium supplementation in PCOS improvement.
Results: In 10 articles being examined, six articles indicated the increase in insulin sensitivity and the decrease in clinical or biochemical hyperandrogenism, four articles showed a significant decrease in body mass index, three articles showed regular menstruation and ovulation, and two articles showed the decrease in triglyceride, total cholesterol, fasting blood sugar, malondialdehyde, high-sensitivity C-reactive protein and glucose content (the increase in the glucose excreted).
Conclusion: Chromium supplementation in females with PCOS decreased fasting serum insulin or insulin resistance and increases insulin sensitivity and it also decreases clinical or biochemical hyperandrogenism. Since at least elemental chromium to show effective metabolism of glucose and lipid is 200 micrograms of elemental trivalent chromium, we suggest to the researchers to use at least this level or more with large sample size and for a long time (more than 12 wk) in future clinical trials. Secondly, we suggested that instead of examining the effect of chromium alone, the synergistic effect of chromium+ carnitine or chromium+ inositol or chromium+ carnitine+ orlistat will be studied on the metabolic biomarkers in women suffering from PCOS in future studies.
Key words: *Chromium, Picolinate, Polycystic ovary syndrome, Systematic review.*

P-134

Is there any association between sperm telomere length and teratospermia?

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Background: Causes of male infertility are abundant and multifactorial including anatomic, endocrine, metabolic and genetics problems. Numerical and morphological sperm defects are categorized in several groups, among them, teratospermia is characterized by the presence of spermatozoa with abnormal morphology over 85% in semen which are frequently incompetent in fertilization function. Genetic factors have long been considered in this field and one of the most recent issues is alteration in sperm telomeres, which are nucleoprotein structures protecting the end of eukaryotic chromosomes, and its contribution to male infertility. In the present study comparison of the sperm telomere length (STL) between teratospermia and normal semen specimen was on the agenda, since to our knowledge direct association between telomere length and teratospermia had not been evaluated previously.

Objective: To investigate if there is any differences between telomere length of teratospermia and normal sperms in men of a similar age span.

Materials and Methods: The total of 60 semen specimens were obtained and categorized in teratospermia and normal samples from Arak Fertility Clinic, Markazi province, Iran. Teratospermia feature of samples was approved by specialists. After genomic DNA extraction, STL was surveyed by the use of quantitative real time PCR (qPCR) and data were analyzed with the help of statistical software.

Results: In order to statistically evaluate the relative telomere length in specimens, telomere to single copy gene (T/S) ratio was calculated for teratospermia and normal specimens. The results significantly indicate that relative telomere length in normal samples are nearly three times longer than this in teratospermia samples ($p < 0.05$).

Conclusion: Amongst various biological factors that affect semen quality, genetic alterations are known as considerable actors. Recently telomeres alterations have been in the spotlight. Several recent studies have reported the suggestive relationship between STL and male infertility. Our results, in line with other previous studies, indicate that the length of telomeres in teratospermia is shorter than that of normal sperm and this alteration might be one of the factors that contribute in incompetency of this kind of sperms. Further investigations on defining relevant molecular processes are highly recommended.

Key words: *Telomere length, Teratospermia, Sperm, Male infertility.*

P-135

Mesenchymal stem cells suppress the c-FOS expression in spinal cord injury induced neurogenic bladder: A preclinical study in rat model

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Background: The normal function of filling and emptying of the lower urinary tract depends on a healthy and intact nerve axis. Spinal cord injury (SCI) blocks signals from the brain micturition center to the urinary sphincters, and lead to the dysfunction of the lower urinary tract. The consequences of the neurogenic bladder (NGB) include kidney failure, urinary tract infections, and urinary incontinence. The current therapies are usually palliative and there is no definitive cure for this disorder. In recent years, the use of stem cells, especially mesenchymal cells, has become a promising therapeutic method for SCI-induced NGB. The *c-Fos* transcription factor is encoded by the primary immediate *c-Fos* genes. *c-Fos* is a proto-oncogene that encodes the Fos protein in the central nervous system and has been identified as an indicator for postsynaptic activation of spinal neurons that receive afferent input from the lower urinary tract. Bladder stimulation increases the number of *c-Fos*-immunoreactive neurons in the periaqueductal gray matter, pontine micturition center and spinal cord. Later to SCI, neuronal activity in urinary neurons increases. Increased expression of *c-Fos* in the bladder indicates neuronal activation in the bladder. Previous experiments show that bladder dilatation or chemical stimulation of the lower urinary tract in rats increases the number and the altered distribution pattern of Fos-IR cells is in the L6-S1 discrete regions, including the lateral and medial dorsal horns (LDH and MDH, respectively), the dorsal commissure (DCM), and the SPN regions.

Objective: To determine the effect of intravesical Bone Marrow Mesenchymal Stem Cells (BM-MSCs) transplantation on *c-FOS* expression in SCI-induced neurogenic bladder.

Materials and Methods: Twenty-four female Wistar rats were randomly divided into 4 groups (each group consisted of 6 rats): the control group which did not receive any intervention. The sham group which underwent laminectomy at the level of T9-T10

vertebrae without any spinal cord damage. Two groups with complete SCI that were dissected on the level of T9-T10 vertebrae after laminectomy using a sterile razor blade. Four weeks after injury, BM-MSCs (1×106/120 µl) were injected into six areas of bladder muscle using a 500 µl insulin syringe. In the negative control group, normal saline with the same volume was injected instead of BM-MSC. Four weeks after cell transplantation, rats were examined for molecular and histological evaluation.

Results: In the present study, a relatively new model of intravesical injection of BM-MSCs was introduced as a minimally invasive method for SCI-induced NGB management in female Wistar rats. The results of Western blot showed that after SCI, *c-Fos* expression in bladder and spinal cord increased, compared to control and sham groups ($p < 0.001$). Following treatment, its expression was decreased significantly. The results of Tukey –post hoc test show that this reduction is statistically significant in Western blot samples of bladder in BM-MSCs group compared to the SCI group ($p < 0.001$).

Conclusion: It can be concluded that the neural communication disorder caused by SCI may severely stimulate the centers which are associated with normal urinary excretion in the brain. *C-Fos* expression was suppressed after transplantation of BM-MSCs.

Key words: *Lower urinary tract, Bladder, Rats, Spinal cord injury, Mesenchymal stem cells.*

P-136
Evaluation and comparison of miRNA-15a loaded liposome and free miRNA-15a in PC3 prostate cancer cell line

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Background: Prostate cancer is one of the most common cancers in men. mircroRNA-15a is categorized as the tumor suppressor miRNAs, but its instability in plasma and low cellular uptake highlight the essential need for an appropriate carrier to deliver this gene to the target tissue.

Objective: The aim of this study was to compare the viability percentage of prostate cancer cells treated with liposomal system containing miRNA-15a vs. free form of miRNA-15a.

Materials and Methods: In this study, miRNA-15a was synthesized on an optimized liposomal system with ratio N / p = 50.1 by incubation at 25°C for 30 minutes. Then prostate cancer cell line (PC3) were treated with non-loaded liposome, free miRNA-15a and miRNa15-a loaded liposome and cell viability was calculated by MTT assay.

Results: The MTT results showed that the viability percentage of PC3 cells treated with none loaded liposome, free miRNA-15a and miRNA-15a were 100%, 97%, and 90% respectively.

Conclusion: According to the results of MTT assay, by loading miRNA-15a on the optimized liposome, its stability and cellular uptake increased and the viability of cancer cells decreased.

Key words: *Prostate cancer, Gene therapy, Liposome, Drug delivery.*

P-137
Down-regulation of TRPV6 calcium channel, in endometrial cells may cause repeated implantation failure

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Background: TRPV6 is an important calcium channel which translocate on the apical surface of epithelial cells by S100A10-Annexin A2 complex. Female TRPV6 knock-out mice are infertile. S100A10 is a calcium binding protein that is expressed in endometrial epithelial and stromal cells. Low expression level of it was seen in the endometrium of infertile women that their uteruses were non-receptive. The highest expression of S100A10 occurs in secretory phase, whereas TRPV6 expression is up-regulated in the proliferative phase.

Objective: The aim of the study was comparison of S100A10 and its associated calcium channels TRPV6 expression between the endometrium of repeated implantation failure (RIF) patients and fertile women.

Materials and Methods: Endometrial samples were taken from RIF women below 40 yr old, with at least three cycles of IVF failure after at least five good quality embryo transfer using piple. Endometrial samples of oocyte donors were mentioned as control group. S100A10 and TRPV6 expression in endometrium of RIF women and oocyte donors were analyzed using Real time PCR.

