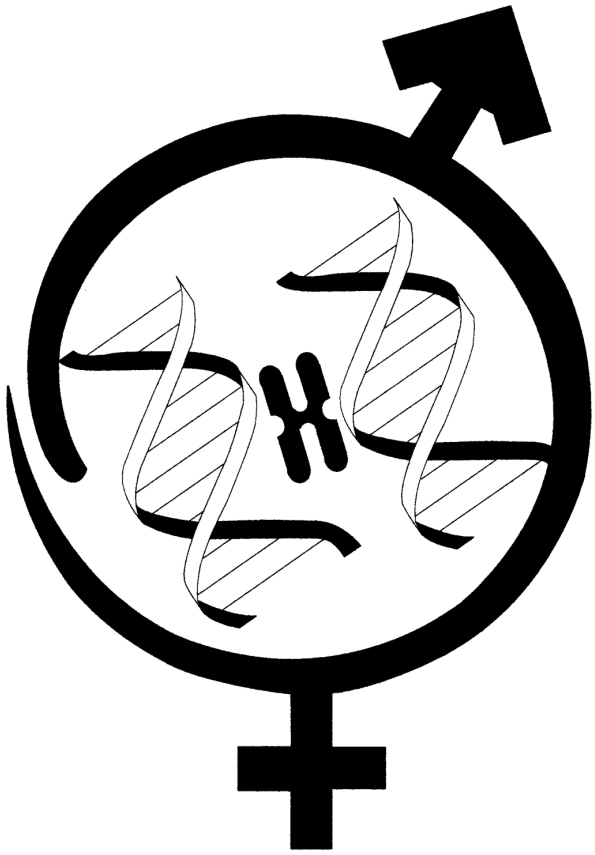
Abstracts of
Royan International Twin Congress

17th Congress on Reproductive Biomedicine
31 August-2 September 2016

11th Seminar on Nursing and Midwifery
31 August-2 September 2016



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Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

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Some of these abstracts have been previously published as full text in other journals. The authors will add more details and supplementary data to their presentations for more discussion in Royan International Twin Congress on Reproductive Biomedicine and Stem Cells Biology & Technology.

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Rouhollah Fathi

Dear Colleagues,

Twenty four years after the foundation of Royan institute by the late and inspirational Professor Kazemi Ashtiani, unalloyed blessing and career manager, and his co-founders in May 1991, that nascent establishment developed into a consequential research institution and is bracing to convene "17th Royan reproductive biomedicine congress" in September 2016 in Tehran.

Many students, researchers and scientists from all around the world actively participated in 16th Royan reproductive biomedicine congress and friendly exchanged their curiosity driven researches and experiences in clinic and laboratory scopes. Endocrinology, Gynecology, Andrology, Genetics, Imaging, Epidemiology and Embryology were the main scientific fields related to infertility and sterility which were presented and discussed in the last Royan reproductive biomedicine congress. The congress wound up by awarding to the most creative and effective research works dedicated to solving reproductive problems under the name of "national and international winners". Moreover, Royan institute as a leading institution in infertility investigation and treatment in Middle East and west Asia; is going to present the cutting edge sciences in Royan annual twine congresses pertaining to promotion of health community throughout the world.

Noted that the dignity and reverence of elites are deeply established in Iranian tradition and ingrained in our Islamic vision, Royan institute awarded Professors Rudolf Jaenisch, Hans R. Schöler and Robert S. Langer as winners of memorable "Kazemi prize".

Royan institute cordially invites prominent and distinguished researchers to attend the 17th Royan reproductive biomedicine congress and exchange the latest achievements in various disciplines relevant to congress goals. We are extending well known Iranian hospitality to all attendees globally and look forward to your active support and participation. It is hoped that reminiscence of this event in Iran, the cradle of civilization and home town of the great scientists such as Avecina and Razi, will linger in your mind forever and the fruition of this congress will provide a bed lock for the appeasement of human sufferings and pain.

Rouhollah Fathi, PhD
Congress Chairman of 17th Congress
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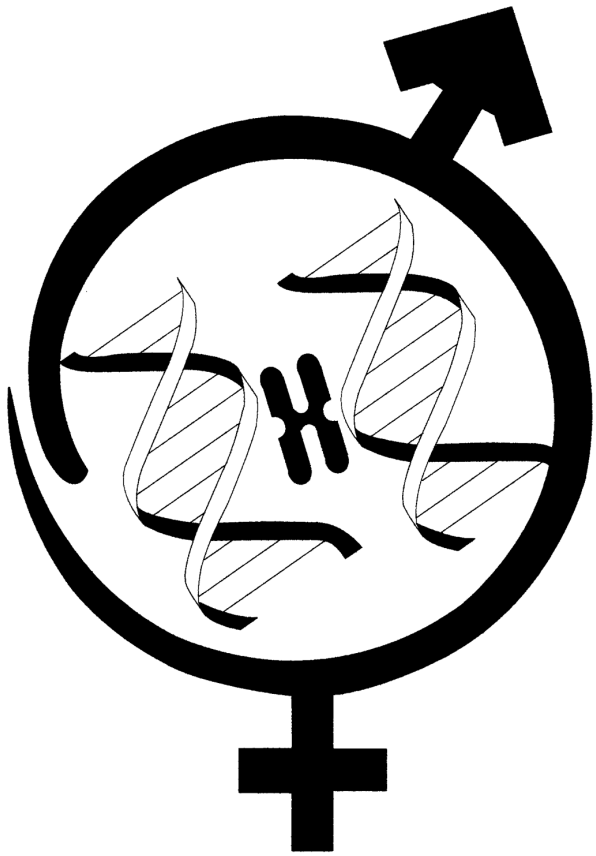

Royan Institute

Tehran, Islamic Republic of Iran

Invited Speakers

Animal Biotechnology

I-1: A History of Ideas on The Origins of Trophoblast

Johnson MH

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Background: To give an historical account of ideas about the origins of trophoblast from the 1950s to the present day.
Materials and Methods: Relevant papers from the 1950s to the most recent were read.
Results: A fundamental problem with ideas about the origins of trophoblast has been their tendency to polarize to one of two extreme positions: either a totally plastic, epigenetic position or a mosaic position.
Conclusion: It is concluded that neither extreme position accurately reflects reality, which is that elements of both are involved.

I-2: A History of The Research Leading to The Birth of Louise Brown

Johnson MH

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Email: mhj21@cam.ac.uk

Background: To give an historical account of the research under taken by Edwards, Purdy and Steptoe between 1969, when Nature published their paper describing IVF in humans, and the birth of Louise Brown in 1978.
Materials and Methods: Archives were consulted and key players interviewed.
Results: We found that more patients (282) were subjected to more procedures (495) than had been realized previously. Moreover, of the women undergoing procedures, 76 (27%) had no chance of pregnancy, as embryos were not being transferred to uteri when they were in the research programme. In total, only five clinical pregnancies resulted from these 495 procedures, of which only two produced live healthy term births.
Conclusion: It is concluded, from examination of several types and sources of information, that the evidence is consistent with Edwards, Purdy and Steptoe behaving ethically in their use of patient volunteers, and that, whilst not undertaking controlled experiments, they did use their up-to-date knowledge of the literature responsibly in their approach to problem solving.

I-3: Globozoospermia from Clinical, Cellular and Molecular Aspects

Nasr Esfahani MH[1, 2]

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Globozoospermia is a rare and severe cause of primary male infertility which is defined by round-headed sperm without acrosome. However, it frequency appears to be higher in countries with higher incidence of consanguinity or communal marriages. Round-headed sperm are not able to fertilize oocytes. Literature studies reveal that in addition to intra-cytoplasmic sperm injection (ICSI), assisted oocyte activation (AOA), is also required to achieve acceptable fertilization rate and clinical outcomes. Low fertilization following routine ICSI, in absence of AOA, has been mainly related to the aberrant expression of sperm-associated oocyte activating factors known as PLCζ and possibly PAWP which are involved in calcium oscillations during oocyte activation. Genetic studies reveal that DPY12 deletion is the main cause leading to formation round head sperm. During this presentation, clinical outcomes of large number of globozoospermia will be present.

I-4: Novel Methods for Purification of Biomolecules Using Modified Magnetic Nano-Resins

Shoghi Kalkhoran E

Email: elhamshoghi@royaninstitute.org

Animal-extracted injectable hyaluronidases have been used safely for many years to facilitate the fertility in human beings. However, concern still exists about the allergic and immunological risks of these products. A novel formulation of recombinant human hyaluronidase, PH20, has been developed as an alternative to these animal-derived hyaluronidases due to lack of allergic and immunologic risks. After producing this recombinant protein, to detect the target protein, like other proteins, the His-tag (6-histidines) is being used as a fusion partner for purification of PH20. In this project modified His-select Nickel magnetic agarose bead was designed and synthesized in Royan Institute and employed successfully for detection and separation of His-tagged PH20 as an alternative to commercial resins
Protein separation based on the properties of modified magnetic nano-resins, is the way that nowadays are widely used in biotechnology. Magnetic nanoparticles, due to their high compatibility, availability, low toxicity, ease of separation and the possibility of magnetic resonance imaging, are the most common categories that are used in biological research. It has been highly demanding to enhance the selectivity of such magnetic nano-resins toward target molecules by modifying their surface with various selected functional groups on the basis of their necessity. Two different modified nano-resins were designed on the basis of magnetic Fe3O4 nanoparticles (NPS). The first one was the synthesis of modified His-select Nickel magnetic agarose beads. In this project, first, highly dispersed magnetic Fe3O4 nanoparticles were synthesized and then polymeric matrix, Sepharose (agarose stabilized with polyacrylamide) was made. Afterwards, polyacrylamide gels were oxidized to carbonyl groups to be able to attach covalently to amino and polycarboxylic ligands. Then, modified sepharose gel was saturated in Nickel solution and finally magnetic nano-resins basis was obtained on the modified agarose beads with a high efficiency together with high selectivity to His-tagged fusion proteins. In the synthesis of the second nano-resins, our current project, new method has been designed to combine two highly effective technologies, synthesizing molecular imprinted polymer coating on the basis of magnetic Fe3O4 nanoparticles (NPS) together with adding nucleic acid sequence (Aptamer) to their structure due to protein recognition and separation. It is supposed that the resulting Apt-MIP would be able to selectively capture the target protein bovine serum albumin (BSA) via recognition cavities. In these two procedures, a magnet was used to separate magnetic nano-resins from the liquid phase and thus the time needed to carry out the experiment, was shortened.

I-5: High Efficient Genome Editing in Pigs for Making Human Disease Models

Zhao J[1*], Zhou Q[1], Yao J[1], Cao CH[1], Huang J[1], Wang X[1], Yuan Z[2], Wang Y[3], Wang H[1]

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Background: Pigs have been extensively used in biomedical research and are an excellent models for human diseases. The efficient and precise genetic modification of pigs would facilitate the generation of tailored disease models and strains with valuable agricultural traits. The emergence of nuclease-mediated gene editing technologies, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeat (CRISPR/Cas9 system), introduced a new era for gene targeting, especially in large animals. Thus develop a strategy independent of SCNT of making precise, high efficient, multiple genes targeting in pig genome will greatly facilitate the development of pig models.
Material and Methods: *In vitro* oocyte maturation, *in vitro* embryo culture cell transfection, microinjection somatic cell nuclear transfer RT-PCR Western blot.
Results: Talens mediated efficient DJ-1 deletion for making Parkinson's disease animal model in total, 5% (2/40), 2.5% (2/80), and 22% (11/50) of the obtained colonies of fibroblast cells were mutated for GGTA1,Parkin, and DJ-1, respectively. Mixed DJ-1 mutant colonies were used as donor cells for somatic cell nuclear transfer (SCNT), and three female piglets were obtained (two were bi-allelically mutated, and one was mono-allelically mutated). Western blot analysis showed that the expression of the DJ-1 protein was disrupted in KO piglets. 2. Efficient CRISPR/Cas9-mediated biallelic gene disruption for making hearing loss disease models and site-specific knockin after rapid selection of highly active sgRNAs in pigs. Assessment of sgRNA mutagenesis efficiencies can be achieved within 10 days from the design of the sgRNA with a single blastocyst genotyping system. The most effective sgRNA selected by this system was successfully used to induce site-specific insertion through homology-directed repair at a frequency exceeding 13%. We further showed that direct cytoplasmic injection of Cas9 mRNA and the favorable sgRNA into zygotes could generate MITF biallelic knockout piglets with an efficiency of up to 100%. 3. One-step generation of triple genetargeted pigs using CRISPR/Cas9 system By co-injection of Cas9 mRNA and multiplexing single guide RNAs (sgRNAs) targeting parkin, DJ-1, and PINK1 genes, respectively, into in vivo derived pronuclear embryos, we simultaneously targeted three distinct genomic loci. In addition, our trio-based whole-genome sequencing analysis suggested that the incidence of off-target events is low.
Conclusion: Combination of TALENs technology with SCNT can efficiently generate bi-allelic KO pigs without the integration of exogenous DNA. Further, we established the protocols for screening high efficient gRNA screen, which could obtain bi-allelic gene knockout, Triple gene targeting at one step with direct injection of one cell zygote. With these techniques, DJ-1 KO pigs, parkin/DJ-1/PINK1 triple targeted pigs for Parkinson's disease, MITF KO pigs for hearing loss disease models has been made.
Keyword: Talen, CRISPR/Cas9, Pig, DJ-1 MITF

Embryology

I-6: Inter-Conversion of Germ Cells and Stem Cells

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I-7: Current Sperm Feezing Techniques and Evaluating Protocols in Animals

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Background: The present study was aimed at reviewing of the current freezing and post-thawed evaluation methods of mammalian sperm.
Materials and Methods: Sperm cells are cryopreserved with some upper technological methods as well as conventional systems. These techniques includes liquid nitrogen vapour, dry ice, dried-freezing, encapsulation, directional freezing methods etc. On freezing with these techniques, successful freezing rates is still changeable. In this review, we explain the detail procedures of these techniques, their advantages-disadvantages and studies performed. As evaluating techniques of post-thawed sperm, we make the focus on motility, fluorescent staining (of sperm viability, acrosome and mitochondria integrities), DNA tests (COMET aand TUNNEL) and electron microscopy of sperm abnormalities and insemination techniques.
Results: In this study, we compare the current techniques of sperm freezing, focussing on freezing protocols. As evaluating protocols, parameters providing the more objective results are discussed.
Conclusion: On sperm freezing, some freezing techniques should be improved for increasing the post-thawed sperm parameters and fertility.
Keywords: Sperm Cells, Freezing Techniques, Evaluating Protocols

I-8: Effects of Cryoprotectants and Trehalose on Ram Sperm: An Electron Microscopic Study

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Background: The present study was conducted to examine the effects of different cryoprotectants (glycerol, G and ethylene glycol, EG) and trehalose (T) on post-thawed ram sperm morphologies.
Materials and Methods: Ejaculates collected from six Merinos rams, were pooled and evaluated at 37ºC. Pooled semen samples were divided into six aliquots, and diluted in a Tris-based extenders containing 5% G, 3% G+60 mM T, 1.5% G+100 mM T , 5% EG, 3% EG+60 mM T and 1.5% EG+100 mM T. Then, they were cooled to 5 oC and frozen

in 0.25 ml French straws. Frozen straws were then thawed individually at 37ºC for 25 s in a water bath for electron microscopy. Field Emission Scanned Electron Microscope (FESEM) was used for examining the thawed sperm. For comparison of the obtained data, the Chi-Square test was used. The differences with values of $P<0.05$ were considered to be statistically significant.

Results: 3% glycerol+60 mM trehalose group provided the highest protection for all sperm morphologies among the groups. All ethylene glycol groups led to higher percentages of undamaged spermatoza, compared to glycerol 5%. The addition of trehalose at different doses in semen extenders tended to reduce the damaged percentages in sperm cells ($P<0.05$).

Conclusion: Trehalose and ethylene glycol supplementation in semen extenders provided a protection of sperm morphologies against cryopreservation injury in electron microscopic examination.

Keywords: Ram Semen, Cryopreservation, Trehalose, Cryoprotectant, Sperm Parameters

I-9: Three Dimensional Culture of Ovarian Follicles

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In vitro culture (IVC) and fertilization of immature follicles is one of the potential uses of cryopreserved ovarian tissue. Follicles IVC have the advantage of eliminating the possibility of reintroducing malignant cells from cryopreserved ovarian tissue back into the patients following treatment and providing a way to harvest more mature oocytes. It also allows direct monitoring and assessment of follicle quality during the maturation period. This approach could be performed in two and three dimensional (2D, 3D) culture systems. Advances in biomaterial engineering resulted in the development of an alginate-based, 3D follicle culture system to maintain the cell-cell and cell-matrix connections.

Although mature oocyte has been acquired from IVC of follicles in 2D system, because of the follicle flattening, cell-cell disruption, moving of proliferating granulosa cells away from oocyte, inappropriate metabolite and paracrine factors exchange, researches were conducted to the 3D culture system that mimics ovarian tissue stroma, resemble in vivo condition and maintaining transzonal projections.

Gel culture, non-gel culture and microfluidic culture are different types of 3D culture system. In gel culture, alginate, matrigel, fibrin and hyaluronan are usually used as matrix for follicles encapsulation. Matrix density (compliance degree with the ovarian stromal tissue), matrix porosity, biomechanical characteristics, gelation properties, supporting follicle growth and survivability, biocompability and shearing modulus are factors determine the rate of follicles development.

In non-gel culture, follicles are suspended with different systems in culture medium, so 3D culture system obtained without the presence of any matrix. Rotating system, serial culture of follicles for day to day, hanging drops and membrane inserts are four types of non-gel culture.

In microfluidic system, encapsulated follicles confront to the directed micro flows of culture medium that are generated in a pre-made chip according to a particular scheme. This technology can be used to produce drops of desired size, including those corresponding to the volume of an individual follicle, which simplifies the further cultivation stages.

Finally, the composition of the follicle culture medium and single or group culture of the follicles are important factors that should be considered in follicles IVC. Follicles group culture mimic ovary condition, but due to the inhibiting factors that secreted from injured or damaged follicles during culture period, other follicles growth can be affected.

I-10: Human Vitrified Ovarian Tissue Transplantation: A Case Report in Cancer Patient

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I-11: Validation of Retinoic Acid as The Master Inducer of Meiosis in Fetal Germ Cells

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Background: We previously published in Science the discovery that retinoic acid (RA) is the key molecule that drives germ cells to enter meiosis in mouse fetal ovaries, and that germ cells in fetal testes are prevented from being exposed to RA by the action of the p450 degradative enzyme CYP26B1 in fetal testes. These findings solved a long-standing mystery, and provided a completely new paradigm regarding how entry into meiosis is regulated in a sex-specific manner. Surprisingly, another group subsequently reported they were unable to detect RA in the developing ovary, and found that germ cells are able to enter meiosis normally in mice lacking ALDH1A2 and -3, two important enzymes for RA synthesis. Since that time, many researchers have questioned the importance of RA in this system. Here, we tested whether the action of ALDH1A1 could account for the anomalous findings.

Material and Methods: ALDH1a1 represents a third potential source of RA in the developing gonads. In this study, we used RARE-LacZ reporter mice, and studied expression of RA-responsive genes by qRT-PCR. We used CYP26B1 knockout mice to experimentally increase endogenous RA levels and test the consequences for germ cell behaviour. We used in situ hybridization, immunofluorescence and Western blotting to examine where and when Aldh1a1 is expressed. We used ALDH1A1 knockout mice to test the consequences for germ cell behaviour, as measured by qRT-PCR and Western blot.

Results: First, we confirmed that RA can indeed be visualized in the developing fetal ovaries, and up-regulates several known target genes there. In CYP26B1 knockout mice, RA was ectopically present in fetal testes, and induced meiotic markers there, but this effect was eliminated in the presence of RA receptor antagonists. Importantly, we showed that ALDH1a1 is expressed in the developing ovary – an important and previously overlooked source of RA. The importance of RA was demonstrated by showing that, in ALDH1a1 knockout mice, entry into meiosis is delayed. Finally, we found that Aldh1a1 is expressed more strongly when RA levels are low, suggesting this gene might be even more important in the absence of ALDH1A2 and -3.

Conclusion: Despite published findings that potentially undermine the role of RA, we have clearly established that RA is present at the right time and place to act as the master inducer of germ cell meiosis in the fetal ovary. Our experiments demonstrate that meiosis induction is mediated by a molecule that is both sensitive to CYP26B1 and acting through RA receptor – only RA fits these criteria. Even though germ cells are able to enter meiosis normally in mice lacking ALDH1A2 and -3, our data clearly implicate ALDH1A1 as the "missing" piece of the puzzle in those experiments. Thus, our data re-establish that endogenous RA is the meiosis trigger in the fetal ovary - an important piece of textbook science..

Keyword: Germ Cells, Meiosis Regulation, Ovary, Knockout Mouse

I-12: Metabolism Measurement Fir Well Quality Embryo Selection

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I-13: Relationship between Implantation Failure and Embryo Proteomics

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I-14: The Oocyte-Secreted Factors GDF9 and BMP15: Species Differences and Paths to Utilization

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Background: To convert the oocyte-secreted factor, human GDF9, which is inactive/latent as the wild type protein, into a form useful for animal reproduction applications.
Materials and Methods: Recombinant protein production and purification. Expression cassettes encoding wild-type hGDF9 and chimeric forms of the GDF9 DNA sequence, all incorporating an N-terminal poly-His tag, were commercially synthesized. These were cloned into the pEF-IRESp expression vector and stable HEK-293T cell-derived lines established. The hGDF9 forms were produced into serum-free production media and the proteins purified utilizing loose Ni2+-IMAC resin. Granulosa cell bioassay. Primary murine mural granulosa cells were plated into 96 well plates in bicarbonate-buffered TCM-199 supplemented with 0.3 mg/mL polyvinyl alcohol, and treated for 24 hours with one of the various GDF9 forms under study. For the final 6 hours of this exposure period the cells were pulsed with [3H]thymidine. Competency related gene expression. Porcine ovaries were obtained from a local abattoir and cumulus oocyte complexes (COCs) were aspirated from 2- to 4-mm diameter antral follicles and put under IVM conditions +/- the different forms of GDF9 under study. After 22 hours of IVM cumulus cells were stripped and RNA isolated. The transcript levels for the competency related genes, HAS2, TNFβIP6 and PTGS2 were determined via rtPCR. Oocyte developmental competence assay. After isolation of porcine COCs and IVM as above (+/- GDF9 variants), 20 COCs from each treatment group were denuded and fertilized with washed porcine sperm. Cleavage rates were determined after 2 days of culture and blastocyst rates were evaluated at day 7.
Results: Four mutant forms of human GDF9 (hGDF9) were produced and purified incorporating amino acid residues found in the mouse GDF9 sequence, a form of the GDF9 protein which is active in the wild-type form. In a granulosa cell bioassay monitoring [3H]thymidine incorporation in primary mouse mural granulosa cells it was found that all 4 mutant forms of hGDF9 were active. When effects on competency related gene expression in porcine cumulus oocyte complexes were investigated, only three of the mutant hGDF9 forms were active. These three forms all caused a 20 fold increase in the expression of the TNFβ IP6 gene. Finally, when oocyte developmental competence was monitored in a porcine low-competence model, it was found that only one of the four mutant hGDF9 proteins caused a statistically significant increase, in this case causing a doubling in the number of blastocysts obtained.
Conclusions: This study indicates that in the case of hGDF9 the Gly391Arg mutation probably increases receptor binding affinity, thereby creating an active protein for a monolayer of granulosa cells in vitro. However, for an improvement in oocyte developmental competence, a second mutation (Ser412Pro), which potentially decreases the affinity of the mature region for the proregion, is also required.

I-15: Cumulin: A New Oocyte-Secreted Factor with Applications in Animal Reproduction and Beyond

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Background: To determine if heterodimerization is the mechanism behind GDF9/BMP15 synergism.
Materials and Methods: Recombinant protein production and purification. Expression cassettes encoding the DNA sequence of each of the different forms of either GDF9 or BMP15 were commercially synthesized. These were cloned into the pEF-IRESp expression vector and stable HEK-293T cell-derived lines established. To produce Cumulin, the GDF9 and BMP15 proteins were co-expressed in the same cell population. The different recombinant protein forms were produced into serum-free production media and the proteins purified utilizing Ni2+-IMAC resin to target the engineered N-terminal poly-His tags. The isolated mature region of a covalent form of Cumulin was obtained via reverse phase HPLC. Granulosa cell bioassay. Primary murine mural granulosa cells were plated into 96 well plates in bicarbonate-buffered TCM-199 supplemented with 0.3 mg/mL polyvinyl alcohol, and treated for 24 hours with one of the various GDF9/BMP15 forms under study. For the final 6 hours of this exposure period the cells were pulsed with [3H]thymidine. Luciferase transcriptional reporter assays. To monitor the capacity of the various forms of GDF9 & BMP15 used in this study to activate SMAD signalling a phospho-SMAD transcriptional reporter assay was utilized. This was carried out either in primary murine mural granulosa cells (using either a SMAD3 or SMAD1/5/8 reporter) or in the human granulosa cell line COV434 (using either a SMAD2 or SMAD1/5/8 reporter). Developmental competence assay. Porcine ovaries were obtained from a local abattoir and cumulus oocyte complexes (COCs) were aspirated from 2- to 4-mm diameter antral follicles and put under IVM conditions +/- the different forms of GDF9/BMP15 under study. After 22 hours of IVM 20 COCs from each treatment group were denuded and fertilized with washed porcine sperm. Cleavage rates were determined after 2 days of culture and blastocyst rates were evaluated at day 7.
Results: It was found that when the appropriate Ser-Cys mutants of human GDF9 and BMP15 were co-expressed it was possible to purify a covalently stabilized heterodimer of the GDF9/BMP15 mature regions which exhibited potent bioactivity on granulosa cells in monolayer culture. The heterodimeric form of GDF9/BMP15, which has been named Cumulin, activates both SMAD signalling pathways, namely the TGF-β/activin and the BMP pathways. The characteristic GDF9/BMP15 synergism was observable in a high level of activation of the TGF-β/activin pathway. Interestingly, even though the isolated mature region of Cumulin was the most potent form on cells in monolayer culture, it was not active on porcine cumulus-oocyte complexes (COCs). However, Cumulin as an isolated pro/mature complex was active on COCs, causing a greater than 2 fold increase in oocyte developmental competence.
Conclusions: The results show that the mechanistic basis of GDF9/

BMP15 synergism is the formation of Cumulin, a heterodimer of these two oocyte-secreted factors. Further, the results support a model of activation for human GDF9 dependent on Cumulin formation. Oocyte-secreted Cumulin is likely to be a central regulator of fertility.

I-16: Ovarian Culture Systems: Focused on 2 and 3 Dimensional Culture systems

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I-17: Optimization of Domestic Animal Sperm Freezing Using Novel Plant-Origin Cryopreservation Media

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Background: Cryopreservation of sperm has allowed to conservation of genetic resources in cryobanks and guarantee of constant commercially of sperm supply for animal breeding program using artificial insemination. We performed several projects for optimization of sperm freezing in bull, ram, goat and rooster. Phosphatydil choline originated from soybean (lecithin) has been assessed in different protocols for substitution of egg yolk in animal freezing media. Demands for replacement of egg yolk in extenders have been increased in recent years due to this concerns that egg yolk contains substances that impede respiration of sperm which may lead to decrease their motility. Moreover, egg yolk increases the risk of microbial contamination that may increase the risk of disease transmission through the transportation of egg yolk-based extenders in the international exchange of stored semen. After replacement of egg yolk by soybean lecithin in extenders, various experiments for consideration of frozen-thawed sperm quality such as microscopic, cellular, biochemical, flow cytometric and epigenetic aspects were applied to evaluate the cryoprotective effects of lecithin.
Material and Methods: Semen were collected from animal in each project (ram, goat, bull, rooster) and then each sperm sample was divided into different groups (according to experimental design in each project) for evaluation of potential effects of different concentrations of lecithin compare to traditional cryoprotectants. Moreover, various antioxidant and additives were assessed along with soybean lecithin. After freeze-thaw of sperm, various parameters such as motion characteristics, viability, membrane integrity, apoptosis, mitochondria activity, fertility potential and pregnancy rate were applied to evaluation the effects of different cryoprotectants for preserving the sperm quality and fertility after cryopreservation.
Results: Overall results in our projects show that for ram and bull sperm freezing, the best results for quality of post-thawed sperm were obtained in extender with 1% lecithin. Moreover, the lower rate of agglutination of sperm was observed in extenders containing lecithin compare to extenders containing egg yolk. For goat sperm freezing, the higher percentages of motility, viability, mitochondria activity and fertility were produced in the extenders with 1.5% lecithin. Also, in goat sperm, lecithin reduced the acrosome damages compare to egg yolk. For rooster, 0.5 % lecithin was enough to produce the highest quality of post-thawed sperm compare to egg yolk. This discrepancy about the optimum levels of lecithin in different species, is related to different capacity of seminal plasma and different size of sperm in ram, bull, goat and rooster. Among different additives for reinforcement of extenders containing soybean lecithin, trehalose and cysteine (ram), and L-carnitine (rooster) had the best reciprocal effects with lecithin.
Conclusion: Results of our project show that substitution of egg yolk with lecithin has beneficial effects for sperm during freezing. Higher results in fertility potential of frozen-thawed sperm in extenders with lecithin, encourages us to develop a commercial extender based on lecithin for future.
Keyword: Sperm Freezing, Lecithin, Mitochondria, Fertility

I-18: Ovary Freezing (Vitrification) and Transplantation to Preserve Fertility

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In over 22 cases of ovary tissue transplantation, results with fresh were no different than with frozen. All patients recovered ovulatory cycling, and 75 per cent became pregnant. There are now 19 healthy babies in our center's experience, and over 100 around the world. Slow freeze protocol is successful, but vitrification is preferable, as we shall explain. There is a massive over recruitment of primordial follicles after the transplant, due to reduced cortical tissue pressure. This principle can be used to coax eggs out of ovaries of women who have already undergone premature ovarian failure.

I-19: Ovarian Minimal Stimulation and Natural Cycle Egg Quality

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It has been shown in a big data analysis of 650,000 SART cycles that the live baby rate is inversely proportional to the amount of gonadotropin used. This is true even after adjusting for ovarian reserve, age, and prognostic category. Yet IVF clinics still keep overdosing patients during ovarian stimulation, thereby ironically diminishing their pregnancy rates. Our multi variable regression analysis shows that minimal stimulation or natural cycle will give a 5 times higher pregnancy rate per egg than hyperstimulation, and at least an equivalent pregnancy rate per transfer and per cycle. In mice it has been demonstrated that hyperstimulation has its negative effect by inducing epigenetic perturbations in the embryo.

I-20: The Y Chromosome

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The Y chromosome contains 60 multicopy genes composed of nine different gene families concentrated in regions of multiple repeat sequences called amplicons arranged to mirror images called palindromes. This pattern is susceptible to deletions caused by homologous recombination with itself, and can explain the presence of small

numbers of sperm in otherwise azoospermic men.

I-21: Developing A Variety of Culture Systems for The Mammalian Ovary: from Oogonia to Ovulated Oocyte

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Tissue culture can be a powerful method for examining tissue growth and development in a highly controlled environment. Culture has proved to be particularly effective for the ovary, where a variety of in vitro methods have been shown able to support ovarian follicle development in a highly physiological manner, with the mouse the most widely used and successful experimental model to date.
The majority of ovarian tissue culture methods use ovarian follicles, from the primordial stage through to fully mature, fertilizable oocytes contained within Graafian follicles. Whole ovary culture allows for primordial follicle growth initiation and development through preantral stages, while fertilisable oocytes can be obtained from individually cultured ovarian follicles or oocyte-granulosa cell complexes. The two techniques can be combined, allowing fertilisable oocytes to be obtained from the oocytes within primordial follicles.
Successful mammalian reproduction requires regulated development of a species-specific number of ovarian follicles to the Graafian stage each menstrual/oestrous cycle. This process of follicle dominance is achieved by the continual development of a much larger number of follicles, most of which then undergo follicular atresia and die. We have used tissue culture techniques to examine interactions between follicles as they grow and develop, determining how these interactions can affect both follicular growth initiation and follicle dominance.
Finally, tissue culture techniques spanning earlier stages of female germ cell development prior to follicle formation have proven more challenging. Our recent work has developed a method that supports female germ cell entry into meiosis, followed by nest breakdown, follicle formation and follicle growth initiation, allowing investigation of a particularly poorly understood area of ovarian biology.

I-22: Use of Mouse Gonadal Tissue Culture for Laboratory Investigations into The Impact of Chemotherapy Treatment

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Cancer treatment with chemotherapy drugs is often gonadotoxic, potentially causing life-long infertility. With the continued increase in long-term survival rates, resulting fertility problems have become of greater concern. This is particularly a problem for survivors of childhood cancer, who can be rendered infertile by treatment before they even reach puberty. While epidemiological studies have allowed many chemotherapy drugs to be grouped into low, moderate or high risk categories for gonadotoxicity, beyond this we know very little about exactly how the drugs damage the gonads, knowledge vital for the informed development of protective strategies.
We are using a range of tissue culture techniques, culturing both ovary and testis, to determine the specific effects of a variety of different chemotherapy agents. Work has examined the cell type affected; the developmental stage of the germ cells most at risk; and the time course of cell death. With few fertility preservation options available, especially for childhood cancer patients, the ideal solution will be if the gonads can be protected from the damaging effect of the chemotherapy drugs: current work is examining the potential of tyrosine kinase inhibitors to protect against cisplatin-induced damage.

Ethics and Reproductive Healths

I-23: Nutrition-Reproduction Crosstalk in Breeder Roosters

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The bulk of studies had shed light on the pivotal role of nutrition in reproduction. Accordingly, several approaches have been introduced in efforts to improve semen quality and the antioxidative capacity of seminal plasma. A major concern in the poultry industry is the production of higher numbers of embryonated broiler hatching eggs to which the contribution of male to overall flock fertility is more than that of female birds, essentially due to the typically lower number of males. Among a variety of factors affecting the reproductive performance in breeder roosters, the nutritional status, especially energy/protein intake, antioxidant capacity, and specific additives have been taken into consideration by many researchers. The paper deals with the significant interaction effects of nutrition and reproduction and introduces nutritional approaches, including dietary antioxidants to improve semen quality, sperm functionality, and reproductive success in the male breeders.
Keywords: Antioxidant, Breeding, Nutrition, Poultry, Semen

I-24: Supplementation of Holstein Cows Diet with Omega-3 Fatty Acids: Effects on Reproduction and Performance

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Beneficial effects of optimizing omega-3 fatty acids intake in overall health, growth and immunity have been documented in human and animals. In high yielding milking cows, these compounds have been suggested to improve pregnancy rate and prevent early embryonic losses. To add to the current data in this area, 140 multiparous Holstein cows were randomly divided into 2 groups immediately after calving and received either Ca-salt of fish meal (containing 16-20% DHA and EPA) or equal amount of palmitic acid (control group). Supplementation rate was 240 g/ animal/ day from calving to 21 days after calving and 120 g/ animal /day from 21 to 150 days after calving. Milk and blood samples were taken on day 90 of the trial from 10 cows that randomly selected from each group and analysed for fatty acid composition. Milk production recorded bi-weekly and reproductive parameters were recorded based on a time schedule. Omega-3 fatty acid supplementation significantly decreased interval from calving to subsequent pregnancy (88 vs. 98.5 days) and increased proportion of cows confirmed as pregnant until 120 days in milk (0.46 vs. 0.37) compared to palmitic acid supplementation ($P < 0.05$). Other

parameters including interval from calving to first artificial insemination, conception rate, milk production and milk somatic cell count were improved numerically; the latter was indicative of improved immune status and health of mammary gland. Results supported the efficacy of feeding omega-3 fatty acids for improving Iranian dairy cow fertility.

Key words: Omega-3 Fatty Acid, Dairy Cattle, Reproduction

I-25: Practical Approaches to The Nutritional Management of Male Infertility

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Epidemiological studies have shed light on a link between sperm quality and lifestyle factors, including dietary habits. Spermatogenic failure, defined as one or more semen parameters falling below the World health Organization (WHO) cut-off for normozoospermia, is the most common form of male infertility. There is a growing interest in the worldwide for modification of lifestyle factors to treat male infertility. Nutritional status and dietary patterns, as major lifestyle factors, are crucial determinants of normal reproductive function.

Data on the association of dietary factors with sperm quality have been accumulating. The role of micronutrients has attracted the attention of researchers to the extent that some studies have investigated the role of micronutrients in the risk of male infertility. However, current understanding of the impact of nutrient intake on sperm quality is still limited because most studies to date have focused primarily on the intakes of vitamin E, selenium, vitamins C, zinc, folinic acid, docosahexaenoic acid, eicosapentaenoic acid, Coenzyme Q10 or a few isolated nutrients and have paid less attention to the contribution of overall nutrient intake to sperm quality. Our recent study shows that nutrient pattern comprising mainly antioxidants, vitamin D, fiber and polyunsaturated fatty acids showed an inverse association with asthenozoospermia. High intakes of fruits, vegetables, poultry, sea foods, skim milk and shellfish as well as low intake of full-fat dairy food intake, sweets and processed meat with especially high saturated fat foods were reported to have favorable association with sperm quality. Furthermore, higher intake of soy foods and soy isoflavones may be associated with lower sperm concentration and higher concentration of blood mercury might be associated with male infertility.

There is a complete lack of appropriate randomized controlled trials that could correlate changes in diet with improvements of male fertility. Hence, future clinical trials in this filed are needed.

I-26: Trans-Generational Effect of Nutritional Signals on Reproduction Status

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Several epidemiological and experimental evidence are now indicating that intrauterine environment, including nutrition, affect subsequent reproduction status that may span more than one generation through potential epigenetic changes.

Studies investigating populations exposed to famine or malnutrition have highlighted the need for adequate nutritional intake for successful reproductive capability. Steroidogenesis as well as folliculogenesis in the offspring ovary appears to be the major targets of nutritional programming typically influenced by protein availability. As primordial follicle pool is established early in life its vulnerability to embryonic condition is not surprising. Results of clinical and experimental studies also show early life adversity, depending to its severity, duration and developmental stage is associated with decline in ovarian follicular reserve, changes in ovulation rates, and onset of puberty. Interestingly, it is shown that nutrition status after birth does not further influence offspring reproductive tempo and reproductive condition is rather dominated by offspring's nutritional history during the prenatal and lactational period.

Early-life events induce highly integrated responses in endocrine-related homeostasis, as shaping GnRH neurosecretory system, resulting in persistent changes in the developmental trajectory producing an altered adult phenotype which can disturb healthy reproduction. For instance polycystic ovarian syndrome phenotype, is known to be modified by factors during prenatal life. Moreover, effects of relative overnutrition as well as the complex interactions between pre- and postnatal nutrition is of high importance, especially in the context of our day's obesity epidemic. Likewise, early malnutrition during different critical developmental time windows may also result in different long-lasting effects on pubertal development in male.

Understanding the impact of nutritional disruptors which may be commonly encountered by pregnant women is necessary for development of new nutritional approaches during pre- and postnatal periods to ensure reproductive health in later life.

Key words: Nutrition, Reproduction, Intrauterine Environment

I-27: Recruiting Egg Donors through *In Vitro* Fertilization (IVF) Clinics and Agencies: Adherence to Guidelines, and Views of IVF Providers

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Background: Many medical, ethical and social issues have arisen concerning egg donation. We thus aimed to examine whether and how *in vitro* fertilization (IVF) clinics and egg donor agencies comply with ethical guidelines, as indicated on their websites, regarding trait-based payment variation, presentation of risks, and minimum recruitment age; and how IVF providers view these issues.

Materials and Methods: We systematically examined 207 websites, of which 102 were egg donor agency or IVF clinic websites that both recruited online and displayed compensation amounts. We also conducted semi-structured in-depth interviews of 25 IVF providers.

Results: Of the 102 sites, considerable numbers were noncompliant with the American Society for Reproductive Medicine's (ASRM) guidelines that prohibit varying compensation based on a donor's traits (34%), and recommend an age of 21 years or older (41%), and presentation of risks alongside compensation (56%). Trait-based payment variation was associated with being an agency rather than a clinic, location in the West, not being endorsed by ASRM or Society of Assisted Reproductive Technology (SART), and referring to ASRM's guidelines about compensation. Of sites mentioning traits, prior donation success was the most commonly paid for trait (64%). Agencies were more likely than clinics to indicate compensation, offer a fee range, set their minimum > USD $5,000, specify preferable traits, cap provider age at ≤ 31, require an education minimum, and allow both parties to meet. IVF providers reported that they often use agencies, to have more supply of eggs, and/or to give patients more choice, but that agencies vary widely in quality – how well they screen, inform and prepare donors for the process. Agencies may not all effectively assess or record how many times donors provide oocytes.

Conclusions: These data, the first to systematically analyze agency and clinic websites, reveal that many do not follow ASRM's guide-

lines. IVF providers often struggle with issues concerning egg donor agencies. These data have critical implications for policy, practice, and research, suggesting needs for consideration of possible changes in guidelines, and/or improvements in compliance regarding ethical and other concerns.

I-28: Age Cut-Offs for Women Using Their Own vs. Donor Oocytes: Challenges Faced by Clinicians

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Background: To study how *in vitro* fertilization (IVF) providers view and make decisions concerning age cut-offs and futility for women using their own vs. donor oocytes – how providers weigh these issues, and whether they establish clear cut-offs, and if so, where.
Materials and Methods: In-depth interviews of approximately 1 hour each were conducted with 35 assisted reproductive technology providers and patients (15 physicians, 10 other health providers, and 10 patients), and systematically analyzed. Additional interviews were conducted with 10 providers and 20 patients.
Results: Providers face several challenges regarding what age cut off, if any, to use for women using their own vs. donors' eggs. Providers confront dilemmas about how rigidly to set age cut-offs, how much autonomy patients should have (especially since many older women may minimize or deny their low odds of success), exactly who should decide, how best to counsel patients, how and to what degree providers should "discourage" older women, what specifically providers should say to older women, whether and how to consider fathers' ages, and whether to accept patients' self-reported ages, which may not always be accurately reported. In making these decisions, clinicians frequently rely on their "gut feelings."
Conclusions: These data, the first to explore how providers make decisions about age cut-offs for women concerning the use of these patients' vs. donor eggs, raise several critical issues. While the American Society for Reproductive Medicine and other professional societies have addressed several concerns, these data highlight additional questions and challenges (e.g., how providers should weigh these other competing concerns, including autonomy vs. beneficence). These data have important implications for practice, policy, research and education.

I-29: Impact of Life-Style on Male and Female Fertility

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Femal Infertility

I-30:Late ART Outcomes

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Presently, the assisted reproductive technologies (ART), which involved the sciences of the manipulation of both oocytes and sperm in the laboratory, are used in the treatment of human infertility widely. Since the introduction of *in vitro* fertilization (IVF), after born of the first child (Louise Brown) by this procedure in 1978, as some research reported, over 5 million babies have been born all around the world by ART. Approximately, in each year more than 200,000 babies are born by ART around the world. During this time there have been rapid advances in ART., the effectiveness of IVF has progressed greatly after the innovation of embryo cryopreservation, and afterwards the more invasive 'technique' of intracytoplasmic sperm injection in 1992. The identification and removal of genetically abnormal embryos have been facilitated by the innovation and development of preimplantation genetic diagnosis method; however, this procedure is more invasive and involves significant manipulation of the embryo. The majority of available data showed that ART singleton pregnancies had a higher risk of poor pregnancy outcomes in compared to those with spontaneous conception. In a recent meta-analysis by Qin et al, fifty cohort studies comprising 161,370 ART and 2,280,241 spontaneously conceived singleton pregnancies were evaluated. The ART singleton pregnancies had a significantly increased risk of pregnancy-induced hypertension, gestational diabetes mellitus, placenta previa, placental abruption, antepartum hemorrhage, postpartum hemorrhage, polyhydramnios, oligohydramnios, cesarean sections, preterm birth, very preterm birth, low birth weight, very low birth weight, small for gestational age, perinatal mortality and congenital malformation. The children conceived through ART might be exposed to higher health risks than spontaneously conceived children; this may be due to the following factors: background biology of sub-fertile couples, multiple pregnancy, related factor to fertility treatment procedure and embryo – endometrium interface.
There is increasing evidence that considered the infertility or subfertility as an independent risk factor for obstetrical complications and poor perinatal outcomes, even without the applying of ART. Multiple pregnancy is the remarkable predictive factor for adverse maternal, perinatal and neonatal outcomes. Couples with good prognosis for success should be thoroughly aware about the significant risks of multiple pregnancies associated with all ART and counseled about the benefits and cumulative pregnancy rate of elective single embryo transfer (eSET) policy and encouraged to using this protocol. Although several million children have been born by using ART treatments, but limited documents are available regarding the longer-term health and development outcomes for these children. It has been supposed that ART procedure may cause to long term adverse consequences, in addition to the documented adverse perinatal outcome and increased risk of congenital abnormalities in the children resulting from ART treatment. These adverse outcomes included: respiratory and allergic disorder, endocrine disorders, ophthalmological and auditory disorders, growth and pubertal development, metabolic and cardiovascular effects and risk of cancer. Although there are considerable researches on these effects, but further studies with a long-term follow-up are required for a definitive statement or accurate conclusion.

I-31: The Role of Progesterone in The Management of Early Pregnancy Failure

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Adequate endometrial development induced by an orderly sequence of estrogen and progesterone production by the developing follicle prior to ovulation and by the corpus luteum thereafter. Ovulation

marks the transition from follicular phase to luteal phase, characterized by the formation of a corpus luteum which now secretes progesterone in addition to estradiol. The lifespan of the corpus luteum is 11 to 17 days (mean 14.2 days) from the time of ovulation to the onset of menses. Blastocysts produce HCG 7 to 8 days after fertilization and if the corpus luteum is not rescued by HCG production from the trophoblast of the implanting pregnancy, it will undergo luteolysis. The reverse is also true for the interdependence of these two entities in that a functioning corpus luteum is essential to early pregnancy survival. In the now classic human experiments of lutectomy in pregnant women, surgical removal of the corpus luteum before 7 weeks of gestation was associated with spontaneous miscarriage in all women and was preceded by a fall in progesterone levels. Supplementation of the pregnancy with progesterone after corpus luteum removal resulted in pregnancy survival, clearly demonstrating the dependence of the early pregnancy on progesterone production from a functioning corpus luteum

In addition to the endocrine support of pregnancy, progesterone has also been shown to exert a beneficial role via the immune system. It has been demonstrated that Th1 system dominance is associated with miscarriage and failed pregnancy. In contrast, successful pregnancies are dependent on the Th2 system becoming predominant. Progesterone exerts its beneficial role by combining with receptors on lymphoid cells that then produce progesterone-induced blocking factor (PIBF) with results in Th2 system domination. The use of Ru486, which blocks progesterone receptors, prevents PIBF production resulting in Th1 system dominance and miscarriage. A similar effect is seen with administration of antibodies to PIBF.

A negative correlation exists between the resistance index in the corpus luteum blood flow and progesterone levels. Thus, higher levels of progesterone are associated with lower resistance index indicating higher blood flow to the corpus luteum. The beneficial effect of progesterone on vascular tone was observed in experiments using 5HT to cause vascular constriction and increased placental vascular tone. This constriction effect was overcome in a dose-dependent fashion by the use of progesterone. Furthermore, the expression of nitric oxide synthetase activity was enhanced in human endothelial cells exposed to progesterone compared to control exposure. The resulting increase in nitric oxide production is associated with a vasodilatory effect thereby promoting blood flow to the uterus and improving pregnancy success. The therapeutic efficacy of progesterone was observed in early pregnancy when the pulsatility index in uterine and spiral arteries was reduced thereby increasing blood flow to the pregnancy and promoting successful pregnancies when sub-chorionic haemorrhage was present.

A systematic review with meta-analysis of progestational agents in women with recurrent miscarriage demonstrated improved efficacy with and absolute treatment effect of 22.9% in the ongoing pregnancy rate.

Progesterone support is necessary to ensure successful pregnancy outcome. The beneficial effect of progesterone takes place a multisystem manner that involves the integration of endocrine, immune and blood flow systems.

I-32: The Role of Metformin in Treating PCOS Associated Infertility

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I-33: Applications and Safety of Infrared Lasers in IVF

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Lasers have been used for several years for assisted hatching and biopsy of embryos in IVF. Following initial trials of numerous wavelengths, the advantages of the near infra-red laser wavelength range 1450 – 1480 nm became apparent. This beam is used in pulses up to 3 msec duration. Strong absorption of the beam in water causes rapid local heating near the beam focus. If the beam focal point is in the embryo zona pellucida, the region near the focal point is rapidly liquefied. This produces a thin region in the zona pellucida. It also weakens intercellular bonds and facilitates extraction of specimens for trophectoderm biopsy.

Since the beam produces highly superheated water during the brief laser pulse duration, safety considerations require that its effects must be localized. The focus must be remote from the embryo cells. In the present discussion the detailed temperature at and near the beam focus is predicted and verified, the size of the affected region is derived, and limits recommended while using the beam with embryos and oocytes. Use of the beam and its limits will be shown with zona pellucida and biopsy cutting.

In the above application the superheated water at the focal point does not vaporize since the pulse duration is too short. Phase change is avoided. However with a modified laser it is possible to cause sudden phase change, resulting in an explosive bubble of water vapor which strongly affects the target. In further applications on cell culture monolayers, the laser causes phase change microbursts, which can cut cell cultures or eliminate unwanted cell components from the culture. The strongly localized limit of beam effects will be described and demonstrated.

I-34: Genetic Aspects of OHSS

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I-35: Fertility Preservation in Cancer Patients: from Bench to Bedside Patients

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I-36: Ovarian Stimulation Protocols in PCO and POF Patients

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I-37: PCO: Non Insight in The Therappy

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I-38: POF: Is Egg Donation The Only Solution?

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I-39: Different Approaches in Management of Implantation Failures

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Repeated implantation failure (RIF) is future of pregnancy following several IVF treatment cycles. However there are no formal criteria defining the number of failed cycles or the total number of embryos transferred in these IVF attempts. But it is better to define RIF as failure of implantation in at least three consecutive IVF attempts in which 1-2 embryos of high grade quality are transferred in each cycles. Lack of implantation may be attributed in part to endometrium, embryo, suboptimal time for transfer of embryo (window of implantation). Many researches were done for detecting the problem. For reevaluation of endometrium, it is better to do Hysteroscopy for finding of unknown pathology in the cavity.
Endometrial injury is another way for increasing the endometrial receptivity. The underlying mechanism of how endometrial injury may improve endometrial receptivity remain unclear however several pathway have been hypothesis such as induces of decasualization, wound healing response, increase in the secretion of cytokines, interleukins, macrophages and all at which are beneficial for embryo implantation. One review by the Cochrane collaboration in 2015 showed the endometrial biopsy or injury in previous cycle of COH may improve the pregnancy rate .But Injury on the day of OPU have adverse effect and reduce the chance.
Sequential day 3 and day 5 transfer. This approach helps us for preventing of cancellation of cycle for Blastocyst transfer and may improve endometrial receptivity.
Blastocyst transfer is another option for better selection of embryos. Especially use time laps for better selection of embryos. PGD or PGS: Array CGH and next generation sequence and sequential comprehensive Chromosome analysis are another option for better selection of embryos and increase rate of pregnancy.
Endometrial Receptivity Array (ERA) has been developed which is capable of identifying the genomic signature receptivity. This diagnostic tool showed that the window of implantation (WOI) is displaced in the patients with RIF. All of these will discuss in the presentation.

I-39: Role of FSH in Metabolic Disturbances in Polycystic Ovary Syndrome

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Background: Granulosa cells play an important role in the development of ovarian follicles by creating a niche for the oocytes, and are responsible for providing several trophic and metabolic factors to the preovulatory oocyte to ensure its successful maturation and subsequent embryo development. Though follicle stimulating hormone (FSH) is a potent and essential hormone for the development and maturation of follicles, the metabolic pathways in preovulatory granulosa cells regulated by it and their implications in PCOS condition are not completely understood.
The main objective of our investigations was to explore the signaling proteins involved in cross-talk between FSH and Insulin/IGF1 signaling pathways and their implications on glucose metabolism in granulosa cells from PCOS women. Our aim was to examine the role of insulin receptor substrates (IRS-1 and IRS-2) in FSH signaling pathways in preovulatory granulosa cells from normal and PCOS women.
Material and Methods: In the ongoing study, 48 normal and 30 PCOS patients were enrolled. Human granulosa cells were isolated from ovarian aspirates for IVF. mRNA expression was studied by Real-time PCR. IRS-2, AKT and PI3-kinase knock-down in rat and human GCs were performed by respective siRNA transfections. IRS-2 promoter and ChIP assays were used to study the role of SP1 in IRS-2 transcription induction by FSH in rat GCs. The transcription factor binding sites (TFBS) in IRS-2 promoter were analyzed by Mat Inspector Genomatix software.
Results: The expression of both IRS-1 and IRS-2 in human and rat GCs were stimulated by FSH through different signaling pathways. FSH-stimulated IRS-2 expression was dependent on cAMP/PKA/SP1 pathway. FSH stimulated the nuclear translocation of SP1, binding to IRS-2 promoter and consequent activation of its transcriptional activity in a cAMP/PKA dependent manner. Knockdown of IRS-2 by siRNA decreased the FSH-stimulated PI3K activity, p-Akt levels, GLUT4 translocation and glucose uptake in preovulatory GCs. FSH-dependent increase in IRS-2 expression was found distinctly impaired in granulosa cells from PCOS women. PCOS patients had normal FSH-stimulated IRS-1 expression in granulosa cells.
Conclusion: FSH specifically increases IRS-2 expression in human and rat GCs. FSH induction of IRS-2 is crucial for the activation of PI3-Kinase, Akt, and glucose metabolism in preovulatory GCs. FSH-stimulated IRS-2 expression was defective in GCs from PCOS women and normal IRS-1 level was insufficient to preserve follicular function in PCOS women. Thus, IRS-2 emerged as a dominant player in PCOS condition. It emerges that molecular defect in this action of FSH in PCOS granulosa cells may cause deceleration of glucose metabolism and follicular growth leading to infertility in PCOS women. Collectively, these results support a therapeutic potential of IRS-2 in the management of infertility in PCOS women.*Funded by Department of Biotechnology, India

I-40: Molecular Defects in Polycystic Ovary Syndrome (PCOS) and Prediction of Infertility in PCOS Patients

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Background: Polycystic Ovary Syndrome (PCOS) is a heterogeneous, genetically complex, endocrine disorder of unknown aetiology in women, worldwide. The syndrome is characterized by a constellation of symptoms including ovulatory dysfunction, hyperandrogenism, and polycystic ovaries. Despite the increasing risk of developing diabetes mellitus and cardiovascular disease in later life of PCOS women, the ovary-specific alterations in gene expression in PCOS women with or without insulin resistance are not completely understood. Molecular abnormalities have been described in theca cells, oocytes and granulosa cells of women with PCOS, however a common molecular link in the development of PCOS is not yet configured.

The main objective was to compare the gene expression profiles in granulosa cells of PCOS women with or without insulin resistance. Further, aim was to find the common defects in oocytes, theca and granulosa cells of PCOS patients, and to evaluate the relation between PCOS susceptibility genes and conception rate in PCOS women.
Material and Methods: In the ongoing study, 20 normal and 45 PCOS patients were enrolled. Human granulosa cells were isolated from ovarian aspirates of normal and PCOS women undergoing IVF therapy. Gene expression was examined by whole genome Microarray (Affymetrix HG-U133 Plus 2), Real-time PCR. and Custom microarray (Agilent).
Results: The whole genome microarray analysis of gonadotropin-stimulated granulosa cells of PCOS patients with and without insulin resistance showed significant differences in the expression of diabetes mellitus, inflammation, and cardiovascular disease-related genes. The pair-wise comparisons highlighted the importance of the study of the two phenotypes of PCOS separately. The expression profiles of theca cells, oocytes and granulosa cells from PCOS women were compared and a common set of genes were identified. Taken together, the translational impact of these data was the development of a gene panel to predict infertility in PCOS women.
Conclusion: The expression profiles of stimulated granulosa cells from PCOS women with insulin resistance (PCOS-IR) and without insulin resistance (PCOS non-IR) indicated molecular differences in these two phenotypes of PCOS. It emerges that these genes may have role in follicular growth arrest and metabolic disorders in PCOS women. There were common defects in the three cell types of follicular compartment. Inherent defect in certain genes linked to metabolic pathways may lead to infertility in PCOS women and consequent poor rate of conception even after IVF therapy. Thus this study is very important and has clinical implications. *Funded by Department of Biotechnology, India

Genetics

I-41: The Impact of Aneuploidies on Implantation Potential

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Implantation failure is correlated with a wide spectrum of factors while role of aneuploidy as a piece of the puzzle is not well defined. Although genome integrity is necessary for healthy live birth, many other factors can prevent implantation and/or later development. Several publications confirm that 24-chromosome preimplantation genetic screening (PGS) increase implantation rate certainly in advanced maternal age as the well described factor for preimplantation, prenatal and postnatal aneuploidy. Comparison of aneuploidies prevalence in these stages indicates a decreasing pattern through development not only in pregnancy trimesters progress but also in cleavage toward blastocyst stage.
Embryos with complex aneuploidies certainly multiple chromosome losses are frequent in cleavage while they are rare in blastocyst and later stages. Looking at products of conception (POC) reveals that single chromosome trisomies are the most frequent, double trisomies have low frequency whilst autosomal monosomy and multiple trisomies are rare. Aneuploidy incidence in PGS and POC is dependent to type of chromosome with relatively similar pattern except some chromosomes. Implantation failure seems more related to postzygotic mitotic errors rather than meiotic ones. Frequency of mosaicism as a consequence of mitotic error reduces in blastocyst compared with cleavage stage but it is still prevalent.
Although chaotic embryos have little chance for implantation and postimplantation development, diploid-aneuploid mosaicism as the most prevalent preimplantation mosaicisim seems to have high developmental capacity in cases of more than 50% diploidy, according to some studies that report live birth probably due to clonal depletion of aneuploid cells following transfer of mosaic embryos; additionally, incidence of mosaicisim in PGS is much more than POC. Regarding high frequency of preimplantation mosaicism, low frequency of mosaicism in POC and mitotic origin of implantation failure rather than meiotic origin, it could be concluded that mosaic embryos with high level of diploid cells have more implantation potential while those with low level of diploid cells are more susceptible to implantation failure.
While many kinds of aneuploidies have implantation potential, 24-chromosome PGS approaches with ability of mitotic errors detection seem to help selection of embryos with higher compatibility for normal postimplantation development.