Results: In the endometrium of RIF women, the expression of S100A10 was significantly higher in luteal phase than follicular phase. But its expression in the secretory phase of the experimental group was not different with its expression in control group. TRPV6 expression in RIF women didn't change during proliferative and mid-secretory phases. Its expression in the RIF women was significantly lower than control group.
Conclusion: In RIF women, very low level of TRPV6 during follicular phase which remains unchanged during luteal phase could be a reason for endometrial cell dysfunction in calcium transfer and failure of embryo implantation. However, in the experimental group, the expression of S100A10 in the luteal phase was similar to control group. It shows independence of TRPV6 to S100A10, in this stage of endometrial cycle.
Key words: *S100A10, TRPV6, RIF, Calcium channel.*

P-138
SPOM as a ART method for treating infertility: A review study

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Background: Assisted reproductive technology (ART) described as the medical procedures used for treating infertility. Infertility, especially in patients with polycystic ovarian disease, is a critical problem as they cannot develop the mature oocyte. One of the methods of ART is stimulated physiological oocyte maturation (abbreviated as SPOM); in this method, instead of removing the mature oocyte for using in IVF, the premature oocyte removed from the ovary and placed in the appropriate medium for maturation which called IVM (in vitro maturation). After in vitro maturation, these oocytes can be used for IVF like mature oocytes.
Objective: In this study, we aim to review the studies about SPOM and discuss the challenges and the different methods used in SPOM and assess the SPOM as a new method for using in human studies.
Materials and Methods: Three databases include Web of Science, Scopus, and PubMed searched by two key words: ART and SPOM. The time range was set from 1992 to 2021, and the irrelevant or duplicated articles removed. The studies assessed include both peer-review and primary studies.
Results: About 50 articles assessed and read, and the parameters which were important in SPOM extracted. Based on the articles' information, the parameters like the challenges which the SPOM faced to it for application in human studies, the advantages and disadvantages of each method extracted.
Conclusion: In this study, we concluded that the SPOM could be a useful approach for treating infertility, especially among PCO patients.
Key words: *SPOM, IVM, ART, PCO.*

P-139
The histopathological effects of maternal caffeine consumption on testis, prostate, seminal vesicle and epididymis of male rat offspring (stereological study)

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Background: Caffeine is an alkaloid of the methyl xanthine group. It is conceivable that caffeine consumption would induce gonadal changes.
Objective: The aim of this study was to assess the impact of embryonic caffeine exposure on rat testis, prostate, seminal vesicle and epididymis.
Materials and Methods: Female rats were divided into three major groups (n = 7) including: A control which only received drinking water. B and C groups received low dose of caffeine (26 mg/kg) and high dose (45 mg/kg) respectively via drinking water during pregnancy and lactation. Structural changes in testis, prostate, seminal vesicle and epididymis were studied by using stereological methods at 21, 60 and120 days of postnatal development.
Results: The result showed the decreases in body and testis weight and testis volume of offspring in group C compared to other groups at all ages ($p < 0.05$). There were significant differences in number of sperm cells of offspring of experimental groups compared to control group in different ages ($p < 0.05$). Number of sertoli, spermatogonia, spermatocyte and spermatid cells in group C showed a significant decrease compared with other groups at all ages ($p < 0.01$). The prostate of caffeine-fed groups presented various degrees of epithelial and stromal hyperplasia and epithelial cell proliferation in the prostatic lobes. In high dose caffeine treated group, epithelium height of seminal vesicle decreased, but it was not significant. The thickness of the muscle layer of seminal vesicles in high dose caffeine treated group increased compared to the control group ($p \leq 0.001$). Significant decreases in epithelium height of tubules of epididymis in high dose caffeine treated group was seen compared with control group ($p < 0.05$). In high dose caffeine treated group diameter of tubules of epididymis decreased ($p \leq 0.001$).
Conclusion: Results showed that maternal caffeine consumption altered the structure of testis and prostate gland, seminal vesicle, epididymis, and spermatogenesis of offspring in adulthood.
Key words: *Caffeine, Testis, Prostate, Seminal Vesicle, Epididymis.*

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P-140

Impending reproductive health problems in adolescent girls during COVID-19 crisis

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Background: Coronavirus disease 2019 (COVID-19) is an ongoing health problem, threatens the overall health, social and emotional wellbeing. Beyond the health problems, economic crises related to the COVID-19 affect reproductive health. Adolescent girls are most vulnerable to face reproductive health problems during COVID-19 pandemic.
Objective: This study aimed to explore reproductive health problems in adolescent girls during the COVID-19 crisis.
Materials and Methods: In this review, a literature review was conducted in the selected databases (Web of Science and PubMed) up to February 2021. The search was performed using the key words of adolescent, reproductive and sexual health, menstrual cycle, and other related key words. The main outcome was investigating the effect of COVID-19 pandemic on different aspects of reproductive health in adolescent girls.
Results: As the reviewed studies indicated, the COVID-19 pandemic may affect the different aspects of adolescents' sexual and reproductive. Economic insecurity and falling into poverty, nutrition and food insecurity, mental health problems during the COVID-19 crisis may lead to difficulties in menstrual hygiene management, changing the menarche age, increasing unintended teenage pregnancies, early forced marriage, sexually transmitted infections, violence, domestic abuse, and finally restriction to access to healthcare.
Conclusion: During COVID-19 pandemic several factors affect adolescent's reproductive health both directly and indirectly. Therefore, it is recommended to extent adolescent's friendly health services during COVID-19.
Key words: *Reproductive health, Adolescent, COVID-19.*

P-141

Evaluation of the fundamental factors, involved in successful culture of human ovarian cells, follicles and tissues: A preliminary step for assembling an artificial ovary

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Background: Todays, the new infertility treatment methods try to mimic the ovarian natural micro-environment by tissue engineering. An artificial ovary tries to preserve fertilization, even with producing oocytes or with releasing steroid hormones. Producing oocytes can be obtained even by follicle culture or by ovarian tissue culture. Many physical and chemical factors are involved in ovarian cells and follicular culture. Based on the current studies, no study has reported the best mediums for ovarian cells, follicles and tissue culture.
Objective: This study aimed to uncover the appropriate media and supplements for in vitro culture of ovarian and cumulus cells (CCs).
Materials and Methods: Cortical, medullar, and hilar cells of human ovary were cultured and their conditioned medium (CMs) were collected. The expression of GDF9 was detected in all the cells. Also, CCs were collected from healthy women, who referred due to male factor infertility. To choose the optimum basal medium, a mixture of ovarian cells was cultured with basal mediums, supplemented with various concentrations of fetal bovine serum (FBS) and human serum albumin (HSA). The cocktails were as follows: [Serum free mediums], [mediums + 10% FBS], [mediums + 20% FBS], [mediums + 1% Alb], [mediums + 2% Alb], [mediums + 10% FBS + 1% Alb], [mediums + 10% FBS + 2% Alb], [mediums + 20% FBS + 1% Alb] and [mediums + 20% FBS + 2% Alb]. The same process was repeated for CCs. Because the CCs need some supplementation, we cultured them with various concentrations of some supplements to choose the best concentration. So, CCs were cultured with various concentrations of L-Glutamine, bovine serum albumin (BSA), HSA, insulin transferrin selenium (ITS), Follitropin alfa® and Pregnyl®. Also, CCs were treated with various concentrations of follicular fluids (FFs) and CMs, too. CMs were collected from ovarian, testicular, adipose and amniotic derived and ovarian carcinoma cells. Then, CCs morphology and proliferation were evaluated.
Results: All the ovarian cells expressed GDF9, as a key factor for ovarian follicular growth. Alfa MEM + 20% FBS and DMEM F12 + 20% FBS were the most suitable cocktails for ovarian and CCs culture, respectively. 20% FBS was superior to 10% for both ovarian and CCs. Also, HSA could not support the growth of ovarian and CCs, alone. The cocktails of mediums with 20% FBS and (mediums+FBS+HSA) were superior to the others. The CMs of ovarian cortical and hilar+medullar cells could lead to higher CCs growth. 17 mM/l L-Glutamine, 24 mg/ml BSA, 20 mg/ml HSA, 10 ng/ml ITS, 300 mIU/ml Follitropin alfa and 3.5 IU/ml Pregnyl led to higher proliferation of CCs.

Conclusion: Ovarian chemical micro-environment is very complex and ovarian follicle growth needs many known and unknown elements like growth factors, which are expensive. We concluded that CMs and serums can support the follicular growth alongside with basal mediums, supplemented with hormones, ITS and L-Glutamine, which are cheaper and more accessible.

Key words: *Artificial ovary, In vitro culture, Ovarian cells, Cumulus cells, Conditioned mediums.*

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Expression of mesenchymal, pluripotent and germ cell markers on the cortical, medullar and hilar cells of adult human ovary

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Background: The paradox of stem cells in human adult ovary is still an open question with a lot of debates. Recent evidences claim the existence of different types of stem cells within the adult ovary from granulosa precursor stem cells, thecal precursor cells and very small embryonic-like cells to ovarian surface epithelial stem cells and female germline (oogonial) stem cells.