I-42: Mitochondrial DNA Level and Oocyte/ Embryo Quality

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I-43: Clinical Utilization of PGS in Recurrent Implantation Failure

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I-44: Beneficial Application of Molecular Cytogenetics in Delineation of Chromosomal Abnormalities Involved In Male Infertility: From Rare To Care

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Background: Chromosomal structural aberrations (deletions, duplications, translocations, inversions, and ring chromosomes) and aneuploidies (extra or missing chromosomes and marker chromosomes) are underlying causes of infertility. Traditionally, cytogenetic analyses of Giemsa-stained metaphase chromosomes are applied to ascertain these abnormalities. However, routine karyotype analysis is not sensitive enough to detect subtle chromosome rearrangements (less than 4 Mb). Identification of submicroscopic aberrations and more detailed molecular profiling of the rearrangements require precise mapping of the breakpoints with methods such as FISH or array CGH (aCGH). In addition, aCGH detects genomic duplications that cannot be identi-

fied by metaphase or even interphase FISH analyses. Besides, it is a technique that was developed for high resolution, genome-wide screening of segmental genomic copy number variations. It allowed for a higher rate of detection of chromosomal anomalies that is especially valuable in cases in which karyotype results cannot be obtained.

Material and Methods: In this project, we report different applications of molecular cytogenetics techniques in order to precisely detect the numerical and structural chromosomal abnormalities, which conventional cytogenetics was unable to perform a conclusive result, in infertile individuals. Some of mentioned chromosomal abnormalities which were detected and confirmed by molecular cytogenetics are 1)Y chromosome isodicentric(Idics) that are associated with male non-obstructive infertility and always occurs as a mosaic with a 45,X cell line and might be misdiagnosed with Y chromosome inversions, 2) complex chromosomal rearrangements (CCRs) in which detection of involved chromosomes and breakpoints are challenging, 3) ring chromosomes as a very rare condition with unknown size deleted segments and 4) mosaic cases in which exact definition of the cytogenetic status as mosaic or non-mosaic and also the number and pattern of cell lines are important for further clinical procedures. In all these cases FISH and aCGH were useful techniques for exact abnormality detection.

Results: FISH was an efficient method for detecting chromosomal abnormalities, which was performed on different kinds of cells. FISH, as a useful tool for an accurate diagnosis and characterization of chromosomal sub-regions, allowed exploring chromosome rearrangements in greater details with chromosome-specific DNA probes in our cases with Idics and CCRs. Moreover, it helped conventional cytogenetics to detects low-percentage mosaicism in a case with mosaic form of Klinefelter's Syndromes and also established the number of chromosomes in each cell line. aCGH could also detect the size of deleted segment in CCRs and ring chromosomes.

Conclusion: By combining high resolution techniques of FISH with aCGH, we have an essential tool to determine whether a complex abnormal karyotype is apparent or not, which is especially important for PGDs and PNDs in affected infertile cases.

Keyword: FISH, Array CGH, Molecular Cytogenetics, PGD, Male Infertility

I-45: Genetic Screening of POFs

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Menopause normally occurs in women during their late 40s or early 50s. However, in almost 1% of cases, it occurs before age 40 years called "premature ovarian failure" (POF). It is a common cause of infertility in women that characterized by primary or secondary amenorrhea, high gonadotropin levels and estrogen level declining in patients. It is known as an enigmatic and heterogeneous disorder, with poorly understood etiology. Presence of anti-ovarian antibodies, chromosomal, enzymatic and iatrogenic anomalies, viral infections and radiations are some of the known causes of this disorder. According to reports, several genetic factors are considered to cause POF syndrome. Genetic etiology of POF can be divided into two categories: cytogenetics and molecular genetics defects. Cytogenetic and molecular genetic studies should be done in order to identify numerical and structural chromosomal abnormalities or gene defects. Chromosomal abnormalities have long been recognized as a frequent cause of POF with widely varying percentages in reported series. The abnormal karyotypes included sex reverse SRY negative, X chromosome mosaicism, abnormal X chromosomes, abnormal autosomes and X-autosome translocation. Genes on the X-chromosome and autosomal genes are two groups of genes involved in these disorders. FMR1 gene that is on X-chromosome is the most important gene related to POF. According to studies, many genes are involved in the development of POF. Some of the genes responsible for POF are often viewed as overview of the genes studied in human or other animals with POF such as: BMP15, FMR2, LHR, FSHR, INHA, FS1, FOXL2, FOXO3a, ER, LIN28A, PGRMC1, POF1, HSD17B, TG, LAMC1, POU5F1, TGFBR3, FOXE1, FOXO4, CITED2, SALL4, CXCL12, PTHB1, Wnt4, BRSK1, HK3, ADAMTS19, NOBOX, FIGLA, KDR, BMPRIB, BMPRII, C1galt1, Mgat1, FGFR. It is clear that this is an area of great research potential. Understanding how ovaries fail may assist women with this disorder by facilitating the development of novel treatments or hormonal replacement therapies. Additionally, such information will provide important clues about optimizing ovarian function in individuals without POF, who are seeking for extension of their reproductive life spans or fertility enhancement by assisted reproductive technology (ART).

I-46: Hira-Mediated H3.3 Incorporation Is Required for DNA Replication and Ribosomal RNA Transcription in The Mouse Zygote

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Background: A successful fertilization event occurs when a sperm cell fuses with an oocyte to form a totipotent zygote and initiates embryogenesis. Sperm DNA is delivered to the oocyte at fertilization depleted of histones and highly packaged by protamines, and therefore needs to reacquire a nucleosomal organization to support development. Genome-wide chromatin reprogramming occurs at fertilization and is thought to center on the paternal genome, under the control of largely unknown maternal factors.

Material and Methods: We used a genetic approach to specifically delete Hira during oogenesis using Zp3-Cre and a conditional ("floxed") allele of Hira.

Results: We report that maternal Hira, a chaperone for the histone variant H3.3, is required for mouse development past the zygote stage. Male pronucleus formation is inhibited upon deletion of Hira due to a lack of nucleosome assembly in the sperm genome. Hira mutant oocytes are incapable of developing parthenogenetically, indicative of a role for Hira in the female genome. Both parental genomes show highly reduced levels of DNA replication and transcription in the mutants. It has long been thought that transcription is not required for zygote development. Surprisingly, we found that Hira/H3.3-dependent transcription of ribosomal RNA is required for first cleavage.

Conclusion: Our results demonstrate that Hira-mediated H3.3 incorporation is essential for parental genome reprogramming, and reveal an unexpected role for rRNA transcription in the mouse zygote. Most studies of reprogramming in the zygote have focused on the sperm genome, and understandably so given the dramatic global chromatin changes that it undergoes. We provide here functional data to support the notion that the female genome is not a mere passenger at this stage but instead undergoes dynamic chromatin reprogramming that is critical for zygote development. Our results also overturn an idea that has stood since the 70's that transcription in the zygote is both minor and irrelevant for development, and that Zygotic Gene Activation (ZGA) only becomes functional at the 2-cell stage, when there is a major burst in mRNA synthesis. We report a critical role for RNA Pol I transcription in the zygote, and show that this transcription is Hira-dependent. Therefore, functional ZGA can actually be considered to begin at the zygote stage, and the component of rRNA transcription is essential for progression to the 2-cell stage. The findings reported here may also be of relevance for human assisted reproduction technologies, because an abnormal 1PN

phenotype similar to that found in maternal Hira mutants is often observed in cases of ICSI that fail to develop past the zygote stage.
Keyword: Zygote, Epigenetic, Reprogramming, Transcription, RNA Polymerase I

I-47: Genetic Aspects of Aging in Female Reproduction

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The reproductive lifespan in women can be defined as the time between the onset of puberty and oocyte depletion at menopause. The first signs of puberty occur around 8–13 years of age. with an Age of Natural Menopause has a broader timing between the ages of 40 and 60 years old. In rare cases, these timings are disrupted with profound social, economic and clinical consequences. Natural fertility declines on average 10 years before menopause. Genome Wide Association and functional studies have identified dozens of highly penetrant mutations associated with reproductive disorders as well as and common DNA variants associated with the timing of puberty or menopause. These findings, have highlighted a diverse range of mechanisms involved in reproductive ageing, implicating core biological processes such as cell cycle regulation and energy homeostasis contributing to the molecular regulation of reproductive aging and disease risk.

I-48: Genetics of Age of Natural Menopause and Ovarian Failure

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The median age of natural menopause is around 51 years and is defined as the permanent cessation of ovulation. Early menopause might occur at 40 years of age while late menopause might happen as late as 62 years of age. Menopause timing has a substantial impact on infertility and risk of disease, including breast cancer. The underlying mechanisms are poorly understood. Menopause is a highly heritable condition and genetic variants may contribute to up to ~50% of the variation in age at menopause. In the last few years Genome Wide Association Studies (GWAS) have been performed on an increasing number of women and DNA variants and have contributed to the identification of a large number of loci and common variants associated with age at natural menopause (ANM). These studies have not yet revealed the entire genetic risk but are suggesting that reproductive performance, age at menopause, and longevity are interlinked through common genetic factors involved in DNA repair and maintenance. Moreover, enrichment of signals in or near genes involved in delayed puberty, highlight molecular links between the onset and end of reproductive lifespan.

Reproductive Imaging

I-49: Diagnosis of Intrauterine Adhesions by Means of Imaging Techniques

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Intrauterine adhesions (IUAs) are presence of fibrotic tissues within uterine cavity resulted from trauma to the basal layer of the endometrium, usually after curettage. However, any uterine surgery or endometrial injury (due to infection or inflammation) may lead to intrauterine adhesions. Adhesions range from minor filmy fibrotic scars without reproductive consequences to severe endometrial destruction which results in menstrual dysfunction and infertility. Therefore, accurate diagnosis and grading of the disease are significant issues, particularly among infertile women.
Several imaging modalities can be used for investigating suspected uterine adhesions containing 2D/3D sonography, 2D/3D sonohysterography (SHG), hysterosalpingography (HSG) and magnetic resonance imaging (MRI). In this lecture, the imaging findings and diagnostic clues for diagnosis and grading of intrauterine adhesions will be described. Lots of unique high-quality 2D/3D sonograms, 2D/3D hysterosonograms and hysterosalpingograms are illustrated in this presentation, using the archive of patients referred to imaging department of Royan institute, Tehran, Iran.
Key words: Intrauterine Adhesions, Sonography, Sonohysterography, Hysterosalpingography

I-50: MRI of Pelvic Floor

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Pelvic floor disorders consist of a spectrum of various pathologies and account for a variety of clinical presentations. Clinical complains include urinary and fecal incontinence, chronic pelvic pain, sexual dysfunction, constipation and genital prolapsed. They make a significant decrease in quality of life and involve up to 30% of middle age women .Basically the first method of investigation is manual exam preferably POPQ . However it is not enough as there is a high recurrence rate after pelvic floor surgeries. MRI with multiple sequences in static and dynamic series can play a major role in definition of multicompartment nature of most of PFDs and can provide a road map for Gynecologist for best surgical planning.

I-51: Pelvic Endometriomas in MRI, Complicated and Atypical Presentations: A Pictorial assay

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Endometriosis is an extra- uterine of endometrial tissues which lead local inflammatory reaction. More than 176 million women in childbearing age worldwide were estimated to have endometriosis. There is an association between endometriosis and infertility and it is seen in 25-30% infertile woman. Patients with endometriosis commonly have symptoms of heavy menstrual bleeding, congestive dysmenorrhea, fatigue, deep dyspareunia, chronic pelvic pain and dysuria.
MRI has provided a simple, feasible and non-invasive tool for diagnosis of clinically suspected patients. Furthermore, it is also useful for both long and short-term follow up in patients with definite diagnosis of endometriosis. MRI features are atypical due to wide variety of signs and location; however, it can be appeared with hemorrhagic regions with different intensity based on hemoglobin concentration and their age. Recent lesions are usually characterized by hyperintense

areas on T1-weighting images, while old lesions are usually hypointense on T1-weighting. Moreover, on T2-weighting images they are appeared with very different intensity but commonly are hypointense. Although MRI has great accuracy in endometriosis identification, some cases finally diagnosed with other pathologies. Owing to atypical imaging findings, endometriosis can be misdiagnosed even by expert radiologists. Hemorrhagic cyst, tumoral benign or malignant ovarian cysts and infiltrative pelvic pathologies are the most lesions that can be diagnosed as endometriosis and vice versa. Therefore, laparoscopic or microscopic examination has remained gold standard for the diagnosis of endometriosis. This pictorial assay aims to demonstrate some possible diagnosis of endometriosis on MRI which was inconsistent with histopathological examination.

I-52: Evaluation of Ovarian Activity Disorders by Transvaginal Sonography (Focused On PCO)

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During the first half of the cycle, small increases in FSH stimulate the ovary to develop a follicle that contains an egg (oocyte). The follicle produces rising levels of estrogen, which cause the lining of the uterus to thicken and the pituitary to release a very large amount of LH. This midcycle "surge" of LH causes the egg to be released from the ovary (called ovulation).
23% of women of reproductive age will have findings of polycystic ovaries . However, only 5%–10% of these women will have classic symptoms of PCOS such as infertility, amenorrhea, signs of hirsutism, or obesity.
Signs and symptoms of PCOS usually begin around the time of puberty (menarche), although some women do not develop symptoms until late adolescence or even into early adulthood. Women with PCOS usually have fewer than six to eight menstrual periods per year. Some women have normal cycles during puberty, which may become irregular if the woman becomes overweight.
In women with PCOS, multiple small follicles (small cysts) may develop in the ovary. Therefore, small follicles (4 to 9 mm in diameter) accumulate in the ovary, hence the term polycystic ovaries. None of these small follicles are capable of growing to a size that would trigger ovulation. TVS is preferred because it often provides optimal visualization of the internal structure of the ovary, particularly in obese patients. Appearance of polycystic ovaries may not be detected at TAS in up to 30% of women with PCOS.The imaging report should be specific and should include ovarian volumes and antral follicle counts, in addition to pertinent findings such as the presence of a dominant follicle or corpus luteum. Two out of three of the following to be diagnosed with PCOS:
-Irregular menstrual periods caused by anovulation or irregular ovulation.
-Evidence of elevated androgen levels. The evidence can be based upon signs (excess hair growth, acne, or male-pattern balding) or blood tests (high androgen levels).
-Polycystic ovaries on pelvic ultrasound.
Regularly menstruating women should undergo scanning during the early follicular phase (days 3–5). Oligo- or amenorrheic women may be scanned at random, or between days 3 and 5 after progesterone-induced bleeding. Sonographic criteria included:One or both ovaries demonstrate 12 or more follicles measuring 2–9 mm in diameter, or the ovarian volume exceeds 10 cc (NO presence of a dominant follicle). Only one ovary meeting either of these criteria is sufficient to establish the presence of polycystic ovaries.
The consensus group also cites the difficulty in distinguishing a polycystic ovary from what has traditionally been referred to as a multicystic or multifollicular ovary, defined as an ovary in which there are six or more follicles, usually 4–10 mm in diameter, with normal stromal echogenicity and described as the common appearance of ovaries in adolescents.
Evaluation of dominant follicle : In evaluation of dominat follicle, Follicular RI and PSV are more important in decision making than the size of the follicle. Increase in perifolliclular vascularity of dominant follicle in theca layer starts developing as early as 8th day of the cycle. Fall in perifollicular RI starts 2 days before ovulation, reaches nadir at ovulation, remains low for 4 days and then with gradual rise reaches 0.5 in mid luteal phase. Vascularity in 3/4th of the folliclar area with RI 0.4 – 0.48 and PSV >10 c.m/s is appropriate.

I-53: MRI of The Breast: Indications and Pitfalls

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Magnetic Resonance Imaging (MRI) is considered an accurate imaging technique which helps us to detect and characterize breast disease and lesions. It provides useful knowledge on following areas: 1) Detection of breast lesions or cancer in early stages of the disease, 2) assessment of the extent of the disease, 3) investigating tissue response to therapies during the treatment period, 4) providing guidance for biopsy and localization if needed, and 5) evaluation of foreign bodies such as breast prostheses.
Role of MRI in the screening of breast cancer has been first released by American Cancer Society (ACS) in 2003. According to their introduced guideline panel, women were categorized at different defined levels of risk. Screening MRI was recommended for women at %20 -25 higher risk of breast cancer.
As derived by European Society of Breast Cancer Specialists (EUSOMA), there are several other indications for performing breast MRI: “staging before treatment planning; screening of high-risk women; evaluation of response to chemotherapy; patients with breast augmentation or reconstruction; occult primary breast cancer; breast cancer recurrence; nipple discharge; characterization of equivocal findings at conventional imaging; inflammatory breast cancer and male breast”. In this presentation, indications, limitations and pitfalls of breast MRI and new guidelines for which will be described.
Key Words: Breast Cancer, MRI, Screening

I-54: Sonographic Screening Examination of The Fetal Heart

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Pelvic floor disorders consist of a spectrum of various pathologies and account for a variety of clinical presentations. Clinical complains include urinary and fecal incontinence, chronic pelvic pain, sexual dysfunction, constipation and genital prolapsed. They make a significant decrease in quality of life and involve up to 30% of middle age women .Basically the first method of investigation is manual exam preferably POPQ . However it is not enough as there is a high recurrence rate after pelvic floor surgeries. MRI with multiple sequences in static and dynamic series can play a major role in definition of multicompartment nature of most of PFDs and can provide a road map for Gynecologist for best surgical planning.

I-55: Endovaginal Sonography of Incompeten cervix

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Transvaginal sonography of the cervix has emerged as the best and safest method for evaluation of cervix during pregnancy . Cervical sonography allows measurements of cervical length which can aid clinicians in identifying women at risk for preterm birth. The use of transvaginal assessments of cervical length can assist in the triage of patients with possible preterm labor. Recent studies also support the use of cervical length measurements as a means of determining appropriate candidates for cerclage placement and progesterone supplementation to reduce the risk of premature birth, further highlighting the importance of this modality in modern obstetric management.

I-56: Male Infertility Imaging

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Infertility issue is still considered one of the most serious problems of affected couples. This problem exists in all communities.
Since 50% of the causes of infertility are directly or indirectly related to male factor or infertile spouse, it is necessary to detect the causes of infertility in men and to make at most endeavor to diagnose infertility factors. The viable treatment procedure is selected when the cause of infertility is well diagnosed.
One of the elements which play role in diagnosis of infertility is imaging technique.
Detection of causes of male infertility requires recruiting various diagnostic methods. For example, specific imaging method is used for congenital abnormalities, whereas conventional radiography is important to detect the causes of infertility. A simple X-ray radiography and radiography with contrast enhancement, gray-scale ultrasound and color Doppler ultrasonography, radioisotope scintigraphy, CT scan and MRI with and without contrast agent, used in the diagnosis of male infertility.
Interventional imaging techniques can even be used in treatment of the infertility.
Recruiting puncture approach for seminal vesicle cysts diagnosis through transrectal ultrasound-guide leads to treatment of obstructive azoospermia. Accordingly, another useful example concerning imaging method in the treatment of men infertility is embolization of dilated veins in high grade varicocele and treatment of varicocele with interventional methods.
Nowadays, there are ongoing research projects that may predict the fertility in male gonads by using imaging methods. In general, considering this point it is possible to follow the restoration of men fertility.
It is advised to use imaging techniques with minimum complications to detect the causes of infertility.
It is hoped that a system of imaging is used to optimize the rate of success in treatment and minimize the relevant costs with high diagnostic sensitivity and specificity.

Oral Presentations

Andrology

O-1: A Prediction Model for Successful Sperm Retrieval in Non-Obstructive Azoospermia Patients Previously Submitted to Testicular Biopsy

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Background: Predictive models (PredMod) of sperm retrieval (SR) in Non-Obstructive Azoospermia (NOA) patients, based on preoperative clinical parameters have obtained only a slight diagnostic accuracy (60,8%; Ramasamy 2013). We sought to evaluate whether including testis histology in a PredMod evaluating also FSH level and testicular volume (orchidometry) would increase its diagnostic accuracy.
Materials and Methods: 356 NOA patients undergone conventional TESE (cTESE) were retrospectively evaluated, based on FSH level and orchidometry (ultrasonographically assessed), and testicular histology (performed by the same pathologist) on samples obtained during cTESE. Binary logistic regression was used to evaluate the diagnostic accuracy of a PredMod built with FSH level, orchidometry and histology, identifying SSR as binary dependent variable.
Results: The mean patients' age was 36.8 years (18-63 yrs). Testicular sperm were retrieved in 158 out of 356 patients (44, 3%). Histological diagnosis was: Sertoli Cell Only Syndrome (SCOS) in 216 patients (60.6%), Maturation Arrest (MA) in 55 (15.4%), Hypospermatogenesis (HYPO) in 85 (23.8%). The binary logistic regression model was statistically significant (x^2 = 96.792, P<0.0001), and correctly classified 72.8% of cases (diagnostic accuracy) with 46.8% sensitivity (95% CI 38.86-54.931), 93.4 % specificity (95% CI 89.03-96.4), PPV 85.06 %, NPV 68.7 %, +Likelihood ratio (LR) 7.13 (95% CI 4.11-12.38), - LR 0.57 (95% CI 0.49-0.66). Only histology was significant to the PredMod, while FSH and orchidometry were not. SR rate was 88.2% in HYPO vs 30.5% (SCO) and 30.9% (MA) respectively (P<0.0001).
Conclusion: NOA patients with known previous histology (because of testis biopsy or failed TESE), in case of histological diagnosis of HYPO would have good chances (88%) of a positive SR by another cTESE attempt, while SCO or MA patients have poor chances (around 30%) of SR with cTESE, and should be addressed to Micro-TESE in order to improve their SR chances.
Keywords: TESE, Sperm Retrieval, Non-obstructive Azoospermia (NOA), Testicular Biopsy

O-2: The Prevalence of Chlamydia Trachomatis Infection in Semen Samples of both Symptomatic and Asymptomatic Infertile Men Referring to Royan Institute, by Using Serological and Molecular Methods

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Background: Chlamydia trachomatis (CT) with damaging effects on sperm quality parameters can often cause infertility in men. The main objective of this study was to determine the prevalence of CT infection in semen samples of both symptomatic and asymptomatic infertile men referring to Royan Institute, by using serological and molecular diagnostic methods.
Materials and Methods: In this case-control study, 465 patients presenting to clinical laboratory of Royan Institute were randomly selected for primary screening and detection of the presence of CT. Among which 93 samples were normozoospermia (Asymptomatic) and other 372 had abnormal parameters (Symptomatic) in semen analysis. ELISA test was conducted as the screening test to detect the presence of anti-CT IgA in patients' seminal plasma. 62 samples (32 symptomatic and 30 asymptomatic) with higher results in ELISA were selected as the case group and 34 asymptomatic samples with negative results randomly were selected as the control group for confirmatory test. The sperm DNA was extracted in order to confirm the presence of CT. PCR method was applied to confirm the serological results by using specific primers for amplification of CT genome.
Results: 62 out of 465 samples had OD>0.200 in ELISA screening test and were selected as the case groups for molecular assay. 34 asymptomatic samples with OD<0.200 in ELISA test were selected as the control group for PCR, as well. In the case groups, 4 out of 32 symptomatic samples (12.5%), and 1 out of 30 asymptomatic samples (3.3%) showed positive in PCR. No PCR positive sample was observed in control group. Furthermore, the comparison of two symptomatic and asymptomatic groups revealed that there was no significant difference between the age (P=0.253) and the semen volume (P=0.447) of the patients. The final results showed that the prevalence of CT in groups with Iranian nationality of symptomatic and asymptomatic infertile patients were equal (1.075%).
Conclusion: CT will lead to pelvic inflammatory disease (PID) and infertility unless it is diagnosed and treated. Screening of infertile men who do not show any clinical symptoms seems inevitable and can be considered as a part of the program of sexually transmitted disease (STD) control. It is concluded that the Anti-CT IgA ELISA test could be introduced as a suitable tool for screening purpose in seminal plasma of infertile men.
Keywords: Chlamydia Trachomatis, Infertile Men, ELISA, PCR

O-3: New Aspect for Varicocele; Correlation with Zn and Fe Induced Cytotoxicity and Biochemical Changes in Testis

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Background: Current study was performed in order to investigate zinc (Zn) and iron (Fe) cytotoxicity in experimentally varicocelized testis and to analyse the relation between heavy metals toxicity and lipid peroxidation with sperm DNA damage, nitrosative and carbonyl stresses, as well.
Materials and Methods: Twenty four mature male Wistar rats were divided into control-sham and test groups. Experimental varicocele (VCL) was induced in all test group animals. Non-VCL-induced rats

were considered as control-sham. The test group were subdivided into three groups based on sample collecting date (2, 6 and 8 months after VCL induction). Zn and Fe distribution in testicles, DNA ladder for sperms DNA fragmentation, testicular total antioxidant capacity (TAC), malondialdehyde (MDA), nitrite oxide (NO) and carbonyl groups (CG) were analysed.
Results: The VCL increased Zn and Fe distribution/accumulation in testicles. The VCL, reduced sperm count, motility and enhanced sperm DNA damage, time dependently (P<0.05). Moreover, the VCL down-regulated the testicular TAC and enhanced the MDA, NO and CG contents.
Conclusion: Our data showed that, impaired blood drainage in varicoceles, increased temperature of the testicles and possible reduction in Zn-regulating proteins expression result in intensive Fe and Zn accumulation in testicular tissue. As known outcome for these ions overload, the DNA damage and lipid peroxidation as well as Sertoli cells barrier breakage occur in VCL-induced animals that enhances the cellular damage. Then, the initiated oxidative stress triggers the previously induced NO oxidation to peroxynitrate. The Produced peroxynitrate, CGs and ROS, in turn, provoke the VCL-induced preliminary damages.
Keywords: Varicocele, Zinc, Iron, Oxidative Stress, Nitrosative Stress

O-4: Comparison of Septin 14 Protein Expression in Normospermic and Teratospermic Patients

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Background: Septins belong to a family of GTP binding proteins that are recognized as novel components of cytoskeleton. In mammals, 14 septin genes have been identified so far. Disruption in the functions of septin has been implicated in the pathology of several diseases, including male infertility. Here, we aimed to study, one of the new members of the septin family, called septin14 which is specially expressed in testis. The objective of the study was to assess the amount of expression of this gene at protein level and its localization in sperms of men with normal sperm morphology and those who have defects in their sperm morphology.
Materials and Methods: The localization of septin 14 protein was studied by Immunocytochemistry by a specific antibody. The expression of this protein was also assessed and compared with Immunocytochemistry technique between patients with normal sperm and those with sperm morphological abnormalities referred to the Royan institute.
Results: The protein expression was detected in normal sperm from head to tail which was highly localized in front of the acrosome and the neck. In teratospermia, however, expression level of septin14 is much less than normospermia. Interestingly, in some of them no evidence of septin 14 expression was seen.
Conclusion: As there was lower amount of septin 14 in teratospermia and these sperms have abnormal morphologies, we can assume that there may be an association between sperm morphology and septin 14. These are preliminary data and should be confirmed with more technical investigations.
Keywords: Septin 14, Immunocytochemistry, Sperm, Male Infertility

O-5: Resveratrol Protects The Testis in Bisphenol A-treated Rats; A Stereological Study

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Background: Bisphenol A (BPA) can endanger reproductive organ including testis. Resveratrol (RES) as a cell protectant component is the key element of grape seeds extraction. The objective was to evaluate the possible protective effect of RES on the BPA -treated rats on the testis structure and function.
Materials and Methods: Sprague-Dawley rats were assigned to seven groups: control, RES (100mg/kg/day dissolved in 1mL of carboxymethyl cellulose), carboxymethyl cellulose, low Bisphenol A (LBPA) (50mg/kg/day dissolved in 0.5mL of olive oil), hight Bisphenol A (HBPA) (100mg/kg/day dissolved in 0.5mL of olive oil), LBPA +RES, HBPA +RES and olive oil. All of the animals were sacrificed after 54 days. Testosterone serum level, semen parameters and testis of stereological structure were evaluated.
Results: Significant abnormalities from the normal range were occurred in testosterone serum level, semen parameters, LBPA and HBPA groups compared to the control rats (P<0.01).
Conclusion: The bisphenol A can alter testicular structure and function. Resveratrol can protect the testis in the Bisphenol A -treated rats.
Keywords: Bisphenol A, Histochemistry,Resveratrol, Testis, Stereology

Animal Biotechnilogy

O-6: Changes in DNA Methylation during *In Vitro* Maturation of Ovine Oocyte Affected by Aging and Melatonin

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Background: Oocyte quality is one of the most relevant factors determining the success of fertilization, which can be affected adversely by post ovulatory aging. Melatonin as a free radical scavenger has been used to enhance developmental competence of oocyte. However, the effect of melatonin on oocyte aging and epigenetic modification has not been fully established. Therefore, this study aimed to evaluate the effect of melatonin and maturation period time on embryo development and epigenetic modification.
Materials and Methods: Ovine cumulus-oocyte complexes (COC) were placed in maturation medium supplemented with 10-6 M melatonin for 24 h and 30-32 h in aged group. Oocytes in control group were incubated in the same condition without melatonin. At least 30 matured oocytes were immunochemically stained to assess DNA methylation and the related fluorescence intensity was analyzed by Image-J software.In order to evaluate the embryonic development, oocytes from IVM-10-6 (n = 127), IVM-Age (n = 121), IVM-Age10-6 (n = 153) and control (n = 115) groups were subjected to parthenogenetic activation and cultured in CR1aa medium for 8 day.
Results: Immunochemical staining revealed that methylation level was higher in oocytes in the IVMage group (24.5) compared to both control (19.8) and IVM-10-6 (16.9) groups. Moreover, significant difference was observed between IVM-Age10-6 (22.6) and IVM-10-6

(16.9). The cleavage rate was not different among all groups; while the blastocyst rate was higher in IVM-Age10-6 (51.4%) group in comparison to the control group (37.1%).
Conclusion: The results of this study demonstrated that supplementation of IVM media with melatonin promoted embryonic development in age group and stimulate global DNA methylation.
Keywords: Melatonin, Ovine Oocyte, Developmental Competence, DNA Methylation

O-7: The role of Epigenetic Modifiers on *In Vitro* Development of Buffalo-Bovine Interspecies Cloned Embryo

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The low efficiency of interspecies somatic cell nuclear transfer (iSCNT) is mostly related to abnormal epigenetic reprogramming. Epigenetic modifiers has been widely used in many species to improve the efficiency of SCNT and iSCNT experiments. Therefore in this study we have tried to investigate the effect of two epigenetic modifiers, Zebularine (DNA methyltransferase inhibitor) and BIX-01294 (histone methyltransferase inhibitor) on *in vitro* development of buffalo-bovine interspecies cloned embryos. The results of MTS assay for the buffalo's fibroblast showed the nontoxic dose for the Zebularine and BIX-01294 started from 40µM and 3µM consequently and the lowest doses, while the flow cytometry results revealed that the effective dose for Zebularine was 20µM which led to a significant decreasing ($P<0.05$) in 5-methylcytocine while the 2µM was the significant dose ($P<0.05$) for decreasing H3K9 di-methylation (H3K9me2). The results of treating the reconstructed embryos with these drugs after oocyte activation for 16 hour showed a significant decreasing in the intensity of 5-methylcytocine in the Zebularine group and H3K9me2 for the group of BIX-01294. Finally, the treatment of reconstructed oocytes with Zebularine and BIX-01294 didn't significantly affect the cleavage rate and blastocyst production ($P>0.05$). These results indicate the ability of Zebularine and BIX-01294 to improve the epigenetic reprogramming of buffalo-bovine iSCNT embryos for a proper epigenetic reprogramming for a competent *in vitro* and *in vivo* development.
Keywords: Zebularine, BIX-01294, Epigenetic Reprogramming, iSCNT, Buffalo, Bovine

O-8: New Aspect for Varicocele; Correlation with Zn and Fe Induced Cytotoxicity and Biochemical Changes in Testis

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Conclusion: Our data showed that, impaired blood drainage in varicoceles, increased temperature of the testicles and possible reduction in Zn-regulating proteins expression result in intensive Fe and Zn accumulation in testicular tissue. As known outcome for these ions overload, the DNA damage and lipid peroxidation as well as Sertoli cells barrier breakage occur in VCL-induced animals that enhances the cellular damage. Then, the initiated oxidative stress triggers the previously induced NO oxidation to peroxynitrate. The Produced peroxynitrate, CGs and ROS, in turn, provoke the VCL-induced preliminary damages.
Keywords: Varicocele, Zinc, Iron, Oxidative Stress, Nitrosative Stress

O-9: The Effect of Vitamin E and Selenium Nanoparticles on Post-Thaw Quality of Rooster Semen

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Background: Sperm freezing is an important technique which is widely used for long term storage of sperm. However, it induces partially irreversible damages to sperm. Accordingly, antioxidant addition to semen extenders has been implicated for cryopreservation of sperm to improve the sperm motility and viability during the freeze–thaw process. A series of experiments have demonstrated that the vitamin E (VitE) can be improve the post-thawed sperm functions in various animal species. Numerous studies have been performed to evaluate the effect of *in vivo* selenium administration on semen characteristics. But the effect of Nno-selenium (Nano-Se) on semen quality during the *in vitro* storage and post-thawing process is not well documented.
Materials and Methods: Semen samples were collected from 12 White Leghorn rooster and pooled, divided into nine equal groups. Extenders were supplemented with either 2 levels of VitE (5 and 10 µg/mL) or 2 levels of Nano-Se (1% and 2%) or combination of both VitE and Nano-Se, and comparisons in response were made with the control group (no antioxidants)after freeze-thawing process. Motility and motion parameters of sperm were estimated by computer-assisted sperm motility analysis (CASA, VideoTesT-Sperm 3.1, St. Petersburg, Russia). Also, viability was assessed by means of the eosin–nigrosin stain method
Results: Using 5 µg/mL VitE and 1% of Nano-Se improved ($P<0.05$) total motility (79.28 ± 3.86 %), progressive motility (18.03 ± 1.02 %) and viability (81.46 ± 2.16 %) of the sperm membrane (77.21 ± 2.12 %) after the freeze–thawing process. Total abnormal morphology of

sperm was decreased (P<0.05) by addition 5 or 10 µg/mL VitE alone or in combined with 1 or 2% Nano-Se.
Conclusion: The combination of low doses of VitE and Nano-Se in rooster extender was more effective on sperm biochemichal parameters compared to VitE or Nano-Se supplementation alone.
Keywords: Progressive Motility, Vitamin E, Nano Selenium, Rooster Semen, Cryopreservation

Embryology

O-10: Sperm Production following Spermatogonial Stem Cells Transplantation in Testicular-Torsion-Detorsion Mice

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Background: Testicular ischemia is the main consequence of testicular torsion, in both clinical and experimental aspects. Preservation and auto-transplantation of spermatogonial stem cells (SSCs) could be a new idea for treatment of infertility in testicular ischemia following testicular torsion.
Materials and Methods: To apply the idea in this study, animals were randomly divided into four groups of control, sham, with torsion, and with torsion followed by transplantation (TT). Isolated SSCs from neonatal mice were cultured and identified by flow cytometry (C-KIT-, INTEGRIN β1+) and RT-PCR for specific spermatogonial cell markers (Oct4, Gfrα-1, Plzf, Vasa, Itgα6 and Itgβ1). SSCs were transplanted upon a 2-hr testicular torsion in the TT group. Cultured cells transplanted into ischemia reperfusion testicle two weeks post-testicular torsion. Eight weeks after SSCs transplantation, the SSCs-transplanted testes and epididymides were removed for sperm analysis, weight, histopathological evaluation and pre and post meiotic gene expression assessment by qRT-PCR.
Results: Our findings indicated that all evaluated parameters (epididymal sperm profile, Johnsen Score, Plzf, Gfrα-1, Scp-1, Tekt-1 expressions and histopathological profile) were significantly decreased following testicular torsion (group 3) in comparison with control group (P≤0.05). However, all above-mentioned parameters showed a significant increase/improvement in torsion transplantation group compared to torsion group. However, these parameters in TT group were significantly lower in the sham and control groups (P≤0.05).
Conclusion: SSCs transplantation could up-regulate the expression of pre- and post-meiotic genes in testicular ischemia, which resulted in improvement of both testicular function and structure after testicular torsion.
Keywords: SSCS Transplantation, Pre and Post Meiotic Genes, Testicular Torsion

O-11: The Time Effect of Induced Mild Oxidative Stress before Freezing on The Human Sperm Performance

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Background: Stress preconditioning of human sperm for cryopreservation Can improve sperm function after freezing .The purpose of this study was to consider the effect of mild oxidative stress period on the frozen-thawed sperm Quality
Materials and Methods: Semen samples were collected from normozoospermic then they evaluated the primary assessment of ROS-TAC score and samples (n=24) with same score of ROS-TAC were selected for experiment. After processing sample with PureSperm each sample was divided into 5 aliquots : fresh group and according to the groups consisting of sperm exposed to NO during the times of 0, 30, 60 and 90 min before cryopreservation.The concentration of NO was applied as 0.01 µM according to previous study. Quality sperm was determined by evaluation of motility and progressive motility (CASA), sperm morphology (Papanicolaou staining) and apoptosis status (Anexin V /PI) after thawing
Results: Data were analyzed using SPSS and the values of P < 0.05 were considered to be statistically significant in comparison with the fresh spermatozoa, there was a significant decrease in all evaluated parameters in the cryopreserved spermatozoa (P<0.001). The higher significant percentage of total, progressive motility, average path velocity and velocity straight linear velocity of frozen-thawed sperm was observed in group with 60 min of pre-freezing sub lethal stress, Moreover, the percentage of of Anexin+/PI+ sperm significantly reduced in this time when compared to the other groups (P<0.01) Also, the percentage of dead sperm significantly increased in the time of 90 min. however, sublethal stress in the time of 0 and 30 min before freezing, had not significant difference in case of sperm quality indices. morphology of sperm was not affected by the oxidative stress treatment time
Conclusion: It seems that applying mild oxidative stress can induce biosynthesis stress-related proteins (HSPs). These proteins reduce activation of the apoptotic cascade and protect cells against oxidative stress.
Keywords: Sperm, Eryopreservation , Mild Oxidative Stress

O-12: Human Follicular Fluid Supplemented with Zinc and Copper Improved Mouse Embryo Development

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Background: Follicular fluid is a unique microenvironment surrounds the oocyte. The most abundant elements found in women follicular fluid are calcium, magnesium, followed by copper, zinc, iron, chromium and rubidium. The amount of trace elements such as zinc and copper in the environment is generally low, but these chemicals can interfere with physiological systems. Blastocyst hatching is a very important physiological phenomenon. Successful blastocyst hatching determines subsequent embryo survival and development. Thus, this study was designed to investigate the impact of human FF supplemented with copper and zinc on embryo quality and blastocyst formation from morula stage mouse embryos.
Materials and Methods: Morula embryos were obtained from 6-8 weeks NMRI female mice after hormonal stimulation by PMSG and HCG by mating with male mouse then embryos were transferred to culture medium supplemented with 4 mg/ml BSA. FF were collected from fertile women and centrifuged at 2500 g for 20 minutes. Cu and Zn level of FF were determined by atomic absorption spectrometry. The embryos were randomly divided in four groups, including: control FF alone, FF with Cu and FF with Zn. Embryos were cultured in medium containing 10% FF supplemented with 4µg/ml Cu or 1µg/ml Zn at 37°C for 48 hours. The embryo quality and hatching rate were assessed.
Results: The embryo quality was assessed based on embryo grading. The percentage rate of grade A embryos was 56, 70, 78 and 85%, also, hatching rate was 44, 57, 64 and 70% in the control, FF alone, FF with Cu and FF with Zn groups, respectively. We observed significant hatching rates and percentage rates of grade A embryos in the FF alone, FF with Cu and FF with Zn groups compared with the control group.
Conclusion: The results of our study showed that with adding copper or zinc to follicular fluid, embryo quality and development significantly enhanced.
Keywords: Embryo Quality, Follicular Fluid, Zinc, Copper

O-13: The Effect of Human Follicular Fluid Supplemented with Zinc and Copper on Vitrified/ Warmed Mouse Embryo Development

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Background: Follicular fluid (FF) provides a very important microenvironment for the development of oocytes that includes many substances which may increase embryo quality. According to the analysis of FF, the chemical constituents of FF have been grouped in the following categories: hormones; growth factors, interleukins; Reactive Oxygen Species (ROS); anti-apoptotic factors; proteins and amino acids; sugars and trace elements (Zn, Cu and Fe). Human FF supplemented IVF medium has been presented to improve in vitro growth to the morula and blastocyst stages. Embryo cryopreservation is an essential part of assisted reproduction. With regard to importance of embryo vitrification and positive effects of FF, we decided to evaluate the effects of human FF supplemented with zinc and copper on embryo quality and blastocyst formation from vitrified/ warmed morula stage mouse embryos.
Materials and Methods: Morula embryos were obtained from 6-8 weeks NMRI female mice after hormonal stimulation by PMSG and HCG by mating with male mouse then embryos were transferred to culture medium supplemented with 4 mg/ml BSA. FF were collected from fertile women and centrifuged at 2500 g for 20 minutes. Cu and Zn level of FF were determined by atomic absorption spectrometry. The embryos were vitrified and warmed according to Kitazato protocol by using the closed pull straws and VIT and THAW Kit, then randomly were divided in four groups, including: control, FF alone, FF with Cu and FF with Zn. Embryos were cultured in culture medium containing 10% FF supplemented with 4µg/ml Cu or 1µg/ml Zn at 37ºC for 72 hours. The quality and hatching rate of vitrified/ warmed embryos were assessed.
Results: The embryo quality was assessed based on embryo grading. The percentage rate of grade A embryos was 47, 61, 65 and 68%, also, hatching rate was 44, 57, 64 and 70% in the control, FF alone, FF with Cu and FF with Zn groups, respectively. We observed significant hatching rates and percentage rates of grade A embryos in the FF alone, FF with Cu and FF with Zn groups compared with the control group.
Conclusion: Our study indicated that follicular fluid supplemented with zinc or copper compensates damages caused by freezing/ thawing process and improves embryo development.
Keywords: Embryo vitrification, Follicular fluid, Zinc, Copper

O-14: Optimization of Domestic Animal Sperm Freezing Using Novel Plant-Origin Cryopreservation Media

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Background: Cryopreservation of sperm has allowed to conservation of genetic resources in cryobanks and guarantee of constant commercially of sperm supply for animal breeding program using artificial insemination. We performed several projects for optimization of sperm freezing in bull, ram, goat and rooster. Phosphatydil choline originated from soybean (lecithin) has been assessed in different protocols for substitution of egg yolk in animal freezing media. Demands for replacement of egg yolk in extenders have been increased in recent years due to this concerns that egg yolk contains substances that impede respiration of sperm which may lead to decrease their motility. Moreover, egg yolk increases the risk of microbial contamination that may increase the risk of disease transmission through the transportation of egg yolk-based extenders in the international exchange of stored semen. After replacement of egg yolk by soybean lecithin in extenders, various experiments for consideration of frozen-thawed sperm quality such as microscopic, cellular, biochemical, flow cytometric and epigenetic aspects were applied to evaluate the cryoprotective effects of lecithin.
Materials and Methods: Semen were collected from animal in each project (ram, goat, bull, rooster) and then each sperm sample was divided into different groups (according to experimental design in each project) for evaluation of potential effects of different concentrations of lecithin compare to traditional cryoprotectants. Moreover, various antioxidant and additives were assessed along with soybean lecithin. After freeze-thaw of sperm, various parameters such as motion characteristics, viability, membrane integrity, apoptosis, mitochondria activity, fertility potential and pregnancy rate were applied to evaluation the effects of different cryoprotectants for preserving the sperm quality and fertility after cryopreservation.
Results: Overall results in our projects show that for ram and bull sperm freezing, the best results for quality of post-thawed sperm were obtained in extender with 1% lecithin. Moreover, the lower rate of

agglutination of sperm was observed in extenders containing lecithin compare to extenders containing egg yolk. For goat sperm freezing, the higher percentages of motility, viability, mitochondria activity and fertility were produced in the extenders with 1.5% lecithin. Also, in goat sperm, lecithin reduced the acrosome damages compare to egg yolk. For rooster, 0.5 % lecithin was enough to produce the highest quality of post-thawed sperm compare to egg yolk. This discrepancy about the optimum levels of lecithin in different species, is related to different capacity of seminal plasma and different size of sperm in ram, bull, goat and rooster. Among different additives for reinforcement of extenders containing soybean lecithin, trehalose and cysteine (ram), and L-carnitine (rooster) had the best reciprocal effects with lecithin.
Conclusion: Results of our project show that substitution of egg yolk with lecithin has beneficial effects for sperm during freezing. Higher results in fertility potential of frozen-thawed sperm in extenders with lecithin, encourages us to develop a commercial extender based on lecithin for future.
Keywords: Sperm Freezing, Lecithin, Mitochondria, Fertility

Ethics and Reproductive Health

O-15: Health Status as Criteria for Access of Infertile Couples to Assisted Reproductive Technology

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Background: Assisted reproductive technology (ART) brought hope for infertile couples to have children but raised many ethical, religious and legal issues. Access to ART is one of the most important issues in this regard that has a wide spectrum from single person and homo sexual to criminal history mental health problems. The basic issue to evaluate this access is welfare of the resulting children. Here in this paper, our questions are: 1. is providing ART services for people who suffer from non-communicable or contagious diseases ethical? 2. What do we have in Iranian Law about access of infertile people with different health status to infertility treatment? 3. What criteria could we include in evaluation of the infertile couples referring to ART clinics?
Materials and Methods: This systematic review study was performed using library resources, law, religious texts, guidelines and declarations both national and international.
Results: On the subject of law, there is nothing about this subject in civil and criminal law and the ethical and legal aspects not addressed in Iranian legal system explicitly. Article 2 of the Embryo Donation Act does not accept donation of embryos to couples with incurable or complicated disease condition. Article 23 of the Family Protection Act, enacted in 1391 prohibits the reproduction in cases of possible damage to the fetus as a result of parents' health condition. European Society of Human Reproduction and Embryology (ESHRE) guidelines recommend that in case of non-communicable diseases, the infertility clinics and doctors are responsible for evaluation of the disability and harm to the resulting child.
Conclusion: The Family Protection Act limits considered safe for a baby and marriage records. This argument can be used to include restrictions in admission of infertile patients. Welfare of the child should be considered but the criteria are unclear. It seems, if possible, prevention of any harm to the resulting children is the basis of the above restrictions.

Keywords: Access, Infertile Couples, Assisted Reproduction, Welfare of Child

O-16: Locus of Control, Anxiety, and Depression in Infertile Patients

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Background: This study aimed to examine the association between locus of control and anxiety and depression, applying multivariate statistical techniques to control for the effects of demographic/fertility variables.
Materials and Methods: Cross-sectional study included 312 infertile patients in a referral fertility center in Tehran, Iran. The Hospital Anxiety and Depression Scale (HADS), the Levenson's Multidimensional Locus of Control Scale were administered to all participants. Hierarchical multiple linear regressions were used to identify factors associated with anxiety and depression.
Results: Controlling for demographic/fertility variables, hierarchical regression analyses showed that internal locus of control was negatively associated with anxiety and depression. Powerful others subscale was positively associated with anxiety, but there was no significant relationship between powerful others subscale and depression.
Conclusion: The findings of this study merit the understanding of the role of demographic/fertility characteristics and locus of control orientations in anxiety and depression of infertile patients to identify beforehand those patients who might be at risk of experiencing high anxiety and depression and in need of support.
Keywords: Anxiety, Depression, Infertility, Locus of Control

O-17: Self-Efficacy Scale and Hope Among Couples Seeking Assisted Reproduction Techniques

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Background: This study aimed to evaluate the hope and self-efficacy infertile couples.
Materials and Methods: In a cross-sectional study, the study sample consisted of three groups of infertile couples; candidate for oocyte donation, embryo donation, and normal infertile; aged at least 18 years and able to read and write in Persian were enrolled at the Royan institute, Tehran, Iran, between 2013 and 2014. Participants were asked demographic and general characteristics and completed the Persian version of the Adult Trait Hope Scale (including hope, pathway and agency subscales) and Sherer's self-efficacy questionnaire. Data on couples were analyzed using paired t test and data on males of females by independent t test.
Results: The mean score of hope subscale was significantly higher in husbands than wives in the normal infertile group (p=0.046). In the normal infertile group, the mean score of pathway was significantly higher in husbands (p=0.032). The mean score of self-efficacy in males was significantly higher than females in embryo donation (p=0.022). No significant difference was seen in the mean score of self-efficacy between wives and husbands in each group.

Conclusion: Considering that this was the first study to examine Snyder's construct of hope and Sherer's self-efficacy scales in special samples of infertile couples, the results suggest that hope may be important in reducing psychological symptoms and psychological adjustment in those who expose the infertility problems, and they follow medical recommendations better through behavioral patterns, which accelerate recovery. It is suggested to hold psychological counseling sessions (hope therapy) during reproduction cycles. There appears to be a critical need for psychology interventions to improve the self-efficacy level for better enjoying reproduction treatment. Efforts should be made decisions on providing comprehensive psychology consultation to all patients during assisted reproduction treatment.

Keywords: Hope, Depression, Anxiety, Stress, Infertility

O-18: Ethical and Psychological Aspects of the New Egg Donation Law in Austria

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Background: As from 2015, a new Law on Reproductive Medicine (Fortpflanzungsmedizingesetz - FMedG) is effective in Austria. The aim of the changes in the new law, was to aerate legal constraints and previous regulations. This opens up new possibilities in the treatment of unintentional childlessness in Austria trying to minimize the IVF-Tourisms to neighbor countries.
Materials and Methods: The new law on egg donation comparing to the previous one carries some advantages and disadvantages for both the patients and IVF-Centers. Giving an exact number of the egg donation procedures before 2015 is not possible. The affected patients rejected to provide the real kind of the assisted reproductive techniques (ART) because of its illegality. After enacting the liberal law, the expectations for practicing egg donation in Austria were very high. Instead the numbers are still discouraging.
Results: In spite of the new possibilities regarding egg donation in Austria, still the majority of the patients prefer to perform this procedure in countries outside of Austria (Czech Republic, Spain and Greece). This might be because of some legal, ethical and psychological aspects in egg donation.
Conclusion: The new law on egg donation in Austria is a step forward towards better reproductive medical care for this group of patients. Still many issues are open which could increase the number of egg donation cycles in Austria. Here the infertility counselors have a sensitive and important role guiding the patients. The Austrian law should be adapted and improved, in doing so it can take example of countries like Iran which are experienced in this field and have liberal laws since many years.

Keywords: Egg Donation, Austrian Law, Ethical Aspects

O-19: The Relationship between "Personal Relation Beliefs", "Attribution Styles" and "Quality of Life" in Infertile Couples Undergoing Assisted Reproductive Techniques

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Background: Beliefs have a key role in shaping and determining the quality of relations between spouses. When an individual faces an uncontrolled event like infertility, it is important how to evaluate it and show appropriate reaction. The purpose of this study was Investigate the Relationship between personal beliefs, attribution style and quality of life in infertile couples undergoing assisted reproductive techniques (ART).
Materials and Methods: A cross-sectional study was conducted on 100 infertile couples referred to Royan Institute, a referral infertility clinic in Tehran, the capital of Iran. Participants completed four questionnaires: A demographic questionnaire, the short form of health survey (SF12), the relationship belief inventory (RBI), and attribution style questionnaire (ASQ). The questionnaires were completed separately for husband and wife and they were assured that the spouses won't see them. Data were analyzed using paired t-test and pearson correlation coefficient. The study was approved by ethical committee.
Results: The mean score of quality of life was significantly higher in husbands than wives (P=0.019). There was a statistically significant positive relationship between personal beliefs and some of its subscales and quality of life in infertile couples (P<0.01). Also, we found a positive relationship between attribution style sub-scale of "positive internal" and quality of life (P=0.033).
Conclusion: There was a relation between personal beliefs, attribution styles and quality of life in infertile couples

Keywords: Quality of Life, Personal Beliefs, Attribution Styles, Infertile Couples, Assisted Reproduction

Female Infertility

O-20: Study on Effects of Iron Oxide Nano Particles on The Size of Ovarian Follicles of Adult NMRI Mouse Strain

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Background: Nanomaterials are particles under 100 nm Diameter. These materials because of their Nanometer size are able to cross from biological barrier like as skin, Blood-brain barrier and placenta. Therefore, The aim of this study was to investigate the toxicity effects of nano iron oxide particles on size of ovarian follicles in adult NMRI mouse Strain *in vivo*.
Materials and Methods: We used *in vivo* techniques to indicate the toxicity effects of these particles. The effect of iron oxide nanoparticles on ovarian tissue were studied three groups: control, sham and experimental doses of 50, 100 and 150 mg/kg. iron oxide nanoparticles were injected intraperitonealy for four days in four estrous cycle of mice(pro-estrus, estrus, met-estrus and di-estrus). After resting for two consecutive cycles, mice were killed and ovarian tissue samples were collected. Finally data were analyzed by SPSS software and tukey test.
Results: In this study size of primordial and Graafian follicles show no significant changes in the ovaries. Size of Primary follicles in experimental group 1 showed significant increasing compared with control group. Secondary follicles and corpus luteum in experimental groups 2 and 3 show a significant decrease in comparison with control group.
Conclusion: The results showed limited effects of nano iron oxide in low concentration while increase iron oxide nanoparticles diameter enhances its accumulation in the cells, finally it causes the loss of cell division regulation and causes cell toxicity.

Keywords: Follicle, Iron Oxide Nanoparticles, Ovary, NMRI Mouse Strain, *In Vivo*

O-21: Increase in Melanocortin 4 Receptor (MC4R) mRNA Expression in Hypothalamic Arcuate Nucleus in Polycystic Ovary Syndrome in Rat

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Background: Obesity is a common symptom in women with polycystic ovary syndrome (PCOS). It has seen about 50% of PCOS women are obese. In addition, alterations in the melanocortin 4 receptor (MC4R) gene expression linked to obesity. MC4R is expressed in the hypothalamus region of the appetite and in general, has a role in regulating of feed intake. Therefore, this study aimed to investigate the MC4R mRNA expression in the arcuate nucleus of hypothalamus after induction of PCOS in rats.
Materials and Methods: Twenty four female nulliparous and primiparous rats divided into two subgroups control and PCOS, respectively. PCOS groups were exposed to constant light for 90 days and all four groups were weighted weekly. After 90 days, histomorphologic alterations of ovary were compared between groups. Furthermore, MC4R gene expression in arcuate nucleus of hypothalamus of the rats using real-time PCR method was assessed. Six adult female rats as a control of real-time PCR test were ovariectomized. The data were analyzed by one-way ANOVA and LSD post hoc test ($P \leq 0.05$, SPSS 22).
Results: Number and size of tertiary follicles in both PCOS groups were more than the controls ($P<0.05$) which confirms induction of PCOS. MC4R gene expressions in both PCOS groups were more than control groups ($P<0.05$). However, parity did not affect in gene expression of MC4R ($P>0.05$). Although, weight loss of primiparous rats was observed in the initial weeks of the experiment; but, after the week 9 of study weight gain in PCOS group was observed until the time of sampling ($P<0.05$).
Conclusion: Increased expression of MC4R after PCOS induction in the arcuate nucleus of hypothalamus, showed the role of this peptide in the pathogenesis of PCOS. Identifying the neuronal pathways controlling feed intake using MC4R and their relationship with PCOS needs further investigation.
Keywords: Polycystic Ovarian Syndrome, Melanocortin 4 Receptor, Constant Light, Rats

O-22: (R)-(+) Pulegone-Induced Damages on Ovarian Tissue; Possible Mechanisms

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Background: Pulegone (PGN), a monoterpene ketone, is widely used for flavoring foods, drinks, and dental products. According to several reports, PGN and its metabolites, piperitenone, piperitone, menthofuran, and menthone exert several cytotoxic impacts in various tissues. Present study was done in order to evaluate the pulegone (PGN)-induced alterations in ovarian aromatization, proto-oncogenes expression and ERα and ERβ receptors expression/synthesis.
Materials and Methods: For this purpose 24 mature albino mice were divided into experimental (received 25mg/kg, 50mg/kg and 100 mg/kg PGN, orally) and control groups. The animals in control group received 2% from Tween 80 as a PGN solvent. Animals received PGN and Tween 80 for 35 continuous days. The mRNA levels of ERα, ERβ, p53, BCl-2 and cytochrome p450 (Cyp19), ovarian angiogenesis, ERα and ERβ expression were analyzed. Moreover, follicular cells apoptosis, follicular atresia, serum levels of estrogen and progesterone along with mRNA damage were investigated.
Results: The PGN at a dose level of 25mg/kg enhanced the Cyp19 and ERα expression, while it reduced mRNA levels of ERα, ERβ and Cyp19 at 50mg/kg and 100mg/kg levels. Moreover, the PGN significantly elevated p53 mRNA and reduced BCl-2 expression. Ovarian angiogenesis was reduced in PGN-received groups and it resulted in increased apoptosis. Finally, the PGN, in a dose dependent manner, elevated follicular atresia and reduced serum levels of estrogen and progesterone.
Conclusion: Thus, chronic exposure to PGN, severely affects ovarian aromatization that impacts the proto-oncogenes mRNA levels by altering ERs expression/synthesis. Ultimately, PGN-reduced aromatization triggers cellular apoptosis and mRNA damage through estrogen synthesis reduction.
Keywords: Ovary, Pulegone, Estrogen Receptor, Cyp19, Apoptosis

Genetics

O-23: Embryo and Oocyte Quality in Polycystic Ovary Syndrome: Analysis of Expression of Peroxiredoxin 2 in Cumulus Cells and Granulosa Cell Apoptosis

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Background: The effect of Hydrogen peroxide (H2O2) and its special antioxidant Peroxiredoxin 2 (PRDX2), in the oocyte and embryo quality of patients with polycystic ovary syndrome (PCOS) is unclear. This study examined for the first time, the alteration of these factors additionally with the apoptosis rate of granulosa cells (GCs) in patients with PCOS.
Materials and Methods: 70 follicles were retrieved from 14 non-PCOS and PCOS women and evaluated individually for the gene expressions of PRDX2 in cumulus cells by Real-Time PCR, the levels of H2O2 in follicular fluid (FF) by ELISA and the GCs apoptosis rate by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The correlation between oocyte and embryo quality by parameters of the study were evaluated.
Results: Results:0.030), decreased expression of PRDX2 in cumulus cells ($p = 0.039$) and also a higher incidence of GC apoptosis rate ($p = 0.001$) which was correlated with PRDX2 gene expression. Both

PRDX2 expression and GCs apoptosis were significantly associated with oocyte and embryo grading.
Conclusion: The findings showed that the lower incidence of GCs apoptosis and greater PRDX2 gene expression in cumulus cells were associated with good quality oocytes and embryos. Further investigations with more sample size suggested confirming these findings.
Keywords: Polycystic ovary syndrome, Reactive oxygen species, Granulosa cells, Apoptosis