Objective: Up to now, the majority of studies have focused on ovarian surface epithelium and ovarian cortical cells, and extend to the medulla less than other parts. No study is on ovarian hilum and medulla cells, in fact. The aim of the present study is to characterize the mesenchymal, pluripotent, and germ cell markers in human ovarian cortex, medulla, and hilum cells.

Materials and Methods: After ethical approval, a segment of human ovarian tissue was collected. Following enzymatic digestion, ovarian cortex, medulla and hilus cells were harvested and cultured. The cells morphology was evaluated. Also, their mesenchymal characteristic was evaluated by flowcytometric analysis of mesenchymal markers (CD29, CD34, CD44, CD105, and CD117) and bone and adipose tissue differentiation markers. The cells karyotyping was assessed on lower and higher passage and the expression of some pluripotent (OCT4, SSEA4) and germ cells markers (DAZL, DDX4, and GCNA) and GDF9 were evaluated by immunocytochemistry.

Results: Three kinds of cells were detected in ovarian cortex. The small cells with morphology of embryonic stem cells, called very small embryonic like stem cells, larger cells or ovarian stem cells and the stromal cells of the ovary. The medulla expressed two kinds of cells: large spindle shape cells and small polygonal cells. The majority of hilum cells demonstrated spindle form, with low number of cells and almost round morphology. The cells of cortex showed spontaneous differentiation into oocyte like cells. Also, the cells of all three parts of the ovary had differentiation abilities toward bone and adipose tissues. The cells of ovarian hilus have greater proliferation capacity than medulla and cortex. They expressed the mesenchymal markers including CD44, CD117, CD29 and CD105, but they were negative for CD34. The cells showed normal karyotyping even after several passages. Although not with the same percentages, the cells of all parts of the ovary expressed OCT4, SSEA4, GCNA and GDF9 and co-localization of DAZL (as male germ cells marker) and DDX4 (as female germ cells marker).

Conclusion: We concluded that adult human ovary has cells with stemness characteristics. Also, despite the most theories that ovarian stem cells are localized in ovarian cortex, our results showed that the cells in medulla and hilus also expressed the stem cells markers, even more than that of the cortex. We propose that human ovarian medulla and hillus cells are as important as cortex and surface epithelium in presentation of stemness ability.

Key words: *Ovarian stem cells, Germ cells markers, Pluripotent markers, Mesenchymal markers.*

P-143

The effect of Covid-19 on female fertility

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Background: Coronavirus disease 2019 (COVID-19), like other infectious viruses, can negatively affect several female reproductive systems.

Objective: We performed this study to investigate the effect of Covid-19 on female fertility.

Materials and Methods: This narrative review reviews the databases of PubMed, Cochran, Library Online Wiley, Google Scholar, Tandfonline, Doaj, Civilica, SID, Magiran, Noormags, Irandoc, IranMedex, Elmnet, and Ricest in English and Persian with the key words COVID-19, Fertility, Infertility and Male selected by MeSH and their Persian equivalents, from 2019 to February 2021. From 52 obtained articles, we finally, reviewed the full text of 7, which had inclusion criteria, and the extracted data presented in the form of a summary of the article.

Results: Among many structural proteins which COVID-19 includes, S protein is able to facilitate the entry of coronavirus into host cells by fusing viral and cell membranes. Basigin, which plays an important role in male and female reproduction, is one of the most important COVID-19 receptors and mediates its entry into host cells. Basigin is also expressed in the uterus and is essential for successful embryo implantation; therefore, disruption or inhibition of Basigin causes weakness in the embryo implantation. In addition, Angiotensin-Converting Enzyme 2 can be found in endometrial epithelial cells and human ovaries and is related to ovarian functions such as steroidogenesis, follicular growth, granulosa-lutein cell apoptosis, oocyte maturation, and ovulation. COVID-19 may infect the ovaries, uterus, vagina, and placenta through overexpression of Angiotensin-Converting Enzyme 2. Also, it may impair women's reproductive functions, resulting in infertility, menstrual irregularities, and fetal distress. The negative effect of COVID-19 on female gametogenesis is not yet certain. Only one study reported the presence of the COVID-19 virus in vaginal fluid. Researches have shown that COVID-19 infection has a lower maternal mortality rate than severe acute respiratory syndrome or middle east respiratory syndrome. Asymptomatic women developed postpartum respiratory symptoms and mother-to-child transmission after delivery. The reproductive system is always exposed to different disorders such as infertility, decreased sperm count, and motility, so medical studies should focus on the possible vulnerability of gamete and next-generation against COVID-19.

Conclusion: There is controversial evidence of the presence of COVID-19 in the seminal plasma of patients recovered or infected with COVID19. COVID-19 has multiple ways of impairing female fertility, although there have not been any cases of infected females with damage to their reproductive systems reported yet. However, the potential risk of COVID-19 infection in female fertility needs to be more assured, and we advise individuals with COVID-19 attempting pregnancy to postpone until the end of treatment. We also recommend that infertile couples take a COVID-19 test before Assisted Reproductive Technology.

Key words: *COVID-19, Fertility, Infertility, Female.*

P-144

Non-toxic concentrations of graphene oxide and its derivatives on ovarian stromal cells showed angiopoietic properties on chick embryo chorioallantoic membrane; An approach for augmented angiogenesis in tissue engineering and tissue transplantation

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Background: Assembling an artificial ovary is new fertility treatment and preservation tactic that mimic the function of the natural human ovary. Its goals are producing oocytes and releasing steroid hormones by the help of biocompatible materials which should minimize inflammation and enhance neo-angiogenesis. A successful angiogenesis process is essential for follicle development, ovarian tissue and artificial ovary transplantation. Graphene is a two-dimensional nanomaterial, mostly used in the form of graphene oxide (GO). It is an anti-apoptotic agent due to the trapping of vascular endothelial growth factor. In the recent years GO and its derivatives are in spotlight for their angiogenic properties and vasculogenesis.

Objective: We aimed to determine which GO derivatives and with which concentrations can better enhance angiogenesis on chick embryo chorioallantoic membrane.

Materials and Methods: Human ovarian cells were harvested. GO and its derivatives including lysine-treated GO (GL), arginine-treated GO (GA), carboxylated GO (GC) and polyethylene glycol (PEG) functionalized GO (Gpeg) were prepared. A mixture of ovarian cells was treated with the concentrations of 50-10000 ng/ml (based on published papers) of the mentioned materials for 48 h. The process was repeated for 8 days in 96 wells plate. As, concentrations close together, led to the same results, so at the next step, the cells were treated with higher concentrations of the mentioned materials (100-220000 ng/ml, based on a pilot study), for 8 days, in 48 wells plate. Since the cumulus cells (CCs) are the most important cells act in the follicle development, so CCs were treated with 100-220000 ng/ml of mentioned materials for 8 day in 48 wells plate, to find which kinds of nano materials and which concentrations are nontoxic for both ovarian and CCs. The MTT assay was used for cell viability. Then, the angiopoietic properties were assessed by chick embryo assay. Non treated cells were used as the control.

Results: The MTT assay after 48 h treatment with 50-10000 ng/ml of mentioned materials showed that 50-400 ng/ml of GO, all concentrations of GC, 200-10000 ng/ml of GL, 900-8000 ng/ml of GA and 50-1600 ng/ml of Gpeg caused higher cell proliferation. MTT assay after 8 days treatment with 50-10000 ng/ml of mentioned materials showed that 50-700 ng/ml of GO, 50-2000 ng/ml of GC, 50-8000 ng/ml of GL, and GA

and 50-2000 ng/ml of Gpeg led to higher cell viability. MTT on CCs showed that 100, 300, 900, 2700, and 8100 ng/ml of GO and 100, 300, and 900 ng/ml of GA had higher cell proliferation. About GC, 100, 300, 900, and 2700 ng/ml had the same results as control. All concentration of Gpeg and GL led to lower proliferation, compare to control. So, 100, 300, 900, 2700, and 8100 ng/ml of GO, 100, 300, and 900 ng/ml of GA, 100, and 300 ng/ml of GC and 100 ng/ml of GL and Gpeg were used for chick embryo assay. We concluded that 900, and 2700 ng/ml of GO, 100, and 300 ng/ml of GA and 100 ng/ml of GC, and Gpeg could enhance angiogenesis on chick embryo chorioallantoic membrane. GL was not so beneficial, also it caused embryo death in some eggs.

Conclusion: GO and its derivatives can act as an angiogenic agents, so it is proposed that they can be used in ovarian tissue transplantation and ovarian tissue engineering to enhance angiogenesis.

Key words: *Graphene oxide, Angiogenesis, Tissue Engineering, PEG.*

P-145

Hydroalcoholic extract of Ephedra pachyclada leaves modifies the ovarian tissue changes caused by cyclophosphamide in female rats

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Background: Cyclophosphamide (CP) is an anti-cancer drug that acts as an alkylation agent after metabolism in the liver. CP has toxic effects on the body's cells, especially the function of reproductive system and infertility.

Objective: The aim of this study was to investigate the effect of Ephedra hydroalcoholic extract on ovarian tissue and hypothalamic-pituitary-gonad axis in female rats treated with CP.

Materials and Methods: In this experimental study, 48 adult Wistar female rats were divided into 6 groups of 8, including control, sham, CP and CP recipient groups along with 250, 500 and 1000 mg/kg of Ephedra hydroalcoholic extract. Ephedra hydroalcoholic extract was fed to animals by gavage. On the twenty-ninth day of the experiment, the serum concentration of LH, FSH, estrogen and progesterone was measured and the number of ovarian follicles was counted. The results were analyzed by ANOVA statistical test at the significance level of $p < 0.05$.

Results: Mean serum concentration of LH and FSH and number of atretic follicles increased significantly in all experimental groups compared to the control and sham groups. But, mean serum concentration of FSH and number of atretic follicles decrease significantly in experimental groups 3 and 4 compared to the CP group. Also, mean serum concentration of estrogen and progesterone and number of primordial, primary, secondary and graafian follicles showed a significant decrease in experimental groups 1, 2 and 3. However, mean serum concentration of estrogen and progesterone and number of primordial, primary, secondary and graafian follicles showed a significant increase in experimental group 4 compared to the CP group.

Conclusion: Due to the antioxidant properties, in a dose-dependent manner, Ephedra hydroalcoholic extract modified the changes caused by CP in female rats.

Key words: *Ephedra, Cyclophosphamide, Ovary.*

P-146

The proliferating effect of some herbal plants on human ovarian and testicular cells

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Background: Traditionally, herbal medicine has been used for the improvement of sexual ability and gonadal function. Some plants are recommended for this purpose, which suggested that they can enhance gamete production, regulate sexual cycle and hormones production and ameliorate inflammation. But their mechanisms and probable side effects on gonadal cells, still should be evaluated.

Objective: In the present study, the cytotoxicity effect of some extract from Iranian domestic plants, which are recommended for improvement of gonadal function, also some plants that have anti inflammatory function were evaluated on ovarian and testicular cells.

Materials and Methods: A biopsy of ovary was obtained through an ovariectomy. Also, a small testicular biopsy was received from testicular sperm extraction surgery. After enzymatic digestion, the isolated cells were cultured. The extracts were prepared from the following herbs: Rosa damascene, Ziziphus jujube, Trachyspermum, Adiantum capillus-veneris, Zingiber officinale, Morus nigra, Ceratonia siliqua, olive page, Aloe vera, Linum usitatissimum, Physalis alkekengi, ginseng, Phoenix dactylifera L, and pomegranate skin. A serial dilution of 8 concentrations was prepared including 2000, 1000, 500, 250, 125, 62.5,

31.25, and 15.62 mg/ml by dissolving in ethanol, Dimethyl sulfoxide and stilled water. The cells were treated with the following concentrations for 48 h. The cytotoxicity analysis was performed, using the MTT kit.
Results: The MTT analyses on ovarian cells showed that after 48, the extract of Ceratonia siliqua, Phoenix dactylifera L and pomegranate skin could enhance the proliferation of cells, compared to control group. Pomegranate skin caused to higher viability and proliferation, even in higher concentrations. Also, about the testicular cells, Phoenix dactylifera L, Ceratonia siliqua and Adiantum capillus-veneris could stimulated the testicular cells proliferation. As well, it should be mentioned that all of the concentrations of the mentioned extracts were effective on ovarian and testicular cell proliferation, significantly. Other extract had negative effect or no significant effect, compared to control.
Conclusion: We concluded that the in vitro treatment with the mentioned herbs can enhance the proliferation of ovarian and TESE derived cells. So, probably they can be proposed as the herbal drugs for testicular and ovarian cells proliferation and improvement of gonad performance, especially in aged persons and patients with gonad insufficiency. Probably they can be used as stimulator of ovarian and testicular cells in vitro growth, which is useful for assembling an artificial ovary. These data are extracted from a preliminary study and still further investigations are needed to uncover the exact efficacy of these herbs. Also, their oral administration should be evaluated on gonadal function.
Key words: *Ovarian cells, Testicular cells, Plant extracts, Cytotoxicity.*

P-147
Effective roles of omega-3, omega-6 and the combination of omega-3 and omega-6 dietary fatty acids on mice semen parameters

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Background: The roles of dietary fatty acids on male reproductive were reported; but to the best of our knowledge different roles of several unique fatty acids and the combination of them on semen parameters have not been addressed yet.
Objective: We investigated the influence of dietary omega-3, omega-6 and their combination on semen quality, body weight and food consumption of mice.
Materials and Methods: We divided 40 mature male NMRI mice into four groups (n = 10/each) in an experimental completely randomized design for six weeks: I. Control group (CTR): gavage with water (0.2 ml/head/day); II. Sunflower oil group (0.2 ml/head/day; gavage) (omg-6); III. Fish oil group (0.2 ml/head/day) (omeg-3); IV. Sunflower oil (0.1 ml/head/day) + Fish oil (0.1 ml/head/day) (omeg-6+omeg-3). The body weight, food intake, and sperm parameters were measured by computer assisted semen analyzer (CASA). All data were analyzed with SPSS software.
Results: Feed intake decreased in groups which were administered sunflower oil+ fish oil compared with the other groups ($p < 0.05$). In agreement with the feed intake behavior, body weight showed a tendency to be lowest in mix group than other groups ($p < 0.05$). However, the highest body weight was recorded in CTR and n-3 groups. The CTR group (7.4 ± 1.05) had a significantly lowest concentration of sperm compared with the other groups (10.1 ± 2.5, 10.4 ± 2.5, and 10 ± 2.03 for omega-6, omega-3 and (omega-6+omega-3), respectively; $p < 0.05$). omeg-3 (67%) showed significant ($p < 0.05$) improved progressive motility compared to the CTR (62%), whereas the omega-6 and omega-6+omega3 groups were in the middle.
Conclusion: Dietary fatty acids can improve sperm quality than control. Although mice sperm have high levels of the omega-6 fatty acids, our findings can be a focus for improvements in sperm quality and motility in fertile animals using only omega-3 sources which confirmed the pivotal roles of omega-3 in sperm.
Key words: *Omega-3/omega-6 ratio, Semen parameters, Mice.*

P-148
Synthesis and optimization of cationic liposomal system for miRNA-15a loading in order to use in prostate cancer treatment

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Background: Prostate cancer is the second most common cancer among men and the fifth leading cause of death in the world. Gene therapy is a new method for cancer treatment. Liposomes are known as carriers for gene delivery, but microRNA instability and poor translating are important challenges in miRNA delivery.
Objective: The aim of this study is to provide an optimized formulation of cationic liposomal system in order to delivery of miRNA-15a as an anti-tumor agent to prostate cancer cell line (PC3).

Materials and Methods: In this study, different formulations of the cationic liposomal system with different content of phospholipid (10, 20, 30, 40, 50%) and positive charge were synthesized, its size and charge were determined by Zeta-Sizer (DLS), then the cell viability percentage of PC3 prostate cancer cell line after treatment with various liposomal systems was evaluated.
Results: Based on the results of the DLS device, the particle size was below 150 nm and zeta potential was in the range of 0 to +15 mV. The MTT results determined that the viability percentage of cells were between 70 to 90%.
Conclusion: The optimal formulation with appropriate size, charge and cells viability percentage which could increase anti-cancer effects of miRNA-15a to PC3 cell line was selected for miRNA-15a delivery.
Keywords: *Prostate cancer, Gene therapy, Nanocarrier, miRNA-15a, Liposome.*

P-149
The impact of paternal age on sex chromosomes aneuploidy, blastocyst rate and quality with pregnancy outcomes

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Background: Postponing childbirth by couples in developed countries is increasing. Paternal age has been ascending between all educational levels, races, and geographic regions. Over the past four decades, the percentage of fathers older than 40 years has doubled, from 4.1% to 8.9%. Therefore, investigating a possible correlation between chromosomal aneuploidy and paternal age, analyzing embryos derived from the frozen and fresh embryo transfer is questionable.
Objective: The present study aimed to evaluate the probable effect of paternal age on aneuploidy, blastocyst rate, embryo development, implantation potential, and pregnancy outcomes in preimplantation genetic testing (PGT) cycle with single blastomere biopsy on the day three by fluorescence in situ hybridization (FISH) for sex chromosomes.
Materials and Methods: The present study included 277 embryos between February 2018 and June 2020. Seventy-six women underwent intracytoplasmic sperm injection with preimplantation genetic testing for aneuploidy using fluorescence in situ hybridization method cycles were divided into four paternal age groups: ≤ 35, 36-40, 41-45, and ≥ 45 yr. Primary outcomes were the rate of aneuploidy, blastocyst, and pregnancy. Statistical analyzes were performed using SPSS software version 23 and the data were analyzed using the $\chi 2$ test. The $p < 0.05$ was considered statistically significant.
Results: Significant differences among four groups in chemical pregnancy ($p < 0.001$), clinical pregnancy ($p < 0.001$), ongoing pregnancy ($p < 0.001$) and live birth rate ($p = 0.22$) were found. There was no early pregnancy loss and clinical pregnancy loss in cycles with paternal age under 35 yr rate ($p < 0.01$). The rate of aneuploidy in sex chromosomes, embryo development in frozen embryo transfer, and fresh cycle were not significantly related to parental age.
Conclusion: We didn't find any significant relationship between paternal age and embryo aneuploidy but an association was found between paternal age and pregnancy outcome in embryos from intracytoplasmic sperm injection cycles.
Key words: *Preimplantation genetic testing, Aneuploidy, Fluorescence in situ hybridization.*