O-24: Sevofluranon Induce Apoptosis Process in Testicular Tissue during Maturity of Male Mice after 7 Days Exposure of Neonatal Mice

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Background: Sevofluranon potential effects on male and female fertility have not been adequately investigated. So, this study investigated Sevofluranon effect on spermatogenesis process in male mature mice after exposure in prepubertal time.
Materials and Methods: 24 neonatal NMRI male mice were classified in 3 groups randomly. Experimental group 1 received 2 litter MAC/30 min Sevofluranon combined 2 litter/min oxygene per day in a single dose. Experimental group 2 received 1 litter MAC/30 min Sevofluranon combined 2 litter/min oxygen during 7 days in a sequential dose. Control group did not receive any treatment. All groups were scarified after 2 months. Testicular tissue was evaluated for cellular and molecular evaluation. Histological assessment, immunohistochemistry and apoptosis process was done by H&E staining and TUNNEL assay. PLZF, Vimentine and Oct4 markers was used in immunohistochemistry. Bax and Bcl2 expression profile was evaluated in testicular tissue by real time PCR. Data was analyzed by ANOVA and Tukey post hoc test.
Results: Our results showed that integrity of testiculat tissue preserved in all experimental groups. Count of spermatogonial cells had a decrease significant difference in group 2. The result of apoptosis assay showed 15%±3 and 9%±2 apoptosis in spermatogonial cells in the group 2 and 1, respectively. Also, Bax/Bcl2 was 4.115 3.654 and 16.4252 in control, experimental group 1 & 2, respectively. This result was significant ($P \leq 0.05$) between groups 2 with other groups.
Conclusion: A single 30 min exposure of 1 litter MAC Sevoflurane in the presence of 2 litter/min oxygen preserve integrity of testicular tissue and lower apoptotic cells during post pubertal testis. Ratio of Bax/Bcl2, apoptotic and germ cell count during 7 days exposure was significant in comparison to one day exposure.
Keywords: Spermatogonia, Sevofluranon, Pre Pubertal, Testicular Tissue

O-25: Reproductive Outcomes of Infertile Men with AZFc Deletion

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Background: A particular region on human Y chromosome long arm, termed the azoospermia factor (AZF), has been known to contain genes pivotal for normal spermatogenesis. Micro/deletions occurred in this locus are associated with infertility and are categorized into three close and non-overlapping subregions: AZFa, AZFb, and AZFc. Based on the evidence on the possibility of finding sperm in testes of AZFc deleted men, conventional or microdissection testicular sperm extraction (MD/TESE) has been suggested to such patients willing to father their own child through Assisted Reproductive Technologies (ART). In this study, we aimed to determine the reproductive outcomes of infertile men with AZFc microdeletion.
Materials and Methods: Medical records of couples with AZFc deleted male partners following ART procedures in Royan Institute were reviewed. All clinical data available was gathered and student's t-test and logistic regression test were used for comparison and statistical analysis.
Results: Of 195 AZFc deleted cases, 113 were azoospermic and 82 had oligospermia. Amongst all available pathologies, Sertoli Cell Only was the predominant trait which was mostly (85.7%) seen in azooseprmic cases. Sperm retrieval rate upon MD/TESE was about 35.5% and 75% in azoospermic and oligoospermic cases respectively. Of those who had their embryos transferred after In Vitro Fertilization or Intra Cytoplasmic Sperm Injection (IVF/ICSI), overall female partners of 17 AZFc deleted patients had clinical pregnancy (rate of 35.4%); 4 pregnancies were the result of testicular sperms and 13 pregnancies were from ejaculatory sperms in oligoospermic males.
Conclusion: Although sperm could be surgically retrieved in both azoospermic and oligoospermic patients, oligoospermic group showed a higher sperm retrieval rate using MD/TESE, followed by a higher pregnancy rate. Accordingly, assessing AZFc deletion may help in estimating MD/TESE or ART success rate in infertile men.
Keywords: AZFc Deletion, MD/TESE, IVF/ICSI

O-26: Expression Patterns of Timp Genes in Preeclamptic Women Using Cell Free Fetal RNA

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Background: Preeclampsia is a pregnancy related disorder. In preeclampsia cytotrophoblastic invasion reduced and it cause incomplete remodeling of spiral arteries. Nowadays noninvasive diagnosis methods are so important and one of their application is early diagnosis of preeclampsia by Cell Free Fetal RNA of maternal plasma. Since balance between TIMP/MMP genes has roles in placental and spiral arteries remodeling. In this study we evaluated TIMP genes expression between preeclampsia and normal women by cffRNA.
Material and Methods: Constant was obtained from patients accord-

ing to low ethical approved. Sample categories in two groups: normal and severe preeclampsia. Whole blood of preeclamptic and normal women in gestational age of 28-32 weeks as well as normal women in 14s and 28s week was collected in EDTA tubes. Maternal plasma was separated and cffRNA was extracted by QIAamp circulating nucleic acid kit. cDNA was synthesized by VILO Superscript Invitrogen kit. The expression of TIMP1,3 genes were evaluated quantitatively by real time PCR method.

Results: Our results have shown that expression level of TIMP1, 3 genes were increased in preeclamtic women in comparison to match controls but results statistically was not significant and we think it is because of low number of samples.

Conclusion: The expression level of TIMP1, 3 in preeclamptic women was higher but not significant in comparison to control group.

Keyword: Preeclampsia, Cell Free Fetal RNA, Expression, TIMP1, 3

Poster Presentations

Andrology

P-1: Comparison and Evaluation of Capacitation and Acrosomal Reaction in Freeze-Thawed Human Ejaculated Sperms Treated With L-carnitine and Pentoxifylline

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Background: Cryopreservation is one of the most effective and acceptable methods use to maintain human fertility before cancer therapy. Unfortunately, this method reduces sperm quality via reactive oxygen species (ROS) production. L-carnitine is an antioxidant enhances spermatozoa motility and protects sperm membrane from ROS damages.The objectives of this study were to evaluate the protective effects of L-carnitine (natural) and Pentoxifylline (artifitial) antioxidants on the plasma membrane integrity of normal human sperms during cryopreservation.
Materials and Methods: 30 normal semen samples were prepared for swim-up procedure. Samples were divided into 3 aliquots; control aliquot (no treatment) and experimental aliquots(incubated in 3.6 mM L-carnitine or Pentoxifylline for 30 min). Sperm motility was assessed according to WHO criteria. All aliquots were cryopreserved by the liquid nitrogen vapor and thawed after 48h. Motility was again assessed. Sperm smears were stained with different lectins (WGA, PNA, CONA) and studied by fluorescents microscopy and flowcytometry. Statistical analysis were performed using ANOVA.
Results: The freez-thawed process significantly decreased sperm motility in all aliquots. Treated aliquots with pentoxifylline contained a significant higher percent of motile sperms compared with control and l-carnitine aliquots. L-carnitine supplementation led to a significant increase in the percentage of non-capacitated sperms compared with control and pentoxifylline-treated samples and in the percentages of acrosomal intact sperms compared with pentoxifylline-treated samples. In addition, L-carnitine supplementation, showed a significant decrease in the percentages of acrosomal-reacted sperms compared with control and pentoxifylline treated samples.
Conclusion: Pentoxifylline enhanced the motility but could not protect the sperm plasma membrane integrity during freeze-thaw process. In contrast, although, L-carnitine improved motility in less extent compared with pentoxifylline, it could protect the plasma membrane as well. Therefore, L-carnitine may improve sperm cryopreservation quality, and as a result it may improve sperm fertility potential.
Keywords: Cryopreservation, Capacitation, Acrosomal Reaction,L-Carnitine

P-2: Evaluation Effect of Blackcurrant on Testis Tissue in Wistar Rat

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Background: The male gametocytes created in process spermatogenesis and disorder in which this part can process cause infertility. Blackcurrant has strong phytochemicals such as anthocyanin. Blackcurrant `s more sugar, the type of fructose does not require insulin to absorb in the body. Omega-3 and omega-6 fatty acids. In this study we evaluation effect it on spermatogenesis.
Materials and Methods: In this study, were used 40 mature male wistar rat weight of 200 ± 20 g. Rats separated in two groups; the control group fed with distilled water and treatment group with blackcurrant `s concentrate (250mg/kg) for 60 days. The weight of rats checked before and after treatment. The rats get unconscious and bleeding from west ventricle has been done (about 3-4ml). Took out testis from traverse incision in the abdominal area and fixed in 10% formalin buffer. In pathobiology laboratory testis tissues washed with normal saline. In survey of spermatogenic cells (spermatogonia, primary spermatocyte, spermatid, sperm, and sertoli cells) compared the amount of each one of cells in experimental groups and control group. All the data studied with SPSS software.
Results: The histology of slides showed; both groups are normal in morphology and dispersal of seminiferous ducts. The treatment group had increasing in spermatogonia, primary spermatocyte, spermatid, spermatozoid in compare with control group. ($P<0.01$)
Conclusion: With using of probes the blackcurrant has androgenic effect and can cause increase parameter which is depended on androgen, poly sperm and the weight of reproductive organs. We hope that the results obtained and more research may help to unfertilized person and also can benefit to prevention of menopause or decreasing the complications of that.
Keywords: Blackcurrant , Rat, Testis Tissue

P-3: The Effect of Morphine Consumption on Sperm Parameters, Protamine Deficiency and the Level of Malondialdehyde in Mice Epididymal Spermatozoa

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Background: Morphine is a natural alkaloid (opiate) occurring in opium poppy. This opioid drug is frequently used for treatment of severe pain because of its powerful analgesic and sedative effects. However, it can be abused because of its high addictive potential. Opiate abuse is considered as one of the problems associated with poor semen production and sperm quality. Therefore, this experimental study was carried out to assess the effect of intraperitoneal injection of morphine on sperm parameters, protamine deficiency and the level of Malondialdehyde (MDA) in spermatozoa aspirated from the tail of mice epididymis.
Materials and Methods: Totally 24 adult male balb/c mice (8 weeks old. 30g) were randomly divided into 3 groups (experimental, sham and control) each containing 8 mice. Group 1 received basal diet and morphine (15 mg/kg/daily, intraperitoneal), group 2 received basal diet and normal saline and group 3 served as control and fed on basal diet for 35 days. Finally, right tail of epididymis of each mouse was cut and placed in Ham's F10 medium for 30 min. Released sperm were used to analyze count(number), motility, viability (eosin-nigrosin staining), morphology (Papanicolaou), protamine deficiency with chromomycin A3 (CMA3) staining and the level of MDA via spectrophotometry based on absorbency at 532 nm with Thiobarbituric acid (TBA) method.
Results: In morphine-treated mice a significant decrease was found

in sperm viability, normal morphology, count and motility compared to other groups (P<0.05). The rate of CMA3-reacted (protamine deficiency) spermatozoa in morphine mice were significantly higher compared to other groups (P<0.05). In addition, in morphine mice there was a significant increase in MDA compared to other groups (P<0.05).
Conclusion: The results showed that morphine abuse disturbs sperm parameters and can result in the production of spermatozoa with less condensed chromatin in mice as an experimental model. In addition, it was showed that morphine injection can increase the level of MDA (as a biomarker of oxidative lipid damage) in mice epididymal sperm.
Keywords: Mice, Morphine, Sperm parameters, Protamine Deficiency, Malondialdehyde

P-4: Comparison of Sperm Apoptotic Markers between Infertile Men with Varicocele and Fertile Individuals

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Background: One third of infertility endured by couples is due to male factors and among these factors varicocele is the most common reason. Varicocele is defined as a palpable elongated, dilated and tortuous testicular pampiniform plexus of veins in the spermatic cord. Although it is not clear that how exactly varicocele causes infertility, heat stress caused by disturbed testicular thermoregulation and increased reactive oxygen species (ROS) in these cases, are proposed to be two main explanations. It is shown that heat stress can initiate programmed cell death known as apoptosis. Therefore, the aim of this study was to compare sperm apoptotic markers (caspase and DNA fragmentation) between infertile men with varicocele and fertile men.
Materials and Methods: Sperm concentration, motility and morphology were analyzed in semen samples of 18 infertile men with varicocele and 14 fertile according to World Health Organization (WHO) 2010 guidelines. Sperm active caspase3/7 and DNA fragmentation were assessed using Flow cytometry and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, respectively. For data analysis, Independent t test statistics analysis was performed between two groups
Results: We observed increased level of caspase 3/7 in infertile men with varicocele compared to fertile individuals. Percentage of sperms with fragmented DNA was also higher in infertile men with varicocele compared to fertile men.
Conclusion: Our results show that varicocele can induce apoptosis in sperms of infertile men. Considering spermatogenesis is disturbed in these individuals. Thus, we conclude that one reason for this disturbance maybe induction of apoptosis in testis.
Keywords: Varicocele, Apoptosis, Caspase, DNA Fragmentation

P-5: Aluminium-Induced Oxidative Stress, Apoptosis and Alterations in Sperm Parameters in Wistar Rats: Recovery Effect of Curcumin

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Background: Reproductive toxicity is a major challenge associated with aluminum (Al) exposure. No studies have evaluated the possible effects of curcumin on Al-induced reproductive dysfunction. Therefore, this study investigated the effects of curcumin treatment on aluminum-induced reproductive damage.
Materials and Methods: In an experimental study, eight male Wistar rats were allocated to five groups to receive no treatment (control), dimethyl sulfoxide (DMSO) or distilled water, curcumin 10 mg/kg body weight (BW), Al chloride 10 mg/kg BW, or both curcumin and Al chloride (10 mg/kg BW each). Treatments were performed by intraperitoneal injections for 28 days. After the treatment period, testis and body weights were recorded. The blood serum was assessed for biochemical analysis as well as the incidence of germ cell apoptosis (by the TUNEL method). Data were analyzed with one-way ANOVA, Tukey's.
Results: Significant reductions in body and testis weight, plasma testosterone and luteinizing hormone levels, sperm count, motility, morphology, and viability, germinal epithelium thickness, seminiferous tubules diameter, superoxide dismutase activity were observed in rats treated with Al. Moreover, Al exposure caused significant increments in the lumen diameter of tubules, TUNEL-positive cells and malondialdehyde levels compared to the control group. However, in rats receiving both curcumin and Al, curcumin significantly reversed the adverse effects of Al on testis and sperm parameters. No significant differences in follicle-stimulating hormone levels and nuclear diameter of spermatogonia were detected between groups.
Conclusion: It can be thus concluded that Al causes reproductive dysfunction by crating oxidative damage. Curcumin, on the other hand, antagonizes the toxic effects of Al and improves the antioxidant status and sperm quality in male rats.
Keywords: Curcumin, Aluminum Toxicity, Reproductive System, Wistar Rat

P-6: Immediate Exogenous Testosterone Treatment by Preventing Cell Adhesion Molecule1 Defect Reduces Spinal Cord Injury Effects on Male Reproduction

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Background: Spinal cord injury (SCI) causes infertility in male patients. This study evaluated the role of cell adhesion molecule1 (CADM1) dynamics on the pathological effects of spinal cord injury (SCI) on male reproductive system. The role of exogenous testosterone during early and chronic periods of SCI evaluated on mice as follows: i) immediately after induced SCI for 7 or 35 days or ii) one week after induced SCI for or 35 days.
Materials and Methods: We evaluated sperm parameters, testis histological architecture, and serum testosterone levels. The expression of CADM1 was semi-quantified by immunohistochemistry. The level of CADM1 gene expression was evaluated by quantified real-time PCR (qRT-PCR).
Results: Different grades of abnormalities in sperm parameters and testis architecture were accompanied by significant reductions in the quantity of CADM1 expression and its gene transcription in the basal and adluminal compartments of seminiferous tubules in both acute and chronic SCI mice. Exogenous testosterone compensated for the decline in serum testosterone levels in the acute and chronic phases. There was a significant reduction in the percentage of apoptotic sperm from the caudal section of the epididymis and short head or abnormal sperm. Sperm motility, Johnson criteria and the number of germ cell layers significantly improved in parallel with an increase in the level of CADM1 transcription and the quantity of CADM1 immunoreactivity in the testis of acute and chronic SCI mice.
Conclusion: Most prominently, the beneficial effect of immediate testosterone treatment was accompanied by profound dynamics in immunoreactivity and transcription level of CADM1.
Keywords: Spinal Cord Injury, Cell Adhesion Molecule, Testis, Testosterone, Sperm Parameters

P-7: Comparing Ameliorative Effects of Melissa Officinalis Hydro-alcoholic Extract with Phenytoin on Epilepsy-induced Damages; Correlation with Testicular Antioxidant and Endocrine Status

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Background: Kindling is a chronic animal model for epilepsy, which could be induced by pentylenetetrazol (PTZ). However, the effects of epilepsy and anti-epileptic drugs on spermatogenesis have not been clarified completely. Phenytoin (PN) is an anti-epileptic drug that by slowing down impulses in the brain controls seizures. Melissa Officinalis (MO) is traditionally used to inducing calmness and to improve cognition. Thus, present study was done in order to compare the effect of PN with MO against PTZ-induced detrimental effects on sperm parameters as main marker for gonadal healthiness.
Materials and Methods: To follow-up this study, mature male Wister rats were divided into control (non-treated), epilepsy-induced (received 40 mg/kg/day from PT, every 48 hrs., for 9 times and 60 mg/kg/day form PT for 10th time, intra-peritoneally) and treated groups. Following induction of epilepsy, the animals in treatment groups received 10 mg/kg/day from PN, MO-alone (100 mg/kg/day) and MO-extract+PN, orally by gavages. The serum level of testosterone, testicular antioxidant capacity (TAC) and sperm parameters (count, motility and viability) were assessed.
Results: No significant alterations in serum level of testosterone were revealed between all groups. However, co-administrating MO-extract and PN up-regulated the epilepsy-reduced TAC level. Moreover, the animals in MO and PN-received groups exhibited remarkably ($P<0.05$) higher sperm count, motility and viability percentage.
Conclusion: According to our data, epilepsy, mainly by down-regulating testicular antioxidant status adversely affects the sperm parameter. While co-administrating MO and PN ameliorates the epilepsy-reduced TAC level. Therefore, improved TAC level in turn enhances the sperm quality.
Keywords: Epilepsy, Melissa Officinalis, Phenytoin, Sperm Parameter, Oxidative Stress

P-8: Radioprotective Effects of Aloe Vera on Mouse Testis Tissue Recovery from X-Ray

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Background: Male plays a key role for genomic instability transition caused by exposure to the next generation. Spermatogenesis in male mice is about 6weeks that damage created by exposure at each stage is different. The main objective of this study was to evaluate the testis damage caused in irradiated male mice at different stages of spermatogenesis cycle and protective role of Aloe Vera in compensating for damage to the testis tissue parameters.
Materials and Methods: Mature NMRI male mice were purchased. There were four treatments including: Control, Aloe Vera, Radiation, Aloe Vera/Radiation. Aloe Vera was injected intraperitoneally in a dose of 200 mg/kg b.w.t for 10 consecutive days before irradiation (5Gy high-energy X ray in whole body). Animals were kept and sacrificed at 1, 2, 3, 4, 5 and 6 weeks post-irradiation for groups I, II, III, IV, V and VI respectively. spermatogenic cell numbers and thickness of germinal epithelium of testis were analyzed.
Results: Different statistical tests revealed that 5Gy of high-energy X-ray created interval in the normal activity of the testis and it reduced in the thickness of germinal epithelium and cell number. In contrast, Aloe Vera treatment improved testis parameters in all groups. In Aloe Vera/radiation treatment in comparison to radiation treatment, partial improvement in tissue damages was seen and the period the damage was repaired was shorter than radiation treatment.
Conclusion: It can be concluded that radiation causes abnormalities in the testis tissue and spermatogenic cell numbers, and Aloe Vera could compensate for the adverse effects of exposure to X-ray on testis tissue.
Keywords: Testis, Aloe Vera, Radioprotector, X-ray, Mouse

P-9: DPY19L2 Gene in Patients with Partial Globozoospermia

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Background: Half of infertility cases are due to male factors. Teratozoospermia is one of the sperm disorders which cause male infertility. Globozoospermia is a rare but sever teratozoospermia disorder. Total globozoospermia is diagnosed by the presence of above 90% round-headed spermatozoa lacking an acrosome in semen analysis. There is also a group of patients that 20-90% of their spermatozoa in semen

analysis lack acrosome which are called partial globozoospermia. Previous studies by other authors and our recent study have shown that in large majority of total globozoospermia patients a homozygous deletion of the DPY19L2 gene is seen. This 200 kb deletion encompasses the totality of DPY19L2 coding sequence. Among all of the other genes that are located in this area DPY19L2 is the one that is dominantly expressed in the testis. The aim of our study was to assess the frequency of DPY19L2 deletion among Iranian infertile men with partial globozoospermia referred to Royan Institute.
Materials and Methods: In this study, 20 men with parial globozoospermia and 24 men with normal spermogram referring to Royan institute were selected and DNA were extracted from their blood samples. The deletion of their DPY16L2 gene was examined using specific primers and PCR technique.
Results: None of patients (<90% of round head sperms) and also control group showed this large deletion.
Conclusion: Considering that deletion of DPY19L2 gene as the main cause reported for total globozoospermia, was not seen in the male with partial globozoospermia. Therefore, it seems that an unknown factor or mutation instead of whole DPY19L2 gene deletion cause similar phenotype in partial globozoospermia.
Keywords: Partial Globozoospermia, DPY19L2 Gene, Male Infertility

P-10: Effects of Sesame Seeds on Sperm Quality in Aging Rats

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Background: Oxidative stress as a consequence of aging can induce infertility in males. Sesame seed is one of phytoestrogenic lignans plants. It has estrogenic and antioxidant effects. Sesame seed containing large amount of sesamin, sesamolin lignans and vitamin E. In this study, we have investigated the effects of sesame seeds on sperm quality in aging rats.
Materials and Methods: This experimental study was conducted on 20 old adult Wistar rats(15-16 months). Rats were divided randomly into 4 groups, experimental (60,30 days) and control (60,30 days) groups. Control groups received standard diet and the experimental groups received 70% standard diet +30% sesame seeds. At the end of experiment, body weight, testis and epididymis weight, measuried. For evaluation of progressive motility,Sperm viability, normal morphology and The total count of sperm, the right tail of the epididym was selected and transferred to Tyrode's Solution.
Results: The results showed that the progressive motility,Sperm viability, normal morphology and The total count of sperm were increase a significantly in 60 days experimental group compared to the other groups.
Conclusion: This study showed, the may using of sesame seed for 60 days improved sperm quality in aging rats. It is probably due to the antioxidant properties of sesame seed.
Keywords: Phytoestrogen, Rat, Sesame Seed, Aging, Sperm

P-11: Assessment of PLC ζ in Infertile Men with Globozoospermia

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Background: Failed fertilization was observed about 1-3 % infertile men after Intra-Cytoplasmic Sperm Injection (ICSI) mainly due to failed oocyte activation. Several sperm factors involved in oocyte activation such as phospholipase C zeta (PLC ζ), Post Acrosomal sheath WW domain-binding Protein (PAWP) and etc. Recently, PLC ζ was introduced as main sperm factor. Therefore, we aimed to compare PLC ζ between infertile men with globozoospermia and fertile men.
Materials and Methods: Semen samples were collected from 15 fertile and 15 infertile men with globozoospermia. Sperm Parameters (concentration, motility and morphology) and expression of PLC ζ were assessed by using Computer Aided Sperm Analysis (CASA) system and Real time PCR, respectively.
Results: The results of this study show that percentage of abnormal sperm parameters were higher in globozoospermic individuals compared to fertile men. In addition, we observed expression of PLC ζ were low in infertile men with globozoospermia compared to fertile men.
Conclusion: We showed that PLC ζ has a crucial role in oocyte activation. Therefore, artificial oocyte activation was suggested for globozoospermic men to obtain high chance of fertilization and pregnancy.
Keywords: Globozoospermia, PLC ζ, Fertilization, ICSI

P-12: Urogenital Tract Infection due to Staphylococcus Aureus in Infertile Male Patients in Tabriz, Northwest Iran

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Background: Urogenital tract infections due to Staphylococcus aureus have been recognized with infertility, bacteria are capable of agglutinating and immobilizing spermatozoa. The aim of present study was to determine the frequency of S. aureus in semen culture isolated from infertile male patients in northwest Iran. In this study.
Materials and Methods: Fluids of 100 infertile men were evaluated. Standard semen analysis was performed according to WHO guidelines. After isolation, identification and determination of susceptibility against important antibiotics, polymerase chain reaction (PCR) was used to identify mecA and tst genes.
Results: Data obtained from the present study shows that 10 (62.5%) of the subjects had abnormal seminal fluid sperm motility and morphology and 3 (18.8%) of the subjects had abnormal seminal fluid density. Whereas after washing with albumin declined to 5 (31.3%), 4 (25%) and 1 (6.3%), respectively. Sixteen (16%) of infertile male patients were colonized by S. aureus. The results of antibiotic susceptibility testing showed except penicillin, other antibiotics have high activity on isolates. Regarding PCR results, mecA sequences were detected in 3 (18.7%) strains, whilst the tst gene encoding TSST-1 was not detected in any of clinical strains. The prevalence of abnormal sperm indices and bacterial infection is high and S. aureus infection should be treated and no longer ignored in the management of male factor infertility.
Conclusion: The prevalence of abnormal sperm cells indices and bacterial infection due to S. aureus is high. In the management of

male factor infertility S. aureus should be properly treated and no longer ignored.
Keywords: Infertility, Staphylococcus Aureus, Seminal Fluid, Meca

P-13: Evaluation of Histopathologic Ghanges of Testicular Tissue Following Consumption of Royal Jelly in Diabetic Male Rats

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Background: Royal jelly (RJ) has been showed antioxidant and antidiabetic effects. This study investigates the assessment of the effects of royal jelly on histopathologic changes of the testes in diabetic male rats.
Materials and Methods: In this experimental study, twenty-eight adult Wistar rats were randomly divided into control (C), royal jelly (R), diabetic (D) and RJ-treated diabetic (D+R) groups. Diabetes was induced by a single intraperitoneal injection of STZ at 50 mg/kg body weight (BW). The R and RJ-treated diabetic groups received daily RJ at 100 mg/kg BW dose for 6 weeks orally
Results: In diabetic group, seminiferous tubules diameter (STsD), Johnsen's score, tubular differentiation index (TDI), spermiogenesis index (SPI), Sertoli cell index (SCI) and meiotic index (MI) significantly decreased and tunica albuginea thickness (TAT) in comparison to the control group. In diabetic rats treated with RJ, diabetes-induced impairment was significantly improved ($P<0.05$).
Conclusion: RJ may be beneficial agent to reduce pathologic alterations of testicular tissue by enhancing antioxidant status.
Keywords: Diabetes, Royal Jelly, Streptozotocin,Testis, Histopathology

P-14: Effect of Royal Jelly on The Testosterone Production in Streptozotocin-Induced Diabetic Rats

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Background: Diabetes mellitus is the most common endocrine disease. It has a significant effect on male reproductive function. Royal jelly showed antidiabetic effects. This study was conducted to investigate the effects of royal jelly on testosterone level of diabetic male rats
Materials and Methods: In this investigation, 32 male Wistar rats were used. After determining their body weights, the animals were divided randomly into four groups: Control and diabetic groups received 1cc of distilled water by oral gavage. RJ and diabetic treated with RJ groups received 100 mg/kg body weight by oral gavage. Diabetes was induced by a single intraperitoneal injection of streptozotosin (60 mg/kg). At the end of the study, all animals were sacrificed at the end of 6 weeks and blood samples collected to estimate testosterone level. Data were analyzed by one-way ANOVA followed by Tukey's test using SPSS version 18 software.
Results: Results showed that testosterone levels were significantly decreased in the diabetic group when compared to control and RJ groups ($P\leq0.05$) and the administration of RJ prevented this decrease in the diabetic treated with RJ group ($P\leq0.05$).
Conclusion: These results suggest that administration of RJ improved in altering the testicular endocrine function such as testosterone release may contribute in diabetes-induced injuries to leyding and sertoli cells.
Keywords: Diabetes, Royal Jelly, Testosterone, Male Rats

P-15: Xanthine Oxidase Activity Associated with Nitrosative Stress in Seminal Plasma of Iraqi Leukocytospermic Patients

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Background: Leukocytes linked directly and indirectly to reactive oxygen species (ROS) formation. Although leukocytospermia is defined as the presence of $\geq1\times10^6$ white blood cells/mL (WBC/mL) in a semen sample, the presence of less than 1×10^6 WBC/mL (low-level leukocytospermia) can still produce a detectable amount of ROS, impairing sperm function and lowering the chances of pregnancy. ROS are extremely reactive oxidizing agents, which a member of the class of free radicals. It was found that xanthine oxidase (XO) can act as pro-oxidant and therefore has a possible function in the initiation of spermatozoal oxidative damage. An additional source of $O2^{\bullet-}$ is the mainly endothelial cell-contained xanthine oxidase (XO). The present study focused the light on the correlation between xanthine oxidase and nitrosative stress.
Materials and Methods: Semen samples were obtained from 150 patients and divided into 3 groups: no seminal leukocytes; group 2, men with low-level leukoctyospermia ($0.1\text{-}1.0\times10^6$ WBC/mL); and group 3, frank leukocytospermia,($>1.0\times10^6$ WBC/mL). After liquefaction of the seminal fluid at room temperature, routine semen analyses were performed. Xanthine oxidase activity was measured fluorometrically. The stable metabolites of NO (nitrite) in seminal plasma were measured by nitrophenol assay. Arginase activity, NO synthase activity, total reactive oxygen species and total antioxidant status were measured spectrophotometrically.
Results: Conservative semen parameters between the three groups were similar. Xanthine oxidase activity, total reactive oxygen species and total antioxidant status, peroxynitrite levels, arginase activity, NO synthase activity and various sperm parameters were compared among leukocytospermic patients. Xanthine oxidase activity, NO synthase activity, total reactive oxygen species and peroxynitrite levels were significantly elevated with increasing leukocytospermia. Conversely, arginase activity was significantly decreased in the leukocytospermic patients.
Conclusion: Patients presenting with leukocytospermia have elevated levels of xanthine oxidase activity in parallel with elevation nitrosative stress levels.
Keywords: Leukocytospermia, Xanthine Oxidase, Nitric Oxide Synthase, Total Reactive Oxygen Species, Oxidative Stress