P-150
Analysis of the influence of preimplantation genetic testing for aneuploidy results with maternal age

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Background: Assisted reproduction techniques and the preimplantation genetic test for aneuploidies help couples with fertility problems to achieve a healthy live birth worldwide. There is a possible correlation between chromosomal aneuploidy and maternal age.
Objective: Evaluating the effect of maternal age on aneuploidy, blastocyst rate, embryo development, and pregnancy outcomes in patients undergoing frozen embryo transfer.
Materials and Methods: This study included an analysis of X, Y, 13, 18, and 21 chromosomes identified by fluorescence in situ hybridization method in embryos from couples undergoing Assisted reproduction techniques and preimplantation genetic test for aneuploidies. The present study included 277 embryos between February 2018 and June 2020. Women were divided into four age groups: ≤ 35, 36-40, 41-45, and ≥ 45 yr. Primary outcomes were the rate of aneuploidy, blastocyst, and pregnancy. Statistical analyzes were performed using SPSS software version 23 and the data were analyzed using the $\chi 2$ test. The $p < 0.05$ was considered statistically significant.
Results: Significant differences among maternal age groups were found in the chemical pregnancy ($p < 0.001$) outcomes. The blastocyst rates ($p = 0.02$), early pregnancy loss ($p < 0.001$), and clinical pregnancy loss ($p < 0.001$) were related significantly with maternal age.

In females with age > 40 yrs. old, there was no euploid blastocyst. Increasing maternal age significantly increases the rate of aneuploidy in sex chromosomes in frozenthawed embryo transfer and fresh cycles ($p < 0.001$).

Conclusion: The present study results found a significant relationship between maternal age and embryo aneuploidy, and showed that the increasing female ages and aneuploidy rate is related together. A negative association was found between maternal age and blastocyst rate, chemical pregnancy, clinical pregnancy, ongoing pregnancy, and live birth rates in the couple from Intracytoplasmic sperm injection cycles.

Key words: *Preimplantation genetic testing, Aneuploidy, Fluorescence in situ hybridization.*

P-151
COVID-19 and male fertility

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Background: The effect of viral infection on male reproductive system is one of the major concern. Recent studies have demonstrated that COVID-19 can damage the male reproductive system by inflammatory cycle caused by a cytokine storm. However, how COVID-19 can affect the male fertility is still controversial.

Objective: This review study was conducted to investigate the effects of COVID-19 on male fertility.

Materials and Methods: To identify the effect of COVID-19 on male fertility, a comprehensive systematic search was carried out in databases such as; PubMed, Web of Science Core Collection, and Scopus using keywords including fertility, male fertility, male reproduction, covid-19, coronavirus, and spermatogenesis. Full-texted, English language and original articles were included in this study.

Results: In total, 9 articles were entered into the study. Bacically, febrile diseases have significant effect on spermatogenesis, sperm concentration, sperm morphology, sperm motility and thus sperm quality. Occurrence of oxidative stress in case of infection with COVID-19, can increase sperm DNA fragmentation and sperm motility is significantly reduced. According to the researches, mild and moderate disease has no effect on spermatogenesis and male fertility.

Conclusion: Although COVID-19 can induce male reproduction system damage, but a significant effect in male fertility awaits more evidence; therefore, it is recommended that men with COVID-19 be evaluated for reproductive function during and after the course of the disease.

Key words: *Male reproduction, Spermatogenesis, COVID-19.*

P-152
Investigation of the effect of antioxidant systems on the male reproductive system

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Background: The misuse of antioxidants as a sperm disinfectant is reactive to the attack of oxygen species. Sperm are highly dependent on these external sources of enzymes during spermatogenesis and their place in the male reproductive system.

Objective: The aim of this study was to evaluate the effect of antioxidant systems on the male reproductive system.

Materials and Methods: This study was conducted in 2021 by searching for the keywords of the reproductive system, men, antioxidants and spermatogenesis in reputable databases such as PubMed and google scholar, which finally found 15 articles, of which 15 articles, 10 articles were used.

Results: Based on studies from various articles, the results showed that adult sperm are considered inactivated in terms of translation and at least contain anti-cancer substances or internal antioxidants, during spermatogenesis and their habitat are highly dependent on external sources of enzymes. The reproductive system in men has the most abundant antioxidant enzymes in semen, enzymes belonging to the family of glutathione peroxidase and peroxyroxine. Oxidants participate in the immunological process and by this regulation regulate reactions; they are a highly protected family of thiol-dependent peroxidases. They were inactivated by oxidation. Peroxyroxine 4 and peroxyroxine 6 have been shown to be abundantly expressed in rat epidermal sperm and are highly oxidized after induction by operating systems such as tert-butyl hydroperoxide (tert-BHP). From glutathione peroxidases and peroxyroxines, substituted enzymatic antioxidants such as superoxide dismutase and catalase work to protect and maintain sperm stored in the male reproductive system at the same time.

Conclusion: Due to the physiological importance of this defence of collective antioxidants, patients with poor sperm motility parameters usually have a deficiency in the level of their semen plasma antioxidants.

Key words: *System, Antioxidant, Male reproductive system.*

P-153
Cytogenetic study of poor quality embryos not transferred in in vitro fertilization compared with control group

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Background: An association between morphology, and geneticintegrity in human embryos is stablished, but this relationship is not absolute.
Objective: This study investigated the correlation between embryo morphological characteristics and chromosomal status in biopsied human embryos, using the array comparative genomic hybridization technique.
Materials and Methods: Preimplantation genetic testing for aneuploidy was performed on Day 3 embryos (n = 120) divided into two groups: 60 'deselected' embryos (unsuitable for transfer or vitrification) versus 60 'selected' embryos (suitable for transfer or vitrification). The morphological grading criteria, including blastomere number, symmetry, percentage of fragmentation rate, and zona pellucida appearance were correlated with array comparative genomic hybridization results.
Results: The incidence of chromosomal abnormalities was significantly higher in embryos with uneven blastomeres, fragmentations, and thick zona pellucida appearance.
Conclusion: In general, embryo selection based of morphological assessment cannot confirm the chromosomal integrity. However, some morphological parameters reflect the cytogenetic status of the deselected embryos.
Key words: *Aneuploidy, Embryo morphology, Fragmentation.*

P-154
Impact of biological and artificial seminal fluids on sperm parameters and DNA status in asthenozoospermic ejaculates

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Background: The chemical composition and physiological properties of seminal fluid (SF) affect sperm quality.
Objective: To investigate the effects of in vitro exposure of artificial seminal fluid (ASF) and biological SF on sperm quality.
Materials and Methods: Asthenozoospermic ejaculates (n = 20) were divided into two aliquots. The first aliquot was centrifuged for obtaining asthenozoospermic SF. The second aliquot was processed with density gradient centrifugation (DGC) and the pellet was diluted separately with following media: (I) ASF; (II) Ham's F10 medium; (III) normozoospermic SF; and (IV) asthenozoospermic SF. Sperm parameters and DNA status were assessed after DGC as well as 2h and 24h after incubation.
Results: The data showed that sperm progressive motility, viability and DNA integrity were significantly higher in group I than control immediately after DGC ($p = 0.009$, $p = 0.003$ and $p < 0.0001$, respectively). At time 2 h, a higher rate of progressive motility was observed in both group I and control compared with group II ($p = 0.0008$ and $p < 0.0001$, respectively). Similar pattern in progressive motility was noticed at time 24 h for both group I and control as compared with groups II and III ($p < 0.0001$). DNA fragmentation index (DFI) was significantly lower in groups II than III and control at time 2 h ($p = 0.0004$ and $p = 0.0001$, respectively). Additionally, DFI of group II was significantly lower compared to groups I, III and control at the 24 h time point ($p = 0.003$, $p = 0.0004$, and $p < 0.0001$, respectively).
Conclusion: Normal SF showed the protective role on sperm DNA structure. Moreover, ASF preserved sperm motility better than biological SF during 24 h; despite being similar to normal SF regarding DNA integrity preservation in short time.
Key words: *Artificial seminal fluid, Asthenozoospermia, DNA fragmentation index, Density gradient centrifugation.*

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P-155
Impact of co-administration of bone marrow stromal cells and l-carnitine on rat damaged ovaries due to chemotherapy

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Background: Despite the great benefits of chemotherapy in treating cancer patients, it has some side effects on ovaries. Cyclophosphamide is one of the most used chemotherapy drugs which directly damages ovaries. It has been observed that transplantation of bone marrow stromal cells (BMSCs), a type of mesenchymal stem cells, may treat ovarian damage after chemotherapy. On the other hand, L-carnitine (LC), as a flavonoid antioxidant, appears to play an essential role in fatty acid metabolism and has beneficial effects on damaged ovaries. In addition, LC has beneficial effects on differentiation and reduction of apoptosis in BMSCs.