P-16: Lipid Profile of Testicular Seminiferous Tubules Following The Administration of Monosodium Glutamate and Quince Leaf Extract in Adult Rats: Histochemical Study

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Background: Monosodium glutamate (MSG) is sodium salt and is L-form of glutamic acid. This agent has toxic effects on human and

animals' tissues. Testicular hemorrhage, the alteration of sperm production and morphology are the most reported changes after administration of MSG. Herbal drugs have gained importance in treatment of several diseases. Flavonoids can inhibit the secretion of inflammatory mediators such as nitric oxide, interleukin XII and tumor necrosis factor alpha. Some studies mentioned the reduction of oxidative reactions following the administration of flavonoids. According to cytotoxic effects of monosodium glutamate on testicular tissue, the aim of this study was to evaluate the protective effects of quince leaf extract, as natural antioxidant and the source of flavonoids, on the reproductive dysfunction induced by monosodium glutamate through histochemical qualitative evaluation of tissue lipid alterations.
Materials and Methods: Monosodium glutamate (30 and 60 mg/kg/day i.p.) and quince leaf extract (500 mg/kg/day p.o.) was administrated separately or in combination form for eight weeks. Frozen sections were performed on formaldehyde fixed testicular tissue samples and stained with" Sudan Black B" method for identification of lipid droplets.
Results: The results showed that, in normal testicular tissue, Sudan black positive lipid droplets were seen in adluminal part of seminiferous tubules and no lipid droplets were seen in interstitial tissue. The most positive reaction was seen in MSG (30 mg/kg) administrated group and the least reaction were seen in (MSG 60+ Ext) group. Treatment of control group with quince leaf extract was lead to reduction in distribution and the size of lipid droplets.
Conclusion: According to the results, it has been concluded that, dose dependent administration of monosodium glutamate can induce some alterations in lipid profiles of testicular tissue which subsequently may lead to generation of various changes in spermatogenesis. Moreover, treatment with quince leaf extract may lead to reduction of lipid profile alterations.
Keywords: Monosodium Glutamate, Quince Leaf Extract, Lipid Profile, Testicular Tissue, Rat

P-17: Correlation of Reproductive Hormone Levels (Testosterone and Luteinizing Hormone) with Epididymal Sperm Quality

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Background: The interstitial tissue of the testis is the site of androgen production. The spaces between the seminiferous tubules are filled with connective tissue. Testosterone secretion by interstitial cells is promoted by the other gonadotropic hormone of the pituitary, luteinizing hormone (LH). The testosterone produced by the interstitial cells is necessary for the completion of spermatogenesis, so both FSH and LH are required for normal spermatogenesis.
Materials and Methods: Testis and blood samples used for this study were collected from forty five healthy adult rams from Hamadan abattoir. The viability, motility, and abnormal morphology parameters of the cauda epididymal sperm was assessed by means of the Eosin-Nigrosin stain method. Serum concentration of testosterone was measured by Enzyme Linked Fluorescent Assay (ELFA) method. Serum concentration of LH was measured by Enzyme Linked Immunosorbent Assay (ELISA). The datas were analysed by SAS software. The level of significance was set at $P<0.01$ and $P<0.05$.
Results: The mean ± SD (standard deviation) concentration of testosterone in blood serum was 1.63±2.19 (ng/ml). The mean ± SD concentration of LH in blood serum was 58.86±52.49 (ng/L). The means ± SD of sperm quality parameters were: mass motility (78.28 ± 11.40%), individual motility (1.97 ± 0.79 score), dead sperm (2.82 ± 4.23%), live sperm (97.17 ± 4.23%), tail abnormality (11.52 ± 4.74%), coiled midpiece (4.15 ± 2.49%), proximal cytoplasmic droplet (1.58 ± 1.75%), detached head (1.98 ± 3.82%), pyriform head (0.40 ± 0.50%), slender head (0.35 ± 0.72%), round head (0.27 ± 0.88%), macro cephalic (0.34 ± 0.58%), micro cephalic (0.22 ± 0.50%), twin head (0.15 ± 0.67%). In this study, no correlation was found between hormones and sperm quality parameters.
Conclusion: It has been clear that testosterone is a necessary prerequisite for the maintenance of established spermatogenesis in testes, and LH acts on testicular interstitial cells to promote the secretion of androgens, primarily testosterone. According to this study, there was no relationship between reproductive hormones (Testosterone and LH) and viability, motility and abnormal spermatozoa.
Keywords: Epididymal, Sperm Quality, Testosterone, LH, Ram

P-18: Evaluation of Genetic Variations in Exon 2 of KIF3B Gene in Infertile Men with Oligoasthenoteratospermia and Immotile Short Tail Sperm Defects

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Background: One of the main causes of male infertility is defect in structure and function of sperm cells. Infertile men with oligoasthenoteratospermia (OAT) defect, have sperms with abnormalities in count, motility and morphology. Patients with immotile short tail sperm (ISTS) disorder have immotile short-tailed sperm with disorganized axonem, and a significant decrease in sperm counts. Numerous proteins are involved in sperm formation and structure. One of these proteins is Kinesin Family member 3B (KIF3B), which recently its essential role in sperm intra-flagellar transport and PCD in male mouse has been demonstrated. So its gene, KIF3B is an appropriate candidate gene in study of human sperm abnormalities. Exon 2 of KIF3B gene, codes one of the main domains (Coild coil Domain) of the protein which is the location for binding to IFT20. The purpose of this study was to evaluate the genetic variations of exon 2 of KIF3B gene in infertile men.
Materials and Methods: In this study, 13 infertile men with OAT and 10 infertile men with ISTS defects were recruited. To study the genetic variations, DNA was extracted from peripheral blood, then PCR sequencing was done.
Results: Sequence analysis results did not identify any mutations or single-nucleotide polymorphisms (SNPs) in exon 2.
Conclusion: Although this preliminary data revealed no mutations or SNPs in exon 2 of KIF3B, due to the high expression of KIF3B gene in testis, and not many studies have been conducted about the exact role of this gene in human male infertility, evaluation of this region in a larger group is recommended.
Keywords: OAT, ISTS, KIF3B Gene

P-19: Laptop Computers May Affect Male Fertility

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Background: Male fertility may be affected by perching laptop computers on the lap, according to a new study.Balancing laptop computers on the laptop raises the scrotum, s temperature
Materials and Methods: This is a review article.
Results: About 15-20% of couples that want to get pregnant aren't able to conceive. Many of those cases trace back to issues relating to the male. Gradually declining sperm production has been noted in recent decades, say the researchers. Elevated scrotal temperatures have been linked to male infertility. Many factors can raise scrotal temperature, including hot baths, saunas, and tight jockey shorts.
Conclusion: Working on laptop computers in a laptop position causes significant scrotal temperature elevation as a result of heat exposure and posture related affects," say the researchers . Is the increase enough to impair male fertility? The researchers can't say for sure . However ,they note that another study showed that sperm concentration dropped by 40% when median daytime scrotal temperature rose by 1-2 degrees Fahrenheit (or 1 degree Celsius).Calling for more studies, they suggest that teenage boys and young men may want to limit their use of laptop cpmputers on their laps.
Keywords: Laptop, Scrotal Temperature, Fertility

P-20: Protective Effect of Thymoquinone on Sperm Parameters Toxicity induced Morphine in Male Mice

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Background: Opioids are the most potent and effective analgesics available and have become accepted as appropriate treatment for acute, cancer and non-cancer chronic pain. Morphine, which is commonly used for the treatment of severe pain, is metabolized essentially in the liver, gastrointestinal tract and kidneys. Thymoquinone petals consist of, glycosides, flavonoids, and anthocyanins. The main goal is to investigate whether the thymoquinone inhibit morphine adverse effects on sperm cells viability, count, motility and nitric oxid and testosterone hormone.
Materials and Methods: In this study, 48 male rats were divided in to 8 groups:control, morphine–treated group (20 mg/kg/day); Thymoquinone -treated groups (4/5,9,18 mg/kg./day); and morphine and Thymoquinone treated group interperitoneal administration for successive 3 days. These mice were randomly assigned to 8 groups(n=6) and sperm parameters (sperm cell viability,count,motility and morphology),testis weight, testis histology , nitric oxide and testosterone hormone were analyzed and compared.
Results: The result of our experimental study revealed that administration of morphine promoted male reproductive toxicity in mice. The intraperitoneal injection of morphine resulted degenerative changes in the seminiferous tubules and reduction in sperm count and motility, decrease of testosterone hormone and tetis weight and impairment of sperm cells evidence for this toxicity. The other side, increasing the dose of thymoquinone significantly boosted motility,count ,normal morphology of sperm cells, seminiferous tubules diameter and testosterone in all groups compared to morphine group.
Conclusion: It seems that Thymoquinone administration could increase the quality of spermatozoa and inhibit morphine-induced adverse effects on sperm parameters. However , further of studies are required for better understanding of the interaction between thymoquinone and morphine mechanism leading to changes of spermatogenesis
Keywords: Thymoquinone, Morphine, Sperm Parameters, Mice

P-21: Comparison of Serum Testosterone Levels before and after Varicocelectomy

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Background: The aim of the present study is to assess the effects of varicocelectomy on the serum testosterone levels and evaluate the improvement of the semen quality in infertile men with varicocele.
Materials and Methods: Comparisons were made between the total serum testosterone levels of 115 infertile men with varicocele with grade II and III with 240 fertile men preoperative. In addition, serum testosterone levels were normalized on the basis of age, grade and testis size. Accordingly, the infertile men with varicocele were evaluated for any improvement in the quality of semen before and after varicocelectomy. The unit of testosterone is expressed as ng/dl. The SPSS 20 software was used to analysis the data. All results of continuous variables were reported as means and Standard Deviation. Statistical significance was set at a P-value less than 0.05.Multiple regression analysis was used to identify potential predictors of the change in testosterone levels before and after surgery.
Results: The mean of patients' age and fertile men were 32.2(523) and 32.8(527), respectively (age rang 21 - 46). The mean of serum testosterone levels before surgery in infertile men with varicocele and fertile men were 567(222) and 583(263) ng/dl, respectively (P=0.558). No statistically significant changes were observed in serum testosterone levels. The infertile men were re-evaluated 3 – 6 month after surgery and the mean of testosterone level was set 594(243). However, the difference does not bear statistical significance P= 0.270. Evaluation of semen quality before and after varicocelectomy revealed the mean of count was 19.1(235) ($\times 10^6$/ml) and after varicocelectomy was 28.9(913) ($\times 10^6$/ml). The change was significant P≤0.001. Concerning other variables no significant differences were observed.
Conclusion: Varicocelectomy may induce a surge in serum testosterone levels in infertile men with clinical varicocele. Although the rise of testosterone was observed, these changes were not statistically significant. We predict that the positive effect is likely caused by improvement of the Leydig cells' function.
Keywords: Varicocele, Varicocelectomy, Testosterone, Infertility, Leydig Cell

P-22: Omega-3 Ameliorates Diabetes-induced Apoptosis on Testicular Tissue; Evidence for p53, Bcl-2 and Caspase-3 Expression

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Background: Previous reports have been shown diabetes adversely affects spermatogenesis and sperm parameters. Omega-3, n-3 fatty acids, is an unsaturated fatty acid, which exerts anti-inflammatory and antioxidant properties. Here in present study, the dose dependent effect of omega-3 on diabetes-induced apoptosis was investigated.
Materials and Methods: To follow-up current study, 32 mature male Wistar rats were assigned into four groups as; control (with no treatment), non-treated diabetes-induced (50 mg/kg sterptozotocin, ip), 300 mg/kg b.w-1 omega-3-treated and 600 mg/kg b.w-1 omega-3-treated groups. Following 45 days after diabetes induction, the testicles were dissected out. The expression of p53, Bcl-2 and caspase-3 were assessed by using reverse-transcriptase PCR and immunohistochemistry, respectively. Finally, the DNA fragmentation ratio of testicles was investigated by using DNA laddering test.
Results: Observations showed a significant reduction in expression of p53 and caspase-3 (at both protein and mRNA levels) in 600 mg/kg omega-3-received animals versus non-treated diabetes-induced group. More analyses for Bcl-2 showed that administrating 300 mg/kg of omega-3 increased Bcl-2 expression versus non-treated diabetes-induced animals. Meanwhile, the expression of Bcl-2 was decreased in 600 mg/kg omega-3-received group. Finally, no DNA fragmentation was revealed in 600 mg/kg omega-3-receieved group.
Conclusion: Our data showed that, omega-3 at dose level of 600 mg/kg b.w-1 reduced caspase-3 expression by enhancing the Bcl-2 expression. However, 300 mg/kg b.w-1 omega-3 reduced the caspase-3 expression as well as DNA fragmentation by up-regulating the p53 expression.
Keywords: Omega3, Diabetes, P53, Bcl-2, Caspase-3

P-23: Study gf Protective Effect of Vitamin E on The Blastocyst Stage Embryo in The Male Mice with Experimental Hypothyroidism

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Background: The variation in the performance of the thyroid gland has the correlation with the defect of the sexual activity and the degeneration of the testis. In several study the improvement of the sperm parameters have been reported when treatment with oral antioxidants is administrated. Vitamin E is effective in reducing adverse effects caused by oxidative stress and it is the basic component of the sperm antioxidant system.
Materials and Methods: In this study protective effect of vitamin E on the percent of the blastcist in the male mouse with experimental hypothyroidism was evaluated. For this purpose 42 adult male albino mouse were divided to 7 groups. all the groups except first, second and seventh groups were the experimental hypothyroidism (induced by propylthiouracil (PTU) in 35 days). The first group was the control group, The second group was injected corn oil (solvent of vitamin E),the third group was only hypothyroidism without any injection, the fourth and fifth and sixth groups were injected 50,100,200 (IU/kg) dose of vitamin E respectively. Seventh group was injected 200(IU/kg) of vitamin E in purpose of positive control. Super ovulation was conducted by intra-peritoneal injection of PMSG(10 IU) and HCG(10 IU) in 10 female mouse. After this period of time (10 -12 hrs after HCG injection) the animals were scarified by dislocation of cervical vertebra. Percent of the blastocyst embryos were evaluated.All of the data were analyzed by SPSS version 19 with bonfferoni test.
Results: Result of the present study indicated that the percentage of blastocyst increased significantly by increasing in the dose of the vitamin E and the third group(hypothyroidism without any injection) has the least percentage of blastocyst
Conclusion: Vitamin E effects on increasing percentage of blastocyst rate in hypothyroidism cases.
Keywords: Vitamin E, Blastocyst, Hypothyroidism, Propylthiouracil

P-24: Effect of Experimental Hypothyroidism on Fertilization Rate in The Male Mice

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Background: The variation in the performance of the thyroid gland has the correlation with the defect of the sexual activity and the degeneration of the testis. Common features to be seen in the wake of hypothyroidism include a decline in the locomotor activity of spermatozoa and abnormalities in maturation of cells of spermatogenesis, which facts necessitate restoration of the thyroid function to prevent disorders of spermatogenesis in the fully-developed organism.In other way transient gestational-onset hypothyroidism affects male fertility by impairing posttesticular sperm maturation process in the epididymis, owing to subnormal androgen(s) bioavailability and androgen receptors expression and functional activity.
Materials and Methods: In this study the effect of experimental hypothyroidism on the percent of the zygote in the mouse was evaluated. For this purpose 12adult male albino mouse were divided to 2 groups. The first group was the control (normal) group and the second group was the experimental hypothyroidism (induced bypropylthiouracil (PTU) in 35 days. super ovulation was conducted by intraperitoneal injection of PMSG(10 IU) and HCG(10 IU) in 10 female mouse. After this period of time (10 -12 hrs after HCG injection) theanimals werescarified by dislocation of cervical vertebra. Percent of the zygotes were evaluatedby considered male and female pronucleous in oocyts.All of the data were analyzed by SPSS version 19 with bonfferoni test.
Results: Result of the present study indicated that the percentage of fertilized oocyts decreased significantly in hypothyroidism group in comparison with control group
Conclusion: Hypothyroidism decreases fertilization rate.
Keywords: Fertilization, Hypothyroidism, Oocyt, Propylthiouracil

P-25: Effects of Morphine on Mean Plasma LH Concentration in D-Lys3-GHRP6-Treated Rats

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Background: D-Lys3 -GHRP-6 is a synthetic peptide which acts as a GHSR-Ia receptor antagonist. It blocks the inhibitory effects of ghrelin on reproductive axis activity. Morphine is an alkaloid which supresses GnRH/ LH release. It increases mean plasma ghrelin concentrations. In the present study the effects of morphine was determined on mean plasma LH concentrations in D-Lys3 –GHRP-6- treated rats.
Materials and Methods: Twenty Wistar male rats weighing 230-250g in four groups(n=5 in each group) received saline, morphine, D-Lys3 -GHRP-6 or simultaneous injection of morphine and D-Lys3 -GHRP-6 via third cerebral ventricle. Blood samples were collected via tail vein. Mean plasma LH concentration was determined by RIA method.

Results: Morphine decrease mean plasma LH concentration compared to saline. Mean Plama LH concentration was higher in the group which received simultaneous injection of morphine and D-Lys3 -GHRP-6 compared to morphine group.
Conclusion: Hypothalamic ghrelin pathway may be involved in the mediating inhibitory effects of morphine on reproductive axis activity in male rats.
Keywords: D-Lys3 -GHRP-6, Morphine, LH Concentration

P-26: Sub-Chronic Mancozeb- Induced Oxidative Changes in The Testes of Mice

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Background: Infertility affects 13-20% of couples around the world. male factors consist 25% to 50% of infertility causes. Exposure to environmental contaminants including pesticides has been considered as one of the main causes of male reproductive toxicity. Mancozeb(MZB), an organocarbamate fungicide that is widely used in agricultural. It has been shown to induce reproductive dysfunctions such as testicular damage. The present study was done for determination of sub-chronic exposure to Mancozeb fungicide on the testis antioxidant systems.
Materials and Methods: Adult male NMRI mice divided into three groups (n= 8 per group). One group was served as control (Group I) and received olive oil. Two groups were given 250(Group II), 500 mg/kg(Group III) MZB in diet, respectively .the regimens were administered by oral gavage once a day for 40 consecutive days. At the end of the treatment period, the animals were sacrificed and the testicular tissue samples were weighed and homogenized in phosphate buffer. The homogenates were used to determine malondialdehyde (MDA) concentration.
Results: There were significant increases in the MDA concentration in the group II and group III compared to the control group. Also MDA levels were significantly higher in the group III.
Conclusion: Our results demonstrate that sub-chronic administration of MZB can damage spermatogenesis through induction of oxidative stress which potentially can cause infertility.
Keywords: Mancozeb, Oxidative Stress, Testes, Lipid Peroxidation, Male Mice

P-27: Protective Effects of Wheat Sprout on Lead-Induced Testicular Toxicity in Rats

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Background: Negative effects of environmental contaminations and occupational exposure to lead on the male reproductive system have been shown broadly. In recent years, use of medicinal herbs in reducing heavy metal toxicities has increased worldwide. One of these herbals, wheat sprout, contains high amount of vitamins, antioxidants and phytoestrogen compounds. This study investigated the effects of wheat sprout extract (WSE) on testicular oxidative stress in rats exposed to lead acetate.
Materials and Methods: Twenty rats were divided randomly into four groups: G1 (control group) received 1 ml/kg/day of normal saline, G2 received 20 mg/kg/day of lead acetate, G3 received 100 mg/kg/day of WSE with 20 mg/kg/day of lead acetate and G4 received 200 mg/kg/day of WSE with 20 mg/kg/day of lead acetate. After 35 days, rats were sacrificed and blood, liver and testicle tissue samples were collected for histomorphological studies.
Results: Findings of lead assessment verified the presence of lead in liver and body of rats exposed to 20 mg/kg/day of lead acetate. Liver lead showed an insignificant decrease in combined lead and WSE (200 or 100 mg/kg/day) groups, compared to that in lead group. Testicular TBARS assessment showed a significant increase due to exposure to lead acetate, compared to that in control group ($P<0.05$). Serum testosterone showed a significant increase in combined lead and WSE (200 mg/kg/day) group, compared to that in lead group ($P<0.001$). Histomorphological studies showed a significant increase in tubular differentiation index (TDI), spermiogenesis index (SI), repopulation index (RI), number of Sertoli cells, and epithelium height and diameter of seminiferous tubules in combined lead and WSE (200 mg/kg/day) group, compared to that in lead group ($P<0.05$).
Conclusion: In summary, results of the current study show that dose dependent WSE significantly prevents testicular toxicity and oxidative stress effects of lead acetate.
Keywords: Wheat Sprout Extract, Lead, Testis, Oxidative Stress, Rat

P-28: Could Nanocurcumin be Considered as Safe Supplementary; Evidences for Apoptosis at Spermatogenesis Level

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Background: Due to high antioxidant properties, the curcumin is widely used in various fields of medication even as commercial Nanocurcumin (NCMN)-contained tablets. However, the dose dependent impact of NCMN on molecular interactions through spermatogenesis has been remained unknown. Thus present study was done in order to analyze the dose dependent effect of NCMN on spermatogenesis during one complete spermatogenesis in rat model of study. For this purpose, the main proteins involving in apoptosis are studied.
Materials and Methods: For this purpose, the animals were divided into 4 control and test groups. The animals in test group were subdivided into 3 groups as; low dose NC-received (7.5 mg/kg b.W.-1), medium dose NC-received (15 mg/kg b.W.-1) and high dose NC-received (30 mg/kg b.W.-1) groups. The expression of Bcl-2, p53 and caspase-3 at mRNA and protein levels were analyzed by using reverse-transcriptase PCR and immunohistochemistry, respectively.
Results: Our observations demonstrated that, NCMN at dose level of 7.5 mg/kg dose not significantly affect the expression of p53 and caspase-3 at mRNA and protein levels. Meanwhile, at higher dose level (15 mg/kg and 30 mg/kg) it significantly ($P<0.05$) up-regulates the p53 and caspase-3 expression at both mRNA and protein contents. More analyses for Bcl-2 showed that the NCMN, in three doses, reduced the expression of Bcl-2 at mRNA and protein levels.
Conclusion: Our data showed that, NCMN at dose level of 7.5 mg/

kg could be considered as safe dose for spermatogenesis. However, at dose levels of 15 mg/kg and 30 mg/kg NCMN promotes apoptosis by up-regulating the p53 and caspase-3 expression.
Keywords: Nanocurcumin, p53, Bcl-2, Caspase-3, Spermatogenesis

P-29: Semen Parameters and Sexual Hormones in The addicted Men following Heroin Abuse

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Background: Although illicit drugs use can be considered a major risk factor for several human diseases, the effects of them on male fertility are controversial. In this research we investigated the association between heroin abuse, semen quality, and sexual hormones in addicted and healthy men.
Materials and Methods: Semen and blood samples were collected from 10 addicts and 20 healthy men. Semen parameters including sperm count, motility, morphology and amount of WBC, RBC and germ cells were evaluated by CASA and special sperm staining. We assayed blood hormones including FSH, LH, Prolactin, Estradiol, and Testosterone by ELISA kits. T tests and nonparametric Mann-Whitney U tests were used to detect significant differences.
Results: Although heroin abuse did not diminish the sperm count in the addicted men, a decrease in sperm motility and an increase in immotile sperms were seen in the experimental group in comparison with controls ($P \leq 0.05$). However, no deference was observed in level of sexual Hormones between groups.
Conclusion: Heroin abuse can be associated with abnormalities semen parameters but not affected on sexual hormones in addicts.
Keywords: Heroin, Semen Parameters, Sexual Hormones, Infertility

P-30: Kinetic of T Cell Response in Testis during Experimental Autoimmune Encephalomyelitis

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Background: Multiple sclerosis (MS) is a demyelinating inflammatory disease of the central nervous system. Experimental autoimmune encephalomyelitis (EAE) is a widely used model for MS. It is known that MS affects women more than men and this has been attributed to sex hormones, and/or sex-linked gene, and more robust immune responses in females. However, it is more severe in males and sexual behaviors/fertility is changed in males affected by MS. The aim of this study was to determine the pattern of T cell response in testis during EAE.
Materials and Methods: EAE was induced in male Lewis rats using guinea pig spinal cord and complete Freund's adjuvant. The animals were evaluated for weight loss and clinical sign of EAE. The animals scored 1 through 5 were anesthetized and perfused transcardially. Then, testes were isolated, snap-frozen in liquid nitrogen, and stored at -80°C until use. Expression of mRNA for IFN-γ, IL-4, IL-17, and FoxP3 was determined as marker of Th1, Th2, Th17, and regulatory T cells, respectively, in testes of animals scored 1 through 5 using real-time PCR where β-actin was used as reference gene.
Results: Expression IFN-γ expression showed an increasing trends from animals scored 1 towards the animals scored 5. IL-17 and IL-4 level did not show a significant difference among the animals at the different score of the disease. However, IL-17/ IFN-γ and IL-4/ IFN-γ ratios both showed a decreasing trend and IL-17/IL-4 ratio remained unchanged. FoxP3 expression was significantly increased as the disease progressed.
Conclusion: IFN-γ has a regulatory role in testis different level of which determines inflammatory/anti-inflammatory condition in testis. Regulatory T cells have increase in number presumably as a compensatory mechanism for decreased activity.
Keywords: EAE, Testis, IFN-γ, FoxP3

P-31: The Effect of Date Palm Pollen on Morphology of Sperm Parameters

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Background: There are many ancient records of herbal medical plants. The phoenix dactylifera date palm pollen is used in the traditional medicine for male infertility. The aim if this study was to determine the effects of orally administrated date palm pollen on sperm parameters of infertile men.
Materials and Methods: In this clinical trial, 30 nonsmoker infertile men whose problem could not be solved surgically were enrolled. They were treated by date palm pollen for 2 months.7 gram of date palm pollen, was solved in drinking milk and administered 3 times a week during the study course. Semen analysis was done before and after the treatment and the results were compared.
Results: The mean sperm count was $12.33 \pm 5.61 \times 10^6$/mL at baseline and $22.03 \pm 12.17 \times 10^6$/mL after the treatment period ($P < .05$). The mean percentage of sperm progressive motility was $14.69 \pm 6.8\%$ before the treatment which increased to $24.01 \pm 11.11\%$ thereafter ($P < .05$). No significant increase was detected in sperm with normal morphology. Fertility rate is 16.6% in these patients. All pregnancies were resulted in term pregnancies.
Conclusion: Date palm pollen seems to improve the sperm count and motility in infertile men. Pregnancy outcomes has been remarkable in this natural treatment. We believe further studies on larger sample sizes are needed to elucidate the potential role and mechanism of action of date palm Pollen in the treatment of male infertility.

Keywords: Palm Pollen, Sperm, Male, Morphology

P-32: The Effect of Rosa Canina Extract on Motility Parameters and Malondialdehyde Concentration on Ram Semen after The Freeze-Thawing Process

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Background: The objective of this study was to investigate the effect of Rosa canina extract as a natural antioxidant on motility parameters and malondialdehyde concentration on ram semen during cryopreservation
Materials and Methods: Semen samples were collected from 5 Ghezel rams. five ejaculates (one ejaculate for each ram) were pooled and diluted with egg yolk Tris- based extenders containing 0 (R0), 100 (R100), 150 (R150) and 200 (R200) µm/mL of Rosa canina etanolic extract. Sperm motility (CASA) and lipid peroxidation (malondialdehyde concentration) were assessed after thawing.
Results: The results showed that R150 extender significantly improved total and progressive motility of post- thawed ram spermatozoa ($P<0.05$).The extender supplemented with 150 µm/mL Rosa canina extract have the best protective effect on freeze-thawed Ghezel ram sperm and significantly improved motility parameters ($P<0.05$). The R200 and R100 groups resulted in the highest and lowest of malondialdehyde concentration, respectively. 100 µm/mL of extract, decreased malondialdehyde concentration proportion to the other groups ($P<0.05$). However, this decrease was not significantly compared to control group.
Conclusion: It was concluded that the Rosa canina extract had an antioxidant potential that makes it useful for addition to semen extenders and that the best results are obtained with Rosa canina extract of 150 µm/mL.
Keywords: Ram, Sperm, Cryopreservation, Rosa Canina

P-33: Correlation between Expression of Neutral Endopeptidase (Nep/Cd10) and Aminopeptidase N (Apn/Cd13) in Normal Human Sperm Cells with Body Mass Index

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Background: Seminal plasma neutral endopeptidase (NEP/CD10) and aminopeptidase N (APN/CD13) are hypothesized to adversely affect sperm motility. Our aim in this study was to investigate the effects body mass index (BMI) changes on expression ratio of two enkephalin degrading enzymes APN and NEP in human sperm cells.
Materials and Methods: Semen and demoghraphic data were obtained from 12 healthy normozoospermic donors after 2–3 days of abstinence according to World Health Organization standards (WHO). Sperm cells isolated by a discontinuous percoll gradient (40–80%) followed up by swim-up techniques. The RNA of percoll-swim-up spermatozoa was isolated with the RNeasy kit. Semen parameters and expression APN and NEP genes by real-time polymerase chain reaction were evaluated. The results were analyzed by performing ANOVA (Tukey's tests).
Results: We found that expression of APN among men with BMI>30 was significantly more than BMI<30. Changes for APN was approximately two- fold (3.96 ± 2.73 vs. 1.72 ± 1.67) whereas for NEP was 5.62 ± 2.25 vs.3.89 ± 3.53.Also, Expression of APN in men with sperm motility rate between 45% and 65% ($3.78 + 2.23$) showed significantly difference than men with sperm motility >65% ($1.16 + 1.39$) ($P \leq 0.05$). Expression of NEP was no significantly different between mentioned groups.
Conclusion: BMI and sperm motility can be associated with aminopeptidase N (APN/CD13) in normal human sperm cells.
Keywords: Semen Parameters, NEP/CD10, APN/CD13, BMI

P-34: Impaired Mitochondrial Energy Production Pathways in Asthenozoospermia

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Background: Sperm acquire motility by the action of a specialized tail structure. In fact, the activation of dynein ATPase by phosphorylation of dynein arms initiates sliding of axenomal microtubules and subsequently propels sperm head. This process has a high requirement for ATP as energy source which is produced either via glycolysis or oxidative phosphorylation. However, there has been some ambiguity regarding the energy production pathway that plays the most critical role in sperm motility. Therefore, we combined high throughput proteome analysis with subcellular fractionation of sperm tail in order to elucidate the mechanism of energy production in asthenozoospermia.
Materials and Methods: This was a case-control study comprising 80 men who attended Royan infertility center. Semen samples were evaluated by computer assessed analyzer and divided into asthenozoospermic group (progressive motility <32%, n=40) and normozoospermic group (progressive motility >32%, n=40). Samples of four individuals were pooled and the tail fractions isolated by sonication and successive sucrose gradient. After confirming tail fraction purity, the extracted proteins were labeled with tandem mass tags (TMTs) followed by shotgun proteomics. Bioinformatic analyses were performed using DAVID. Candidate proteins were further validated by Western blot analysis.
Results: We detected 2145 proteins in the tail fraction of human sperm where 189 and 280 proteins were respectively up and down regulated in asthenozoospermic patients compared to normozoosper-

mic donors. The main down-regulated proteins were involved in energy production pathways. 72 down regulated proteins were localized in mitochondria, participating in electron transport chain complexes.
Conclusion: While both glycolysis and oxidative phosphorylation pathways are required to generate ATP for sperm motility, it turns out that mitochondrial energy production pathways may be impaired in asthenozoospermia.
Keywords: Glycolysis, Oxidative Phosphorylation, ATP, Sperm Motility and Asthenozoospermia

P-35: Effects of Sesame Seeds on The Level of Testosterone, LH and FSH in Young Adult Male Rats

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Background: The testosterone, LH, FSH, age, environment temperature, feed and medicine are effective on spermatogenesis and fertility. Sesame seed is one of phytoestrogenic lignans plants. It has estrogenic and antioxidant effects. Sesame seed containing large amount of sesamin, sesamolin lignans and vitamin E. Antioxidants, directly/ indirectly, increase the fertility rate. The purpose of this study is to evaluate the effects of sesame seeds on the pituitary- gonadal axis hormones in young adult male rats.
Materials and Methods: This experimental study was conducted on 20 young adult male Wistar rats (ages 4 months). Rats were divided randomly into 2 groups, experimental and control groups. Control groups received standard diet and the experimental groups received 70% standard diet +30% sesame seed for 60 days. At the end of experiment, the rats After measuring body weight, right and left testis were anesthetized and blood samples were taken. The level of, testosterone, LH and FSH measured by ELISA.
Results: The results showed that the level of testosterone and LH in the experimental group had a increased significantly compared to control group. There was no significant difference in level of FSH between groups.
Conclusion: The sesame seeds is positive effecton the pituitary- gonadal axis hormones and Increase the level of testosterone and LH in young adult male rats.
Keywords: Rat, Sesame Seed,Testosterone, LH, FSH

P-36: Mean Serum Ghrelin Concentrations following Central Injection of Kisspeptin and Peptide234 in Male Rats

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Background: Kisspeptin neuropeptides are synthetized in the arcuate nucleus of the hypothalmus. They stimulate reproductive axis activity. Peptide 234 is GPR54 receptor antagonist and it blocks the stimulatory effects of kisspeptins on the GnRH/LH release. Ghrelin is synthetized in the stomach and hypothalamus. It decreases the GnRH/LH release. The goal of the present study was to investigate the influence of peptide 234 on kisspeptin effects on mean serum ghrelin concentrations.
Materials and Methods: Fiftheen Wistar male rats weighing 220-250g in three groups(n=5 in each group) received saline, kisspeptin or simultaneous injection of kisspeptin and peptide 234 via third cerebral ventricle. Blood samples were collected via tail vein. Mean serum ghrelin concentration was determined by ELISA method. The data were analyzed by one- way ANOVA followed by post hoc Tukey test.
Results: Kisspeptin may decrease mean serum ghrelin concentration compared to saline. Peptide 234 may suppress the effects of kisspeptin on mean ghrelin concentration compared to kisspeptin group.
Conclusion: Hypothalamic kisspeptin pathway may be a central mechanism to control the reproductive axis activity via controling ghrelin secretion.
Keywords: Kisspeptin, Peptide 234, Ghrelin Concentration

P-37: The Effect of BSO-Induced Oxidative Stress on Ultrastructure of Testis and Sperm Fertility in Mice

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Background: There are evidences that oxidative stress play an important role in male infertility. The aim of this study is to investigate the effect of BSO-induced oxidative stress on ultrastructure of testis and sperm fertility in mice
Materials and Methods: In this study 30 adult male and 10 adult female mice are used. Male mice were divided into3 groups. In control group, mice did not receive any chemical. In experimental group, mice received 2mmol/kg BSO, 35days, IP injection. In sham group,mice received solvent of BSO(0.9% saline). After BSOtreatment, mice in 3 groups were sacrificed. Their testes were dissected, fixed in 2% glutaraldehyde, post fixed in 2% osmium tetroxide. Finally, embedded in pure resin, prepared for ultrastructural study. For determination GSH, GPX, SOD, CAT, MDA and testosterone level, blood was obtained from heart. Female mice were superovulated and killed 24 hours later. Oocytes were collected and inseminated with sperms for assessing fertilization rate. Data were analyzed by ANOVA and post hoc tests
Results: Determination of oxidative markers showed that the concentration of Catalase, GPX,MDA, SOD,GSH in experimental group, in comparison to other groups is reduced, while MDA level increased ($P<0.001$). Also serum testosterone level was decreased. Transmission electron microscopy was revealed in experimental group, in comparison to control group, cellular organels were decreased. Furthermore, detachment of basement membrane from cytoplasmic membrane and a large number of vacuolar spaces in experimental was observed. Finally we found, BSO administration caused a decrease in sperm fertilization capability
Conclusion: It is concluded that exposure to oxidative stress induced by buthionine sulfoximine (BSO) could affect ultrastructure of testis and sperm fertility.
Keywords: Oxidative Stress, Buthionine Sulfoximine, Testis, TEM, IVF

P-38: Expression of Adenosine Monophosphate-Activated Kinase, AMPK, in Human Spermatozoa and Its Possible Regulatory Ef-

fect on Cryopreserved Human Spermatozoa

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Background: Biochemical and physical modifications during the freeze-thaw process adversely influence the restoration of sperm energy-dependent functions required for fertilization. Adenosine activated protein kinase, AMPK, is a cell energy sensor and regulator of cell metabolism that has not been yet assessed in human spermatozoa. Our aim thus was to investigate; (i) the expression and intracellular localization of AMPK protein; (ii) its effects on fresh spermatozoa properties; (iii) its possible role in regulating post- thaw functions of human spermatozoa.
Materials and Methods: Spermatozoa from normozoospermic men were incubated in the presence or absence of different concentrations of AMPK inhibitor, Compound C (CC) or the AMPK activator, resveratrol (RSV) for different times and then were cryopreserved.
Results: AMPK is expressed in human spermatozoa and essentially localized in the whole flagellum and the post-equatorial region in the head. Viability of fresh spermatozoa was not significantly affected by the presence of CC/RSV. However, although CC caused a potent inhibition of spermatozoa motility parameters, RSV did not induce negative effect, except a slight reduction at 25 µM for 1 hour. Furthermore, RSV significantly increased AMPK phosphorylation and decreased reactive oxygen species (ROS) and incidence of apoptosis in frozen-thawed semen. It was not able to restore the decrease in sperm viability and motility parameters following cryopreservation. In contrast, CC showed opposite effects to RSV on AMPK phosphorylation, ROS, apoptosis, and motility parameters.
Conclusion: This study highlights the differential effects of AMPK modulators (CC/RSV) on crucial sperm processes such as motility, intracellular ROS, and apoptosis which can be altered upon freeze-thaw process, indicating the importance of AMPK activity for human sperm functions to accomplish their ultimate role of fertilization.
Keywords: Cryopreservation, Human Spermatozoa, AMPK, Motility, ROS

P-39: Doxycycline Can Suppress Mouse Sperm Cell Apoptosis and Testicular Injury Induced by Experimental Unilateral Testicular Ischemia-Reperfusion

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Background: Testicular ischemia-reperfusion (IR) can result in germ cell apoptosis and spermatogenesis disruption. The main objective of this study was to explore the effects of doxycycline (DC), an antibiotic with anti-oxidant and anti-apoptotic properties, on epididymal sperm apoptosis and testicular injuries following experimental unilateral IR in mice.
Materials and Methods: Experiments were performed on four equal groups each comprising six adult male mice. Following anaesthesia, IR was induced by clamping left testicular vessels with an atraumatic microvascular clamp for 30 minutes in IR group. In IR+DC group, in addition, mice received DC (2.5 mg/kg per day) intraperitoneally for 3 days starting from the day of induction of experimental IR. Vehicle-treated control group and DC-only treated group were also included. After 35 days, fluorescently-labeled annexin V binding assay and Johnsen's criteria were used to detect apoptotic sperms and categorize the spermatogenesis in the ischemic and contralateral sides, respectively. The data were analyzed by one-way analysis of variance followed by Tukey test for post hoc comparisons.
Results: Sperm cell apoptosis in both the ischemic and the contralateral epididymides increased significantly after IR. Moreover, IR resulted in histological damages in the ipsilateral and contralateral testes. Notably, treatment with DC improved IR-induced negative changes in sperm apoptosis and spermatogenesis.
Conclusion: These findings provide evidence that DC treatment may have potentially protective effects against long-term reproductive injuries following IR.
Keywords: Ischemia-Reperfusion, Doxycycline, Testis, Sperm, Apoptosis

P-40: Effect of A Cissampelos Capensis Rhizome Extract on Capacitation, Ca2+-fluxes and Acrosome Reaction in Human Spermatozoa

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Background: Cissampelos capensis is a South African indigenous medicinal plant traditionally used to treat a variety of conditions including male infertility. However, few studies have investigated the effects of this plant or its extracts on human spermatozoa. Therefore, this study aimed at investigating the effects of fractions of Cissampelos capensis rhizome extract (CRE) after fractionating using a methanol gradient on ejaculated human spermatozoa *in vitro*.
Materials and Methods: Methods: This study encompasses HPLC analysis by using a C18 column to isolate 4 CRE methanol fractions (F1, F2, F3, F4). HPLC profiles were obtained for different seasons of harvesting (Autumn, Winter, Spring and Summer). Human spermatozoa from a total of 55 semen samples were washed with human tubular fluid medium supplemented with bovine serum albumin (HTF-BSA) and incubated for 2 hours with 20 µg/ml progesterone (P4) followed by incubation with different concentrations (0, 0.05, 200 µg/ml) of CRE fractions (F1, F2, F3 and F4) and control (without extract) for 1.5 hours at 37°C. Samples were analyzed for calcium homeostasis, capacitation, sperm motility, reactive oxygen species (ROS) modulation, DNA-fragmentation as well as acrosome reaction. For Ca^{2+} flux studies, a high-throughput fluorescence Ca^{2+} flux assay was used.
Results: The summer season exhibited a significantly higher effect compared with the other seasons. The F1 fraction from the summer harvest showed significantly higher values for calcium homeostasis, capacitation, ROS-positive sperm, DNA-fragmentation, acrosome reaction and hyper-activation compared to all other fractions; with the

P4-stimulated samples generally having higher values.
Conclusion: The characterization of the biological effects including pinpointing the targets in the biochemical pathway of capacitation and induction of acrosome reaction in human spermatozoa, apparently by triggering sperm intrinsic ROS production leading to sperm capacitation and acrosome reaction induced by P4.
Keywords: Capacitation, Calcium, Acrosome Reaction, DNA Fragmentation, ROS

P-41: Simvastatin Treatment Exerts Reproprotective Effect in A Mouse Model of Unilateral Testicular Ischemia-reperfusion

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Background: Ischemia-reperfusion (IR) is a main etiology of spermatogenic cells dysfunction following testicular torsion. In this study, we assessed the effects of simvastatin (SIM), a potent hypolipidemic drug with antioxidant and anti-inflammatory activities, on bilateral testicular damages following experimentally-induced unilateral testicular IR in mice.
Material and Methods: Adult male mice were divided into four groups (n = 6, each). Following anaesthesia, IR was induced by clamping left testicular vessels with an atraumatic microvascular clamp for 30 minutes in IR group. In IR+SIM group, in addition, mice received SIM (20 mg/kg per day) orally for 3 days starting from the day of induction of experimental IR. Vehicle-treated control group and SIM-only treated group were also included. Johnsen's scores were recorded in the ischemic and contralateral testes five weeks postoperatively.
Results: Grading of histopathological alterations in the seminiferous tubules showed that SIM treatment significantly increases spermatogenesis in both testes compared with IR group.
Conclusion: Administration of SIM could enhance spermatogenesis and improve reproductive functions after IR. However, further studies to uncover the protective efficacy of SIM in human subjects are necessary.
Keyword: Ischemia-reperfusion, Simvastatin, Testis, Histology, Mice

P-42: E.coli Fim H, Pap and Afa GenesRelation with Male Infertility

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Background: Urinary infections are among important factors in the male infertility. Amongst bacteria, Escherichia coli plays a large role in the destruction of the spermatogenesis. Identification of genes and virulence factors of the E. coli involved in the male infertility would be valuable tools in the prevention and treatment of the male infertility.
Materials and Methods: 100 semen samples were analyzed and cultivated. Applying biochemical analysis the types of the pathogens were identified. E. coli samples were cultivated for DNA extraction and PCR was used for detecting and identifying the prevalence of fim H, pap and afa genes.
Results: Among the 100 samples, 53 were contaminated with the pathogens including 18.86% E. coli, 33.33% Staphylococcus, 45.09% Streptococcus, and 5.88% Klebseilla. The E. coli containing samples has shown sperms with abnormal motility and a morphology of the abnormal sperms. Applying PCR, analysis of the genes has shown that 100% of the E. coli isolates have fim H and pap genes, however, non of samples had afa gene.
Conclusion: Results shows that among the 53% contaminated samples with the different types of the pathogens 10% was E. coli. E. coli is an important genital pathogen, knowing the status, extent, and prevalence of the pathogenic genes, including adhesins is valuable in the control and treatment of the male infertility. In this regard observing fim H and pap genes in the E. coli infected infertile males strongly establishes a relationship between these two genes and the infertility in males.
Keywords: E. coli, Genes (fim H, pap and afa), Infertility, Urinary Infection

P-43: Comparison of Sperm Chromatin Structure and DNA Methylation before and after Varicocelectomy

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Background: Percentage of DNA damage is high in infertile men with varicocele due to high level of oxidative stress. Damaged DNA is less predisposed to DNA methylation. Varicocelectomy can overcome DNA damage but it is not clear that whether this surgery could improve sperm DNA methylation. Therefore, we compared sperm chromatin structure, DNA methylation and sperm parameters before and after varicocelectomy.
Materials and Methods: Sperm parameters (CASA system), DNA fragmentation (TUNEL staining), protamine deficiency (CMA3 staining), oxidative stress (DCF staining) and global DNA methylation (Flow cytometery) were evaluated before and 3 months after varicocelectomy in 30 infertile men with left-sided varicocele (grade II &III).
Results: Unlike sperm parameters, DNA fragmentation, protamine deficiency and oxidative stress significantly improved after surgery, percentage of sperm motility and global DNA methylation insignificantly improved after surgery.
Conclusion: DNA is hypo-methylated in infertile men with varicocele and susceptibility to DNA damage is increased. Further studies need to confirm this result in large population.
Keywords: Varicocele, DNA Methylation, DNA Fragmentation, Protamine Deficiency, Oxidative Stress

P-44: Investigating Effects of Aqueous Root Extract of Mondia Whitei in Human Sperm Functionality

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Background: Mondia whitei commonly known as "White Ginger" is a highly acclaimed medicinal plant that is extensively used by traditional medicine practitioners across Africa in the treatment of sexual dysfunction. Yet, scientific evidence to support the therapy is minimal and those that are published possess ambiguity. To date only one study reporting the effect of the root extract of M. whitei on human sperm motility is available. The aim of the study was to determine the in vitro effects of M. whitei in human sperm functions
Materials and Methods: A total of 60 (28 healthy donors and 32 infertile patients) semen samples were collected. Sperm were washed using human tubular fluid medium supplemented with bovine serum albumin (HTF-BSA) and incubated for 1 hour at 37˚C with different concentration of M. whitei (0, 0.0185, 0.185, 85, 18.5 and 185µg/ml). A sample without M. whitei served as control. Sperm cell motility, vitality, reactive oxygen species production (ROS), mitochondrial membrane potential (MMP), capacitation, acrosome reaction and DNA fragmentation were assessed.
Results: Total motile and the percentage of sperm with intact MMP showed significant dose-dependent increases in both groups (patient and donor). Moreover, the percentages of progressively motile sperm, ROS-positive spermatozoa also revealed significant increases while a trend towards reduced sperm DNA fragmentation could be observed in the patient but not the donor group. Yet, sperm vitality, capacitation and acrosome reaction and kinematic parameters were not affected.
Conclusion: Phyto-chemicals found in M. whitei root extract increase total motility, progressive motility and intact MMP in a dose-dependent manner. However, it does not trigger sperm intrinsic super oxide production that leads to DNA fragmentation.
Keywords: Mondia Whitei, Infertility, Human Spermatozoa, Sperm Functionality, Traditional Medicine

P-45: Effects of Alfalfa on Sperm Motility and Sperm Viability in Young Adult Rats

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Background: Alfalfa is one the phytoestrogen plants and an important part of the diet of livestock animals. Alfalfa a variety effects on the reproduction of animals. Some believe that the alfalfa is reduced fertility in animals. The aim of the this study was to investigate the effects of alfalfa on the sperm parameters in rats.
Materials and Methods: This experimental study was conducted on 20 young adult Wistar rats (3-4 months). Rats were divided randomly into 4 groups, experimental (60,30 days) and control (60,30 days) groups. Control groups received standard diet and the experimental groups received standard diet(80 g) +fresh alfalfa(300 g). At the end of experiment, body weight, testis and epididymis weight, measuried. For evaluation of progressive motility,Sperm viability, normal morphology and the total count of sperm, the right tail of the epididym was selected and transferred to Tyrode's Solution.
Results: The results showed the progressive sperm motility and sperm viability in the experimental groups had increased significantly compared to the control groups.
Conclusion: Alfalfa not only had no negative effects on sperm parameters but also have positive effects on rat sperm parameters.
Keywords: Alfalfa, Epididymis, Phytoestrogen, Rat, Sperm

P-46: Association between Alterations of Akap4 Binding Domain Coding Region and Immotile Short Tail Sperm Defect

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Background: One kind of sperm abnormality that leads to men infertility is immotile short tail sperms (ISTS). In this defect, fibrous sheath (FS) and axoneme are disorganized, the sperms tail is short and the sperms are immotile. Assembly of the FS and other cytoskeletal structures that is dependent on specific protein-protein interactions. In this regard, AKAP4 provide a framework for the transport and assembly of other proteins comprising the FS. AKAP4 by domains interacts with a number of other proteins in the fibrous sheath. One of these is AKAP3, another important component of the fibrous sheath.
Materials and Methods: In this study, 32 infertile men with ISTS defect (with more than 80% short tail sperms in at least two spermograms) and 50 normozoospermic men as control were enrolled. After DNA extraction, primers were designed for target segment of AKAP4 gene, and then PCR sequencing was done.
Results: Sequence analysis results did not identify any mutations or single-nucleotide polymorphisms (SNPs) in exon 5. But, we found a deletion that involved the potential AKAP4/AKAP3 binding regions in a patient under study while the presence of these PCR products was detected in fertile men and other infertile men with ISTS.
Conclusion: Although our data did not significant different between patients and control group, but based on the key role of AKAP4 in sperm tail formation, study in a larger population and determination of levels of mRNA and protein from the AKAP4 gene may lead to more accurate results.
Keywords: Male Infertility, Short Tail Sperm, AKAP4 Gene

P-47: Body Mass Index in Relation to Reproductive Hormones of Infertile Men

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Background: Failure to conceive while using assisted reproductive techniques, suggests the need for further analysis of additional semen parameters. Regarding hormone roles in spermatogenesis, examination of the relationship between body mass index (BMI) and reproductive hormones among infertile men, is one of the important keys in the field of male infertility diagnosis.
Materials and Methods: In this research, 219 infertile men undergoing assisted reproductive treatment were evaluated with male hormone levels including follicle-stimulating hormone, luteinizing hormone and testosterone of the different BMI (18.5-24.9, 25-29.9, and >30 kg mm), between 2012 to 2014. Statistical analysis was performed using SPSS version 20. The effects of BMI on the hormone

levels have been done using multinomial logistic regression.
Results: It was found that factors such as body mass index (BMI) influence testosterone levels. So that, a relationship between increasing paternal BMI and decreasing testosterone level was observed (95%CI: OR of 0.048 to 0.277 and 0.229 to 1.229 for <24.9 and 25-29.9, respectively) as opposed to the medium level of this hormone and BMI >30.
Conclusion: Physical factors such as BMI change male reproductive hormones and these hormonal changes could correlate with sperm quality and assisted reproductive outcomes. If so, some cases of subfertility may be preventable.
Keywords: Male Reproductive Hormone, Infertility, Body Mass Index, Physical Factor

P-48: Evaluation of Correlation of Food Intake and Semen Quality

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Background: Human semen quality have been deteriorated during the past decades. Approximately 30% of all couples in Iran experience infertility in their reproductive years. Therefore, this study was designed to evaluate the effect of food intake on semen quality, in order to find risk factors of male infertility.
Materials and Methods: This case-control study was conducted in Infertility Center of Isfahan Shahid Beheshti Hospital, in July to December 2015. The food intake pattern in infertile men (case group) was compared to healthy individuals without any kind of infertility problems (normospermic) (control group). Two groups were formed on the basis of seminal quality and following World Health Organization (WHO) criteria. The frequency of food consumption was measured by Food Frequency Questionnaire (FFQ) which registered on a scale with five values, ranging from no consumption to repeated daily consumption. The questionnaires were completed by the same interviewer, and a different questionnaire recorded information on weight, height, blood pressure, waist circumference, lab tests (fasting blood glucose and triglyceride) , and smoking habit.
Results: As obtained, some dietary intakes were significantly lower in case subjects including cheese (1.88 vs 2.66, P=0.02), different kinds of bread (1.98 vs 3.1, P=0.001), rice (1.64 vs 3.18, P<0.001), sausages and salami (0.06 vs 0.24, P=0.05), fast foods (0.08 vs 0.34, P=0.02), citrus fruits (1.62 vs 2.6, P=0.001), different kinds of fruits (1.72 vs 2.52, P=0.009), tea (3.22 vs 4.06, P=0.012), coffee (0.42 vs 1.3, P=0.003).
Conclusion: We found that some dietary intakes were significantly lower in case subjects including cheese, different kinds of bread, rice, sausages and salami, fast foods, citrus fruits, different kinds of fruits, tea and coffee. A large scale study is needed to come to clinical guidelines and recommendation for obstetricians.
Keywords: Food Intake, Semen Quality, Infertility

P-49: Testicular Torsion Treatment as A Male Reproductive Health Concern

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Background: Testicular torsion is a true urological emergency and its annual incidence in men/boys younger than 25 years is 1 in 4000. This acute condition occurs most often (40-65%) in the neonatal period and around puberty. Testicular salvage rates following appropriate diagnostic and surgical managements range from 42 to 88%. The main pathophysiological mechanisms underlying bilateral testicular damages following torsion-detorsion have been shown to be related to ischemia-reperfusion.
Materials and Methods: In this systemic review study, 40 papers in English data bank including: Science Direct, PubMed, Wiley and Google Scholar have been investigated from 2000 until 2016. The keywords were testicular torsion, ischemia- reperfusion and male reproductive system.
Results: Semen analyses as well as oxidative stress, histopathological and blood parameters evaluations after testicular torsion surgical treatments in experimental studies have shown marked abnormalities in both testes compared to normal standard values.
Conclusion: Based on critical roles of reactive oxygen species overproduction and inflammatory responses in the bilateral testicular malfunction following torsion invasive treatment, post-surgical administration of safe anti-oxidant and anti-inflammatory agents could be beneficial.
Keywords: Torsion, Ischemia-reperfusion, Testis, Infertility

P-50: The Effect of Antioxidant Combinations on In Vitro Semen Quality in Ghezel Rams

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Background: The objective of this study was to evaluate the effect of Antioxidant Addition including: Rosa canina, Salvia Sahendica, Salvia officinalis, Cornus mas, Stachys schtschegleevii to semen extender on in vitro Semen Quality in Ghezel Rams.
Materials and Methods: Progressive motility, viability, plasma memebrane integrity, total antioxidant capacity and enzymatic antioxidant (GPx) were examined. Ejaculate samples were collected with artificial vagina from five Ghezel rams during non- breeding reproduction season. Semen was diluted with a soybean lecithin based-extender containing group1,without antioxidants, group 2 antioxidants in low concentration (50 µm/mL) and group 3 antioxidants in high concentration (200 µm/mL). Diluted semen was cooled to 5º C. Semen was investigated after 24, 48 and 72h.
Results: Progressive motility and viability in control group were higher than two treatment groups. The total antioxidant capacity in low antioxidants supplemented group was significantly higher than other groups but in enzymatic antioxidant significant changes were not observed.
Conclusion: Antioxidant supplementation of lecithin based-extender improved total antioxidant capacity and plasma memebrane integrity fresh sperm during storage at 5º C for 48h. Sperm storage time had a significant effect on sperm quality. Based on this study, there were no combinations more efficient at combating semen quality than control group.
Keywords: Sperm, Antioxidant, Extende

Animal Biotechnology

P-51: Protective Effect of Genistein Against Morphine-Induced Damage on Nitric Oxide and Testosterone in Male Mice

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Background: Fertility is considered as a life conservative phenomenon among married couples which can be obliterated by various conditions affecting both males and females. In the other hand addiction is a problem which increasingly developed among the various populations throughout the world, and there are evidences that addiction may affect the hypothalamous-pituitary-gonadal axis and sexual functions. Natural products including plants, minerals and animals have been the basis of treatment of human diseases. The predominant phytoestrogen in soy and derived products is the isoflavone Genistein. Genistein has antioxidant properties. The main goal is to investigate whether the Genistein could preventive Morphine adverse effects on nitric xide level and and testosterone hormone blood serum.
Materials and Methods: In this study, various doses of Genistein (1, 2, and 4 mg/kg) and Genistein plus Morphine were administered interaperitoneally to 48 male mice for 30 consequent days. These mice were randomly assigned to 8 groups (n=6). he animals were anesthetized 24 hours after the last injection. By cardiac puncture method blood from sacrificed mice were collected into sterile collection vials and preserved in the 6 temperature of 37 ºC for 30 minutes and was centrifuged to obtain the serum (3000 g for 15 minute). Serum samples were directly frozen at -70 ºC until biochemical analyses. Serum testosterone concentrations were measured by ELISA (Abcam 108666, USA) method. NO concentration in the blood serum was determined with the Greiss method. Samples' optical density (OD) was measured by ELISA reader at the wavelength of 540 nm.
Results: Morphine caused a significant decrease in the testosterone hormone compared to Saline group (P=0.00). In addition, the testosterone hormone increased significantly in Genistein (P<0.05) and Genistein plus Morphine in all groups administration compared to Morphine group (P=0.024). The mean of Nitric oxide in blood serum increased significantly in Morphine group administration compared to Saline group (P=0.00). Also, the mean of Nitric oxide in blood serum decreased significantly in Genistein (P=0.00) and Genistein plus Morphine in all groups compared to Nicotine group (P=0.00).
Conclusion: The results of current research showed that Genistein could enhance the sperm count owing to the growth and proliferation of germinal cells resulting from testosterone increase and reducing effects of ROS production. Also , suggest the protective potential of Genistein especially at low doses studied against toxic effects of Morphine -treated male mice.
Keywords: Genistein, Morphine, Nitric oxide, Testosterone