Objective: The aim of this study was to investigate the effects of co-administration of BMSC + LC on ovarian function, structure and apoptosis after creating a chemotherapy model with cyclophosphamide in rat.
Materials and Methods: Forty female Wistar rats were intraperitoneally injected with cyclophosphamide for 14 days for chemotherapy-induced ovarian destruction. Then, the rats were randomly divided into four groups: I. control group, 25 µl of culture medium was directly injected into the bilateral ovaries, II. BMSC group, 2×10^6 BMSCs suspended in 25 µl of culture medium were directly injected into the bilateral ovaries, III. LC group, 200 mg/kg of LC was injected intraperitoneally one day before until seven days after chemotherapy, IV. Co-administration of BMSC + LC group, injection of BMSCs, and LC were performed together. Four weeks later, the function of the ovaries was evaluated by measuring the levels of serum estradiol (E_2) and follicle-stimulating hormone using the enzyme-linked immunosorbent assay kit, the structure of the ovaries was evaluated by counting the number of ovarian follicles at different stages using hematoxylin and eosin staining, and apoptosis was investigated by evaluating the expression of ovarian B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax) proteins using western blot were assessed.
Results: Co-administration of BMSC + LC was more effective in repairing damaged ovaries than the effect of their separate administration. Co-administration of BMSC + LC increased E_2 and decreased follicle-stimulating hormone levels compared to the control group ($p < 0.001$). The number of follicles was higher in the co-administration of BMSC + LC group compared to the control group ($p < 0.001$). Co-administration of BMSC + LC increased Bcl-2 protein level, decreased Bax protein level and increased Bcl-2/Bax ratio ($p < 0.001$).
Conclusion: The effect of co-administration of BMSC + LC is probably more effective than the effect of their separate administration on the recovery of damaged ovaries by chemotherapy with cyclophosphamide in rat.
Key words: *Bone marrow stromal cells, Carnitine, Chemotherapy, Ovary, Regeneration.*

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P-156
Achieving targeted nano-carriers for use in simultaneous injection and delivery of a combination therapy system (drug and gene) for cancer cells

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Background: One of the most common causes of death in humans is cancer. Each year, out of every 100,000 people in the world, about one hundred to three hundred people die from this disease. One of the new methods for treating cancer is targeted nanocarriers.
Objective: Achieving targeted nano-carriers for use in simultaneous injection and delivery of a combination therapy system (drug and gene) for cancer cells.
Materials and Methods: This review, we searched in PubMed, Scopus and, Web of Sciences database from 2000 to 2020 with "nano liposomal drug delivery" key word and used 17 full text of 35 in this abstract.
Results: In general, the design of nanosystems in drug or gene delivery involves several important fields: 1) Nanocarriers: particle size is very important in delivering nano-drug. Because of not only the biochemistry of the body is targeted but also the size of nanoparticles is very suitable for cells. The biological systems are in nanoscale too. For treat a disease, it's necessary to acting on the same dimensions of the active ingredients in the disease, which have the same nanoscale. 2) target finders: target detectors Nanoparticles have specific surface molecules. They bind drugs to cells with specific receptors based on their specific surface molecules. They can even facilitate the uptake of nanocarriers by cells. In this way, the efficiency of drug delivery and the effect of the drug is higher and fewer doses are used.
Conclusion: Targeted nano-liposomes can transfer therapeutic drugs and genes to specific cells.
Key words: *Targeting, Nano-liposomal, Drug delivery, Gene delivery.*

P-157
A review of intimate partner sexual violence in COVID-19 pandemic

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Background: The outbreak of SARS-CoV-2 (COVID-19) and quarantine due to this is greatly challenging for families, especially women. Partner sexual violence against women can endanger the physical and psychological health of women, especially their reproductive health, family and community health.

Objective: The objective of this study was to review the effect of partner sexual violence against women during the COVID19 pandemic.
Materials and Methods: The present study was a narrative review in which researchers conducted computer searches on Google Scholar, PubMed, magiran, SID, Science Direct, and ProQuest databases using key words of COVID-19, partner sexual violence, domestic violence and women related to the subject of the study from 2019 to now. Researchers have read the full text of the article and related reports and finally presented it in the form of a summary of the article.
Results: The findings of the present study have led to the classification of content into three general categories. The first category was the impact of COVID-19 pandemic violence on women's mental health including: anxiety, major depression, post-traumatic stress disorder, job loss and financial insecurity, social isolation, lack of support, harm from threatening behavior and speech, and feelings of humiliation. The second category was the impact of COVID-19 pandemic violence on physical health including: beatings, bruising, bleeding, tooth damage, rupture of the eardrum and increased substance abuse. Finally, the third category of the impact of COVID-19 pandemic violence was sexual health including: unwanted pregnancy, unsafe abortion, unsafe sex, sexually transmitted diseases, and neglect of female sexual needs.
Conclusion: Due to the increasing incidence of violence in the corona pandemic, the urgent need to develop and implement treatment options with regard to all aspects of mental, physical and sexual in women is of particular importance. The findings of this study can be available for experts to design therapeutic interventions against violence.
Key words: *COVID-19, Partner sexual violence, Domestic violence, Women.*

P-158
Complications of COVID-19 and male fertility: Literature review

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Background: Coronavirus disease 2019 (COVID-19) is a disease with respiratory signs, but it causes complications for other organs, as well. The virus can damage all the cells that express Angiotensin-converting enzyme 2 (ACE2). Studies have detected virus RNA in semen and testis of patients and the signs of orchitis and testicles discomfort confirmed the male reproductive system (MRS) damages. But, in some cases, the disease is more complicated with probable long-term damage to MRS. The reason may be due to the factors that affect both MRS and COVID-19.
Objective: Therefore, the present study aimed to list the complications, which have adverse effect on COVID-19, MRS and male fertility.
Materials and Methods: We reviewed all published papers (till December 9th, 2020), from Google Scholar, PubMed and Scopus, including original articles, reviews, guidelines, letters to editor, comments on guidelines, editorials, clinical trials and case reports. All published reports were screened using the following words: SARS-CoV-2, corona virus, COVID-19, "severe acute respiratory syndrome coronavirus 2", "2019 ncov", male reproduction, testis, semen, sperm, male factor infertility, fertility treatment, male reproduction, obesity, smoking, vitamins, oxidative stress, fever, stress, supplements and ACE2.
Results: Some interfering factors for both COVID-19 and MRS are listed as follows: 1) Vitamin D deficiency is destructive for both male fertility and COVID-19. It has positive correlation with acrosome reaction and sperm motility. 2) Vitamin C has positive effect on immune system. Its deficiency is a risk for severity of COVID-19. It can improve both sperm parameters and DNA fragmentation. 3) Supplementation like vitamin E, Zinc, and omega-3 play a supportive role in COVID-19, due to antioxidant and immunomodulatory properties. 4) Smoking is a risk factor for both COVID-19 and male fertility. Smokers' cells up-regulate ACE2 expression. They show a higher percentage of severe cases of COVID-19. The rate of both smoking and COVID-19 in men is higher than women. But, there is a paradox on the correlation of smoking and COVID-19. Some paper suggested the protective effect of smoking for COVID-19; while, the majority decline this issue. 5) Physical and psychological stress increase cytokine. In COVID-19, together with the physical stress, hospitalization causes a level of psychological distress. Stress is a negative factor for MRS, too. 6) The viral infections, through fever and cytokines production, have a negative effect on spermatogenesis and MRS. Cytokine storm is a hyperinflammatory syndrome, characterized by fever, hypercytokinemia and multi organ failure. COVID-19 induce changes in cytokines profile which implicate for male fertility. 7) Fever and hyperthermia reduce sperm quality. It has considerable deleterious impact on spermatogenesis. 8) ROS production in COVID-19 and oxidative stress interferes the male fertility. The mechanism of reproductive damage in COVID-19 is through the ROS. 9) Obesity is independent risk factor for COVID-19 and male fertility.
Conclusion: The above-mentioned factors may interfere with COVID-19 and male fertility, preliminary by ROS production, spermatogenesis failure and sperm DNA fragmentation.
Key words: *COVID-19, ROS, Smoking, Vitamins.*

P-159

Fabrication of miR-16-1 carrier lipid system with the aim of affecting prostate cancer cell line (PC-3)

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Background: Prostate cancer in the men is one of the most common types of cancer. This cancer has a poor prognosis and is diagnosed usually in advanced stage. One of the new methods in cancer therapy is drug delivery system. Liposomes and niosomes are nano carrier that can improve cellular uptake of drug and genes and another material like mi RNAs. Tumor suppressor miR-16-1 target tumor tissue and specially the Bcl-2 oncogene.

Objective: Our aim in this study was to increase targeting cancer cell with miR-16-1 liposomal system.

Materials and Methods: The lipid system was synthesized by thin hydration film method using neutral and charged phospholipids with a percentage of 70: 30: 20: 3. In brief, phospholipids and cholesterol were dissolved in chloroform. After homogenization organic solvent was removed by rotary evaporator (Heidolph, Germany) at 50 °C until a thin-layered film formed. The dry lipid films were hydrated by adding phosphate-buffered saline (PBS, pH = 7.4) and obtain the liposomal suspensions. Then to reduce the vesicles' mean size, the prepared vesicles were sonicated for 15 min using a micro tip probe sonicator. The charge and size of nanoparticles were analyzed by Zeta Sizer and the Zeta Extractor. In the next step, miR-16-1 was loaded on the system by incubation method for 45 minutes at 25°C.

Results: The size and charge of the lipid were below 100 nm and below 12 mV before loading and below 150 nm and near 0 mV after loading.

Conclusion: Accordingly, the results quantitatively and qualitatively indicate the loading of miR-16-1 on the lipid system, which can be effective in targeting prostate cancer cells in the next phase.

Key words: *Prostate cancer, Gene therapy, Liposome, miRNAs, Drug delivery.*

P-160

Study of differentiation potential of human amniotic fluid-mesenchymal stem cell in neural tissue regenerative

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Background: One of the applications of human amniotic fluid mesenchymal stem cell is repairing nerve tissue. New studies have been conducted on the application of these cells in neural differentiation under different conditions including different concentrations of growth factors, different concentrations of amniotic fluid and various scaffolds. The results have been satisfactory and promising. Various factors have been optimized to improve the results of differentiation into neural tissues in these studies.

Objective: The purpose of this study was investigating the potential of human amniotic fluid mesenchymal stem cell in regenerative medicine special in neural tissue regenerating.

Materials and Methods: Evaluation of various studies performed in vitro and in vivo so far suggests the high differentiation potential of amniotic fluid mesenchymal stem cells. Considering the fact that the neural differentiation of these cells has received a lot of attention recently, the present study provides a complete overview of published research on the use of these cells in neural differentiation and neural tissue regeneration.

Results: Finding of review show amniotic fluid mesenchymal stem cell can alter morphological characteristic and become neural-like cells, which stimulate the expression of neuronal markers. Amniotic fluid stem cells showed a more primitive phenotype than the potential for differentiation of other stem cell sources, which could potentially be suitable for cell-based therapy in reconstructive medicine for neurodegenerative diseases.

Conclusion: According to the high potential of these cells in differentiation nerve tissue, as well limited studies on Human amniotic fluid mesenchymal stem cell for differentiation into neural tissue have been performed by the same research team in this obtained promising result.

Key words: *Neural differentiation, Amniotic fluid, Human amniotic fluid mesenchymal stem cell (AF-MSCs).*

P-161

Quantification of a breast cancer-related miRNA using an electrochemical biosensor: Application of a novel graphene/gold/quantum-dot nanocomposite

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Background: Breast cancer is one of the second most common cancer diagnosed in women. Most of the cases are diagnosed at late stages in which treatments are less effective. Therefore, there is a global challenge finding novel ways to early detect all types of cancer including breast cancer. In this way, conventional methods suffer with low selectivity and sensitivity. However, molecular biomarkers such as miRNAs, which are small non-coding RNAs, are emerged in modern medicine as feasible detection method. Their up- and down-regulation in tissue or even blood samples can be a sign of cancer development.
Objective: MiR-155 have been known as one of the biomarkers for breast cancer detection that is up-regulated in early stages of the disease.
Materials and Methods: Here we developed a novel electrochemical nanobiosensor for quantification of miR-155 in patient serum. We have used nanocomposite of graphene oxide and graphene quantum dots that are decorated with gold nanourchines. The nanocomposite was characterized using Fourier-transform infrared spectroscopy (FTIR) method and the modified electrode was characterized using scanning electron microscope (SEM) imaging, cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) methods. The final readout signal of the electrochemical label was recorded using differential pulse voltammetry (DPV).
Results: In results, the characterization methods showed the fabrication steps efficiency. In addition, the results of selectivity assay showed that the nanobiosensor is able to detect the target miRNA sequence from some non-complementary sequences (including one-base mismatch, three-base mismatch, completely mismatch, and also mixture of non-complementary and complementary sequences. Additionally, the results of real sample assay are similar to the synthetic samples, with no significant difference.
Conclusion: The nanobiosensor showed very high selectivity towards the target miRNA-155 compared to the non-specific targets including one- and three-base mismatched miRNA-155. In addition, the wide linear range of the nanobiosensor with low detection limit is promising results that makes the nanobiosensor a potential choose for future medical applications of breast cancer detection and screening.
Key words: *Biosensing techniques, Electrochemistry, Breast cancer, Nanostructures, MicroRNAs.*

P-162
rs1256049 in *ESR2* gene is not associated with poor ovarian response in assisted reproductive technology

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Background: In vitro fertilization (IVF) is a common approach for infertile couples with different etiologies. In a standard IVF cycle, exogenous follicle stimulating factor (FSH) is administered for ovarian stimulation. Numerous studies have been performed to determine individual variability in the ovarian response to gonadotropins in routine stimulation protocols. Different factors including hormonal FSH anti mullerian hormone, functional like antral follicle count (AFC), or genetic markers have been studied for optimizing individualized dose of exogenous gonadotropin in available protocols. Mutations in the genes coding for FSH receptor, luteinizing hormone receptor, estrogen receptor (ESR) and anti mullerian hormone receptor may influence ovarian response.
Objective: Considering the vital role of estrogen in ovulation, we aimed to investigate the SNP in position 1082 G/A (rs1256049) of the beta-estrogen receptor gene (*ESR2*) gene that may be correlated with an altered response to FSH in IVF cycles.
Materials and Methods: In this study, 200 women with poor ovarian resesponse and 40 women with good response were studied in terms of polymorphism RsaI 1082 G>A of *ESR2* gene. Different genotypes of this polymorphism (GG, AG, AA) were determined using RFLP-PCR technique and using RsaI restriction enzyme.
Results: Statistical analysis was performed using SPSS software and appropriate statistical tests. Finding showed that there was no significant difference between the genotypic distribution of the under-studied polymorphism and the allele frequency with in and between groups.
Conclusion: Our finding suggests the polymorphism 1082 G > A of ESR2 gene is not related to poor ovarian response. However, the mechanism involved in this relationship is still unknown and considering the polymorphic nature of the ESR2gene (around 1800 SNP), more studies are needed to ascertain this relationship. In fact, more understanding of these genomic variants would help to managing controlled ovarian stimulation protocol, individually. Generally, the study of SNPs of the reproductive hormone receptor genes is a remarkable field of research that could provide us with new evidence about the way each woman responds to exogenous gonadotropin administration during ovulation induction.
Key words: *Polymorphism, Beta-estrogen receptor, Poor ovarian resesponse, RFLP-PCR, RsaI.*

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Polymorphism of *ESRI* (XbaIG/A) as a genetic agent in women with poor response to controlled ovarian hyperstimulation

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Background: Physiological function of ovarian regulates by reproductive hormones including estrogen. Estrogen is a steroidal hormone and its actions in ovary mostly occur through its binding to intracellular receptor α and β. *ESR1* (α) gene include many polymorphic sites (SNPs) located along various regions of it that control expression and function of this receptor.
Objective: This paper aimed to investigate the association of XbaI A/G (rs9340799) with poor ovarian response in Iranian women undergoing IVF treatment referred to Yazd Reproductive Sciencse Institute.
Materials and Methods: To do so, a group of 40 women with normal response ovarian and a group of 209 women with poor response ovarian in IVF cycles were included. Genomic DNA extraction was performed with Blood DNA Extraction Kit (Favorgen Co.). Using PCR-FRLP technique and XbaI restriction enzyme SNP in -29 G/A site of *ESR1* gene were genotyped.
Results: Our finding show polymorphisms in *ESR1* (rs9340799) was significantly different between women with normal and poor response ovary considering AG+GG and GG+AA ($p = 0.005$) genotype but was not statisticaly signification regarding to AA+AG ($p \leq 0.05$).
Conclusion: The study of SNPs of the ESR1 gene is an interesting field of research that could provide us with new facts considering the way each woman responds to standard stimulation protocol in IVF cycle.
Key words: *Alpha-estrogen receptor, Polymorphism -29 G>A (rs9340799), Poor ovarian resesponse, RFLP-PCR.*

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What are antenatal care needs of women who conceived through assisted reproductive technologies? A mixed methods literature review

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Background: Nearly 5% of newborns in Europe are nowadays born following assisted reproductive technologies (ART), a steadily increasing number. Women who conceived through ART might experience more stress and anxiety during pregnancy compared to women who conceived spontaneously, and their mother-infant attachment might be delayed. Therefore, ART-women might have additional antenatal care needs.
Objective: To provide an updated review of the available evidence on the antenatal care needs of women who conceived through ART.
Materials and Methods: By means of a mixed methods literature review, we systematically searched for literature to identify journal articles published till May 2019 in five databases. The following medical subject headings terms were used: "Pregnancy", Pregnant women", "Reproductive techniques", "Assisted, Prenatal care", "Qualitative research" and "Midwifery". After evaluating 876 abstracts, 33 eligible studies were assessed for methodological quality appraisal using the Mixed Methods Appraising Tool. 11 articles were included in this study: 6 qualitative studies and 5 quantitative studies.
Results: Analysis of the included studies resulted into four themes: 'General health', 'Anxiety', 'Maternal-fetal attachment' and 'The experience of pregnancy after fertility treatment'. ART-women reported lower social and physical functioning scores and elevated levels of anxiety compared to women who conceived spontaneously. The results concerning mother-infant attachment in relation to method of conception were inconclusive. ART-women reported difficulties adjusting to pregnancy and experienced paradoxical feelings regarding their pregnancies. Moreover, they want maternity caregivers to pay more attention to their history, more psychosocial support, routine check-ups and ultrasounds. Lastly, women who are transferred from the fertility clinic to local maternity services may experience a care gap between discharge from the clinic and having their first appointment or ultrasound at the local maternity services.
Conclusion: The results of our study indicate that women who conceived through ART could indeed have additional antenatal care needs. Most importantly, ART-women want their occasionally conflicting feelings to be acknowledged by caregivers. Therefore, caregivers should invite these women to talk about their history and to express their care needs in order to individualize their care plan.
Key words: *ART, Pregnancy, Midwifery.*

P-165
The effect of long-term cryopreservation on live birth rate

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Background: The duration of cryopreservation storage could be important for embryo survival, and therefor it can have some impacts on chemical pregnancy and live birth rate.
Objective: This retrospective study was conducted to evaluate the impact of cryopreservation storage duration on embryo survival, chemical pregnancy and live birth rate.
Materials and Methods: This retrospective study was carried out over a period of 8 years, from 2011 to 2019 in Novin Infertility Treatment Center on a total of 48 patients. The first vitrified-warmed cleavage embryo transfer was performed 3 months after oocyte retrieval

and was resulted in live birth pregnancy (group 1). The second transfer was performed after duration of 5 to 8 years on each member of pervious group (group 2). The results of these transfers were also collected. There was no significant difference between these patients regarding age, body mass index, quality of embryos and semen parameters of male partner. The IBM SPSS V.26.2019 was used for data analysis. A $p < 0.05$ was considered statistically significant.

Results: These results indicates that the length of storage time did not have a significant effect on post-thaw embryo survival ($p = 0.65$). There were also no significant impact on chemical pregnancy ($p = 0.23$) and live birth rate ($p = 0.2$). Our outcomes demonstrate that clinical pregnancy, miscarriage, twin pregnancy and live birth rate were similar between group 1 and 2 (82% vs 71%, 8.6% vs 16%, 8% vs 10.2% and 62.5% vs 43.7% respectively).

Conclusion: Cryo storage duration did not adversely effect on post-thaw survival or pregnancy outcome. The pregnancy rate in the first type of thawed embryo transfer is slightly higher, which is not statistically significant. This may be due to increased maternal age at the second thawed embryo transfer (after 5-8 yr) and not for the reason of adverse effect of long term freezing.

Key words: *Embryo survival, Cryopreservation storage, IVF, Pregnancy rate, Long term freezing.*

P-166
Non-invasive preimplantation genetic testing advantages and disadvantages

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Background: It is essential to identify quality and healthy embryos for transfer in in vitro fertilisation successful. Research has shown that traditional morphological methods are not sufficient for diagnosis. The best embryo can select the best embryo with preimplantation genetic testing. With PNG be tested the severe inherited conditions or for chromosome abnormalities. It needs to biopsy from the embryo for genetic testing, includes separating one or more cells from the embryo, or collecting blastocoel fluid and embryo culture medium. Because of the risks of cell biopsy from embryos, it is necessary to find non-invasive methods for PNG. The research has shown, we can use genomic DNA in blastocoel fluid and embryo culture medium. It may be open a new way for selecting the best embryo.

Objective: This review tries to evaluate the results of research about non-invasive pre-implantation genetic testing. The most popular method is detecting and analyzing cell-free DNA in embryo culture medium for genetic testing. Of course, there is a long way to go to use this method for PNG. This article tries to examine its technical and biological problems.

Materials and Methods: Original research and review papers about non-invasive pre-implantation genetic testing were sourced by searching PubMed and Google Scholar databases until February 2021. The search included keywords: 'spent culture media'; 'cell-free DNA'; 'non-invasive pre-implantation genetic testing'; 'blastocentesis'; 'blastocoel fluid' and 'pre-implantation genetic screening'.

Results: Available data suggested that blastocoel fluid and embryo spent culture medium, samples provide DNA suitable for genetic analysis and are a potential tool for preimplantation genetic testing. Embryonic DNA could be detected in the embryo spent culture media and blastocoel fluid. Primery studies have been successful in molecularly examining cell-free DNA, but the amount and quality of available DNA has varied. Reports of similarity in the results of free DNA genetic testing and embryo biopsy have been reported differently.

Conclusion: The reports have shown that results of trophectoderm biopsies may be different in cytogenetic data. It is likely for embryonic mosaicism or DNA contamination. It was said that aneuploid embryonic cells are removed from the embryo. Therefore, DNA distributes in the spent culture medium and blastocoel fluid. Of course, this point needs to check out more. The hard part is isolating and amplifying DNA to make an accurate clinical diagnosis. Some factors are distorting, and there are contaminants in this work, but generally, it is important and necessary that we have a non-invasive pre-implantation genetic testing.

Key words: *Cell free DNA, Embryo, Spent culture medium, Blastocoel fluid, Preimplantation genetic test.*

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Thrombophilic factor analysis of 234 followed-up couples with recurrent pregnancy loss

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Background: Recurrent pregnancy loss (PRL) is a common disorder that occurs in 5% of pregnancies and is defined as the loss of two or more consecutive pregnancies before 20 weeks of gestation. various causes such as thrombophilia might increase the risk of RPL.

Objective: This study aimed at testing the association between thrombophilic factors with anticoagulant treatment in Iranian women with RPL.

Materials and Methods: In this study thrombophilic factors of 234 couples were evaluated using strip assay and PCR sequencing for six important causes of hereditary thrombophilia including factor V Leiden (FVL), factor VR2, factor II, PAI-1, MTHFR 677C>T and 1298A>C in couples. Then we followed up females for two years. any evidences, medications and outcome of next pregnancies were registered by completing questionnaire. Finally, data was analyzed by SPSS software.

Results: The mean age of females was (29.32 ± 5.348). There were homozygote and heterozygote 20210G>A mutation of factor II in females 0.4%, 2.1% and male 0.9%, 0.9% respectively. Also the results in homozygote and heterozygote form for 1691G>A mutation of factor V Leiden were in females 0%, 3% and in males 0%, 1.3% and for 1299H>R mutation of factor VR2 were 1.7%, 15.8% and in males were 1.3%, 12.4% sequentially. The medication and percentages for treated women were as followed: Folic Acid alone was administered 22 cases, Folic Acid plus ASA 80 mg was administered for 32 cases, Folic Acid plus injection of anti-coagulant drugs was administered for 27 cases, the combination of Folic Acid, ASA 80 mg and injection of anti-coagulant drugs was administered for resulted in 105 cases, ASA 80 mg plus injection of anti-coagulant drugs was administered for 5 cases. Only 4 women of 43 women (9.3%) who never received medication achieved live birth. The best outcome was success in combination therapy (Folic Acid, ASA 80 mg and injection of anti-coagulant drugs) that 67 cases of those achieved live birth (63.8%).

Conclusion: This study showed genetic counseling followed by thrombophilic factors evaluation and finally proper medication may be effective in the treatment of women with RPL. Best pathway of treatment leading to the successful child delivery was combination therapy (Folic Acid, ASA 80 mg and injection of anti-coagulant drugs) and rate of success pregnancy were significant by using this treatment. (p = 0.00).

Key words: *Recurrent pregnancy loss, Thrombophilic factors, Follow up.*

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