P-52: Fluoxetine Induces Hypospermatogenesis in Adult Rats

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Background: Fluoxetine (FLX) application as a selective serotonin reuptake inhibitor (SSRI) drug has been accompanied by untoward side effects including reproductive dysfunctions. This study analyzed the influence of FLX treatment on spermatogenic process in adult rats.
Materials and Methods: Mature male Wistar rats were randomly assigned into experimental and control groups. The experimental group subdivided into two groups, which received 5 mg/kg/day and 10 mg/kg/day FLX, orally for 48 days. Testicular samples were collected 24 hours after the last treatment and mean testicular biopsy scores (MTBS) were determined to categorize the spermatogenesis.
Results: Treatment with FLX caused spermatozoa maturation arrest in a dose-dependent manner as evidenced by significant decreases in MTBS.
Conclusion: These findings suggest that there is a high degree of male infertility risk associated with use of FLX, bringing about the necessity of more researches about the precise mechanisms of SSRI antidepressants-induced spermatogenic failure.
Keywords: Fluoxetine, Spermatogenesis, Testis, Rat

P-53: Induction of Early Puberty following Dose Dependent Administration of Monosodium Glutamate in Adolescent Rats: Epididymal Sperm Analysis

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Background: Monosodium glutamate (MSG) is a food additive which acts as preservative or enhancer of palatability. Male infertility, obesity and hypogonadism have been reported following administration of MSG in adult rats. The aim of this study was to evaluate the possible effects of MSG on the puberty in adolescent rats.
Materials and Methods: 25 days old rats were divided into three control, low dose MSG (6 mg/kg/day) and High dose (60 mg/kg/day) MSG groups. MSG was administrated orally for 40 days. At the end of study, the animals were euthanized and epididymal sperm analysis was performed.
Results: The results showed that, high dose of MSG significantly led to increase of total sperm count and sperm motility in comparison to other groups. Low dose of MSG was not affecting the production of spermatozoa.
Conclusion: In this study, the results of sperm analysis indicated that, despite of previous studies shown that, some alterations in spermatogenesis have been observed following use of MSG in adult rats, the administration of monosodium glutamate in adolescent rats during the period before sexual maturity dose dependently can lead to induction of early sperm production.
Keywords: Adolescent Rats, Early Puberty, Monosodium Glutamate, Sperm Analysis

P-54: Optimization of Sperm Mediated Gene Transfer: Effects of Incubation Times for Post-Transfected Sperm Quality

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Background: Recently, a method of producing transgenic poultry is using the transmission technique with sperm. Current study was done to determine the effects of different times of incubation for gene transfer in cock of sperm and exogenous gene on sperm quality of post-transfected sperm.
Materials and Methods: Genetic engineering methods were applied for the ligation of human βFSH to the PcDNA 3.1+ and following

cloning. Semen samples were collected from six roosters and were prepared by twice washes to prevent DNase activity. Spermatozoa transfected with linearized PcDNA3.1+/ βFSH vector as following groups: 1) Fresh sperm without transfection processing. 2) Incubation of sperm and DNA at the time of 0 3) Incubation of sperm and DNA at the time of 30 min 4) incubation of sperm and DNA at the time of 45 min5) of incubation of sperm and DNA at the time of 60 min. After transfection, total and progressive motility, velocity parameters (VCL, VSL, VAP, LIN and STR), membrane integrity, viability and DNA uptake by sperm were assessed.
Results: PCR analysis detected the presence of βFSH in the rooster sperm .Treatments had significant effects on the viability, motility, progressive motility, membrane integrity, velocity of post-transfected sperm (P <0/05). Moreover, increasing the time of incubation from zero to 60 minutes, significantly reduced the viability, motility, progressive motility and membrane integrity of post-transfected sperm (P <0/05).
Conclusion: The highest significant percentage of motility (90/2±1/25), viability (73±1/55), progressive motility (58.6±1/57) and membrane integrity (71/3±1/24) of sperm were observed at the time of 0 and 30 min after transfection. (P <0/05).
Keywords: Transgenic Poultry, PcDNA, βFSH, Viability, Motility

P-55: Effect of Coenzyme Q10 Supplementation on Sperm Motility, Oxidative Stress Index and Plasma Membrane Integrity of Ram Semen

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Background: Co-Q10 is only lipid transport in mitochondrial respiratory chain. In addition, coenzyme Q10 is a fat property like so release of phospholipids at cell membrane and protects the sperm plasma membrane (SPM). CoQ10 acts as an antioxidant to prevent the lipid peroxidation to stabilize cell membranes and preserve cell functions.
Materials and Methods: The purpose of this study was to assess the role of Q10 on sperm motility, level of oxidative stress marker, monitored by lipid peroxidation and plasma membrane integrity of frozen-thawed Ghezel ram semen. It has been shown that several antioxidant including the mitochondrial cofactor increase endogenous antioxidants or mitochondrial bioenergetics. Ejaculate samples were collected with artificial vagina from five adult rams and diluted with a lecithin-based semen extender containing co-Q10 (0.5, 1, 2, 2.5 mM) and without antioxidants (control). Diluted semen was cooled to 4ºC and frozen in 0.25 ml straws, prior to being stored in liquid nitrogen. Semen parameters were investigated after thawing.
Results: Malondialdehyde levels did not change with addition of co-Q10 in extenders compared with the control group. Motility improved on 0.5 and 1 mM co-Q10 level. Plasma membrane integrity increased at level 0.5mM and decreased in 2.5 mM co-Q10.
Conclusion: In conclusion, the results were lead to ameliorate quality of sperm using 0.5 mM coq10 level studied in this experiment.
Keywords: Semen, Co-Q10, Motility, MDA

P-56: Effect of Co-Q10 on Total Antioxidant Capacity and Enzymatic Antioxidants of Ram Semen

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Background: Coenzyme Q10 is a lipid soluble antioxidant that participates in electron transport during oxidative phosphorylation in mitochondria protection against oxidative stress. The supplementation of a cryopreservation extender with antioxidant has been shown to provide a cryoprotective effect on ram sperm quality.
Materials and Methods: The aim of the present study was to evaluate the effect of co-Q10 (Q10) on total antioxidant capacity (TAC) and study their r association with enzymatic antioxidants. Total antioxidant capacity (TAC), glutathione peroxides (GPx) and superoxide dismutase (SOD) was evaluated in ram semen. Semen samples were collected by artificial vagina from five mature Ghezel native breed rams (3 and 5 yr. of age). Samples were pooled, diluted with lecithin-based semen extender without any antioxidant (control) or supplemented with different concentrations of Q10 (0.5, 1, 2 and 2.5 mM), at a final concentration of 200×10^6 sperm/mL. Samples freezed and stored on liquid nitrogen. Finally, samples thawing on 37ºC for 30 second.
Results: No significant differences were observed in TAC and GPx between control and treatments. This experiment SOD were increased (P<0.05) by supplementation of 2.5 mM Q10.
Conclusion: The present study show that coenzyme Q10 supplementation can decrease the destructive effects of the free radicals on 2.5 mM level.
Keywords: Semen, Q10, Total Antioxidant Capacity, Enzymatic Antioxidants

P-57: Beneficial Effects of L-Argentine on Frozen-thawed Fertility of Rooster Sperm

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Background: Fertility of post-thawed rooster sperm is not desirable for artificial insemination in commercial stocks. The purpose of this study was to optimize the procedure of rooster sperm freezing via supplementation of extenders with L-arginine. Antioxidant capacity of L-arginine may reduce the oxidative stress during cryopreservation of sperm via scavenging the reactive oxygen species. Moreover, L-arginine has crucial role for metabolism of Nitric Oxide (NO) that can induce intercellular pathway to protect sperm against apoptosis. In this study, different concentrations of L-arginine (0, 2, 4 and 6 mM) have evaluated on the rooster sperm parameters after freezing-thawing.
Materials and Methods: Semen samples were collected form 4 Ross roosters and then pooled to eliminate the individual differences. Pooled sperm then divided into 4 aliquot parts to dilute according to following groups: 1. no additive, 2. 2mM, 3. 4mM and 4. 6 mM of L-arginine in extenders. For freezing procedure, diluted sperm was aspirated into straws and equilibrated at 4oc for 30 minutes and then cryopreserved according to our previous study. Motion characteristics, viability, membrane integrity and mitochondria activity were assess after thawing to consider the optimum concentration of L-arginine.
Results: Results showed that higher motility of sperm was observed in group containing 2 and 3 mM L-arginine (58.2 ± 1.5, 61.7 ± 1.5) compare to other groups. For progressive motility, there was no significant different between groups. The highest percentage of membrane integrity and mitochondria activity were obtained in group containing 4mM cysteine (47.3 ± 1.5, 62.7 ± 1.7, respectively). For viability, L-arginine had not significant effect on the frozen-thawed sperm.
Conclusion: L-arginine is suitable additive to be more investigate for cryobiology of rooster sperm. It may enhance or preserve the quality

of rooster sperm during freezing-thawing.
Keywords: Cryopreservation, L-Arginine, Rooster Sperm, Motility, Mitochondria Activity

P-58: Expression Pattern and Functional Characterization of NANOG During Goat Pre-implantation Development *In Vitro*

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Background: Embryonic stem (ES) cells represent a valuable model for investigating early embryo. The pluripotent and self-renewal status of embryonic stem cells is maintained by a complex system of molecular signaling pathways and transcription factors. NANOG is one of transcription factors which are among the key regulators in this system. The molecular features and transcription regulation of the NANOG gene in domestic animals are not well investigated.
Materials and Methods: To examine the function of NANOG in goat ES cells, first we investigated expression NANOG in preimplantation goat development with spatio-temporal pattern and it was analyzed in regulation of development by Real time RCR then we performed a goat knockdown of NANOG and determined the effects of elimination zygotic expression of NANOG on the cellular differentiation. We collected *In Vitro* fertilized goat embryos in zygote stage and injected NANOG and SCR siRNA into each zygote, and cultured until the blastocyst stage. We assessed cleavage and blastocyst formation rates in uninjected controls, SCR - and siRNA-injected embryos.
Results: In the present study, pattern of NANOG mRNA expression was observed in the goat preimplantation embryos. For *In Vitro* fertilized embryos, the transcript of NANOG could be detected from oocytes to blastocyst stage, highest levels of NANOG transcript was detected at the 2 to 8-cell and it continues during preimplantation development. Cleavage and blastocyst rates in the siRNA-injected group had lower effect than both the control group and the SCR group at 2-8 cells and blastocyst .Embryos lacking NANOG show abnormalities in the number of ICM, or total cells in the blastocyst.
Conclusion: Based on our findings we propose that maybe NANOG is required *in vitro* for establishment and maintenance of pluripotency and cell division in early developing embryos.
Keywords: NANOG , Short-Interfering RNAs (siRNAs), Goat, *In Vitro*

P-59: Protective Effect of the Crocin on Sperm Parameters in Mice Exposure with Paraquat

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Background: Paraquat (PQ), N1, N paraquat-dimethyl 4, 4 dipyridine, well-known used herbicide has been reported for its adverse effect on reproduction potential. Considering the PQ-dependent oxidative stress, current study was done in order to evaluate the protective effect of crosin, as an antioxidant agent, on PQ-induced detrimental impact on sperm parameters. For this purpose, the sperm count, motility and viability were assessed.
Materials and Methods: To follow-up current study, 28 mature male mice were divided into four Control group (received saline normal 0.1 ml per day intraperitoenally) and test groups as; crocin-alone-received (200 mg/kg bw-1, ip), Paraquat - alone - received) 5 mg/kg bw-1, ip(and PQ+crocin received groups. All animals were administrated for 35 days. The sperm count, percentage of live sperm (assessed by Eosin & Nigrosin staining), percentage of motile sperm were evaluated. All data by using benferroni test in SPSS software.
Results: Observations revealed a significant reduction in percentages of sperm count, motility and¬ viability in PQ-received group versus control animals($P<0.05$). Meanwhile, co-administration of crocin with PQ remarkably up-regulated the spem count, motility and viability compared with PQ-alone-received animals($P<0.05$).
Conclusion: Our data showed that crocin at dose level of (200 mg/kg bw-1/day, ip) significantly inhibits the PQ-induced detrimental effect at sperm level by ameliorating the sperm quality.
Keywords: Crocin, Paraquat, Mice, Sperm Parameters

P-60: Epigenetic Alteration Of CHD5 Gene Regulatory Region in Infertile Men with Impaired Spermatogenesis

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Background: Spermatogenesis is an intricate biological process that transforms diploid spermatogonial stem cells into haploid spermatozoa in seminiferous tubules of testis. The post-meiotic phase of sperm development is replacement of histones with sperm-specific protamines, in order to repackage the genome into the highly compact chromatin structure of mature sperm. CHD5 is identified as a master regulator for the histone-to-protamine replacement. The aim of this study was to evaluate the epigenetic profile of CHD5 gene in testis samples of infertile men with impaired spermatogenesis.
Materials and Methods: This is a case control study. Testes tissue samples were collected from 30 infertile men referred to ROYAN Institute, in four groups including: complete maturation arrest at spermatid level, oligoasthenoteratozoospermia, Sertoli cell only syndrome as negative control and hypospermatogenesis as positive control. Constant was obtained from patients according local ethical approval. Chromatin imunoprecipitation (ChIP) coupled with real-time PCR was performed to evaluate the incorporation of acetylated/methylated lysine 9 of histone 3 (H3K9) as activation/repression epigenetic marks, into regulatory region of CHD5 gene.
Results: ChIP data relieved significant hypoacetylation of CHD5 regulatory region in complete maturation arrest, oligoasthenoteratozoospermia and Sertoli cell only syndrome groups; whereas, a significant hyperacetylation was detected in the same position in positive group. Also in the complete maturation arrest and Sertoli cell only syndrome groups, methylation level of H3K9 was more than acetylation level compared to control group.
Conclusion: Our study showed hyper-acetylation and hypo-methylation of CHD5 promoter is in correlation with activating this gene in spermatogenesis. The finding implies a significant association between epigenetic silencing of CHD5 gene and impairment of spermatogenesis infertile men.
Keywords: Male Infertility, Spermatogenesis, Epigenetic

P-61: Study of Protective Effect of Vitamin E on The Fertilization Rate in The Male Mice with Experimental Hypothyroidism

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Background: The variation in the performance of the thyroid gland has the correlation with the defect of the sexual activity and the degeneration of the testis. In several study the improvement of the sperm parameters have been reported when treatment with oral antioxidants is administrated. Vitamin E is effective in reducing adverse effects caused by oxidative stress and it is the basic component of the sperm antioxidant system.
Materials and Methods: In this study protective effect of vitamin E on the percent of the zygote in the male mouse with experimental hypothyroidism was evaluated. For this purpose 42 adult male albino mouse were divided to 7 groups. all the groups except first, second and seventh groups were the experimental hypothyroidism (induced by propylthiouracil (PTU) in 35 days). The first group was the control group, The second group was injected corn oil (solvent of vitamin E),the third group was only hypothyroidism without any injection, the fourth and fifth and sixth groups were injected 50,100,200 (IU/kg) dose of vitamin E respectively. The seventh group was injected 200(IU/kg) of vitamin E in purpose of positive control. After this period treatment time super ovulation was conducted by intra-peritoneal injection of PMSG(10 IU) and HCG(10 IU) in 10 female mouse. After this time (10 -12 hrs after HCG injection) the animals were scarified by dislocation of cervical vertebra. Percent of the zygotes were evaluated by considered male and female pronucleous in oocyts.
Results: That the percentage of fertilized oocyts increased significantly by increasing in the dose of the vitamin E and the third group(hypothyroidism without any injection) has the least percentage of fertilization.
Conclusion: Vitamin E effects on increasing percentage of fertilization rate in hypothyroidism cases.
Keywords: Vitamin E, Fertilization, Hypothyroidism, Propylthiouracil

P-62: The Effect of Green Tea Extract in Soybean Lecithin-Based Semen Extender on Prevention of Apoptosis after Post-Thawed of Ram Sperm

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Background: Green tea (Camellia sinensis) is a well-known antioxidant, contains several polyphenolic components with antioxidant properties, but the predominant active components are the flavanol monomers known as catechins, where epigallocatechin-3-gallate and epicatechin-3-gallate are the most effective antioxidant compounds. Green tea (containing EGCG) induces an antiapoptotic pattern of gene expression thereby modulating cell survival. This study examined the effect of different concentrations of Green tea extract in a Tris-based extender containing soybean lecithin on Prevention of Apoptosis after post-thawed of ram semen.
Materials and Methods: Semen were collected from four mature Ghezel ram (3 and 4 years of age), of superior genetic merit and proven fertility. A total of 20 ejaculations (5 ejaculates for each ram) were collected twice a week from each ram using an artificial vagina, during the breeding season (autumn). A Tris based extender containing 1% soybean lecithin and 7% glycerol was prepared and then divided to 4 aliquots. Then, different concentrations of 0, 5, 10 or 15 mg/l of green tea were added to extenders. The diluted semen was gradually cooled to 4 °C for 2 hours. The cooled semen was loaded into 0.25-mL French straws (IMV, L'Aigle, and France). Apoptotic cells of ram semen were evaluated after the freezing-thawing process by an Annexin V-FITC detection kit.
Results: The percentage of live spermatozoa in 10 mg/l (51.0±2.8) was significantly higher compared to 15 mg/l and the control group (33.8±2.0, 32.1±1.7, respectively) ($P<0.05$). The percentage of apoptotic spermatozoa was significantly lower in 10mg/l (22.8 ± 2.4) compared to 15 mg/l and control group (32.9±.6, 37.0 ± 3.4, respectively). Finally, there was no significant difference in the percentage of dead spermatozoa among treatments.
Conclusion: We conclude that inclusion of a Green tea extract in a soybean lecithin-based diluent leads to enhanced potential of cryopreserved ram sperm cells probably by preventing apoptotic cell death.
Keywords: Ram, Green Tea Extract, Soybean Lecithin, Annexin-V, Sperm

P-63: Functional Characterization of Oct4 during Goat Preimplantation Development *In Vitro*

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Background: POU transcription factors are involved in transcriptional regulation during early embryonic development and cell differentiation.Oct-4, a member of this family, has been characterized as a regulator of ES cell pluripotency.
Materials and Methods: To study the role of Oct4 during the early development of Goat embryos, we designed siRNA to target Oct4. We began by injecting this siRNA into IVF goat zygotes
Results: Maternal OCT4 expression remained high level in oocytes. Once cleavage, its expression gradually decreased until 4-cell stage, when embryos developed to 8-cell stage, and the expression level increased gradually till blastocyst *in vitro*; the rate at which cleavage and blastocysts development were unchanged compared to noninjected or scramble-injected controls. Embryos lacking Oct4 did not show abnormalities in the number of TE, ICM, or total cells in the blastocyst.
Conclusion: We conclude that oct4 is not required for blastocyst formation during goat development; nevertheless, it is possible that it is necessary for maintaining ICM and TE integrity which need more assessment.
Keywords: Oct4, Knockdown, Goat embryo

P-64: Effect of Rosa Canina on Doxorubicin-Induced Testicular Toxicity in Mouse Testes

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Background: Doxorubicin is one of the most popular chemotherapeutic drugs used in the treatment of several malignancies. Spermatogenic cells constitute one of the body tissues that are susceptible to doxorubicin induced oxidative stress and apoptosis. Therefore, its clinical use is accompanied by its severe reproductive toxicity especially disturbing male fertility. Application of Rosa canina extract as an anti-inflammatory agent with antioxidant activity is necessary to reduce toxicity. The aim of this study was to investigate whether doxorubicin -induced testes toxicity could be prevented by using the Rosa canina extract.
Materials and Methods: Male NMRI mice were treated with vehicles, DOX alone (3 mg/kg, i.p. on day 7, 14, 21), R. canina extract alone (100 mg/kg and 200 mg/kg, i.p. for 28 days), R. canina extract plus DOX (each dose given 1 hour post R. canina). Assessment of testicular toxicity was done by recording changes in morphometrical parameters.
Results: Histological assessments in doxorubicin treatment showed significant decrease in the number of spermatogonia, primary spermatocyte, round and elongated spermatid and sertoli cells. In addition, the combined treatment of R. canina extract with doxorubicin improved the adverse effect of doxorubicin in two doses on testes.
Conclusion: These finding suggest that the R. canina extract compensates the adverse effects of doxorubicin on germ cells in mouse testes.
Keywords: Rosa Canina Extract, Doxorubicin, Germ Cells, Mice

P-65: DNA Uptake in Rooster Sperm: Effects of Methyl-Beta-Cyclodextrin on Post-Transfection Sperm Quality

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Background: In recent years, sperm has been selected for researchers as a vector for production of transgenic animal this interest is mainly related to the possibility of massive production of transgenic animals using post-transfected via artificial insemination. Therefore, using the optimized protocol and suitable transfectant need to be survived.
Materials and Methods: Methyl-beta-cyclo dextrin (MBCD) was considered as a transfectant to remove Cholesterol from phospholipid bilayer membrane of Sperm For higher efficiency of DNA uptake to the sperm cells. So, different concentrations of MBCD (0, 1, 2 and 4 mM) were analyzed in Lake Solution for transfection of erogenous genetically construction to the rooster sperm. Genetic engineering methods were applied for the ligation of hG-CSF to the PcDNA 3.1+ and following cloning. Semen samples were collected from six roosters and were prepared by twice washes to prevent DNase activity. Spermatozoa transfected with linearized PcDNA3.1+/hG-CSF vector as following groups: Treatment 1: washed sperm without DNA (Control), Treatment 2: washed sperm incubated with DNA, Treatment 3: washed sperm incubated with DNA plus 1mM MBCD, Treatment 4: washed sperm incubated with DNA plus 2mM MBCD and Treatment, 5: washed sperm incubated with DNA plus 4mM MBCD at 37°C for 30 minutes. After transfection, total and progressive motility, velocity parameters (VCL, VSL, VAP, LIN and STR), membrane integrity, viability and DNA uptake by sperm were assessed.
Results: PCR analysis detected the presence of hG-CSF in the rooster sperm. MBCD at the concentration of 1mM improved the percentage of motility (98/9 ± 0/81), membrane integrity (64 ± 1/64) and viability (76/2 ± 1/65) compare to other groups.
Conclusion: Moreover, a significant reduction in quality indices of post-transfected sperm was observed compare to pre-transfection. This event may be related to the intrastructural and structural damages to the plasma membrane. However, more study is required for illusion of the molecular mechanism for absorption of sperm by DNA.
Keywords: Sperm Mediated Gene Transfer, Transgenic Chicken, Motion, Velocity Parameters

P-66: Antioxidant Effect of Ascorbic Acid on Freezability of Ram Semen after Freeze-Thawing Process

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Background: This study was conducted to investigate the antioxidant effects of Ascorbic acid on freezability of ram semen diluted with extenders after freeze- thawing process
Materials and Methods: Five Ghezel rams were used. Semen was collected by artificial vagina twice a week. The spermatological characteristics of the collected semen samples were determined. Pooled and diluted semen was cooled slowly to +4°C over 2 h. Cooled semen was divided to 5 groups. Each of the 5 semen groups was diluted different of Ascorbic acid (0, 0.05, 1, 1.5, 2 mg/mL). Diluted semen was packaged in 0.25 mL French straws. The semen in straws was frozen in liquid nitrogen vapor. Sperm membrane functionality (HOST), antioxidant enzymes containing: Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and total antioxidant status (TAS) were evaluated after thawing
Results: Extender 1.5 mg/mL yieled the highest results for membrane functionality. The plasma Glutathione peroxidase, Superoxide dismutase and total antioxidant status significantly ($P<0.05$) increased in extenders containing different levels of Ascorbic acid when compared to control groups . There was significant difference in the spermatological characteristics on 1.5 mg/mL of ascorbic acid after freezing-thawing ($P<0.05$)
Conclusion: Extender containing 1.5 mg/mL of ascorbic acid was the most adequate combination to achieving post- thawing sperm parameters and our results support it.
Keywords: Ram, Ascorbic Acid, Cryopreservation, Antioxidant

P-67: Effect of Bovine Serum Albumin Supplementation in Tris-Soybean Lecithin based Extender on Semen Quality of Chilled Ram Epididymal Spermatozoa

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Background: Post-mortem recovery of viable epididymal spermatozoa is the last chance for preservation of genetic resource of valuable

animals. Improving sperm quality during liquid storage for several days after semen collection is an important challenge for artificial insemination programs of ovine species. To achieve this goal, different extenders and supplementations have been tested. The supplementation of antioxidants such as BSA (bovine serum albumin) could protect sperm efficiently. Therefore, the aim of this study was to evaluate the effects of different concentration of BSA in tris-soybean lecithin based extender on ram epididymal sperm functional characteristics, nucleus status and lipid peroxidation level during liquid storage at 4ºC for several days.
Materials and Methods: Spermatozoa were collected from one epididymis of each pair of 22 testicles of Zandi's rams and diluted in tris-soybean lecithin based extender was supplemented with 0, 2.5, 5, 7.5, 10% of BSA. Spermatozoa motility, viability, plasma membrane integrity, chromatin condensation, nuclear protamination and malondialdehyde level were assessed by computer-assisted sperm analysis, eosin-nigrosin, hypo-osmotic swelling test, aniline blue, chromomycin A3 and thiobarbituric acid reaction, respectively at 0, 24, 72 and 120 hours after refrigeration at 4ºC. Data were analyzed by SPSS software.
Results: Sperm viability were improved by supplementation of 10% BSA during all storage time and membrane integrity at 120 hours compared to control ($P<0.05$). While sperm motility, chromatin condensation, nuclear protamination and malondialdehyde were not significantly different between all groups ($P>0.05$). Although, sperm progressive motility was higher at 120 in control group ($P<0.05$).
Conclusion: Addition of 10% BSA in tris-soybean lecithin based extender could improve epididymal sperm viability and membrane integrity during refrigeration for several days. However sperm motility, chromatin condensation, extent of nuclear protamination and malondialdehyde were not affected by BSA supplementation.
Keywords: Ovine, Chilled Preservation, Epididymal Sperm, BSA

P-68: Optimizing Vitrified Ovine Oocyte Survival and Development with Non-Equilibrium Cryopreservation Method

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Background: High viability of vitrified oocyte requires the implement of vitrification under conditions that ensure sufficient permeation of cryoprotectants and generate an environment which is able to avoid toxic and osmotic injury to oocyte. Optimal vitrification condition was obtained by examining in detail the time of equilibration process. Thus, we conducted a study to elucidate the optimal oocyte equilibration condition by examining time of equilibration during vitrification.
Materials and Methods: *In vitro* matured COC was vitrified by an equilibration process for a given time (3, 7, 15 min). Immediately after completion of the equilibration process, 3 to 7 oocytes were placed in each of the cryotop with a small amount of medium and were plunged into liquid nitrogen. After warming, COCs were parthenogenetically activated. Yield and quality of embryo development between experimental groups were assessed.
Results: It was observed that partial dehydration during brief exposure (3 min) to cryoprotectants yields high survival of cryopreserved oocyte. This study provides evidence that if the time of exposure to the cryoprotectants is too long (7 and 15 min) it can alter the oocyte developmental potential.
Conclusion: Non-equilibrium preservation with minimum volume excursion is recommended to be used for vitrification method, instead of equilibrium preservation.
Keywords: Vitrification, Non-Equilibrium Preservation, Oocyte, Developmental Potential

P-69: Preimplantation Development of *In Vitro* Fertilized Goat Embryo Produced by Frozen or Fresh Saanen Sperm

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Background: Saanen goats are most popular dairy breed in Switzerland, due to their high productivity and ease of management. Their milk generally has a butterfat content of 3-4%. *In vitro* fertilization (IVF) and subsequently embryo transfer are important technologies which can improve genetic gain /variation by using sperm from high genetic selected sires. In this regard, we used IVF technique using fresh or frozen Saanen semen for investigate fertilization potency and *in vitro* development of oocytes extracted from slaughterhouse goats ovaries.
Materials and Methods: Cumulus-oocyte complexes (COCs) were matured *in vitro* and fertilized by fresh or frozen Saanen sperm. *In vitro* produced embryos were cultured until blastocyst stage in synthetic oviduct fluid at 38.5°C, 5% CO_2, 5% O_2 and maximum humidity.
Results: The results indicated that there were no significant differences between cleavage (86.41% ± 1.89 vs. 85.6% 9 ± 6.71) and blastocysts rates (34.09 ± 4.1% vs. 37.36 ± 5.27%) among fresh or freezing groups. We also assessed the "quality" of blastocyst using differential staining to recognize blastocyst mean total cell number. The result showed no difference between mean blastocyst total cell numbers of embryos that fertilized with fresh or frozen semen (173 ± 27 vs. 197 ± 19).
Conclusion: Saanen fresh or frozen semen successfully fertilized *in vitro* matured oocytes from different Iranian goat's breeds and produced good quality blastocyst which can be used for embryo transfer.
Keywords: Saanen Goat, Fresh and Frozen Sperm, IVF, Embryos

P-70: Effect of Vitamin E and Selenium Nanoparticles on Post-Thaw Oxidative Status of Rooster Semen

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Background: Optimization of the management of rooster breeder need for efficient methods of semen storage such as semen cryopreservation. Indeed, avian spermatozoa are more susceptible to lipid peroxidation (LPO) by reactive oxygen species (ROS) during *in vitro* handling and storage of sperm. To date, several clinical and experimental studies have examined the effect of antioxidants on rooster sperm parameters after cryopreservation. Despite this large body of literature, the effect of these additives on sperm antioxidant capacity and activity has not been mentioned.
Materials and Methods: Semen samples were collected from 12 White Leghorn rooster and pooled, divided into nine equal groups.

Extenders were supplemented with either 2 levels of VitE (5 and 10 µg/mL) or 2 levels of Nano-Se (1% and 2%) or combination of both VitE and Nano-Se, and comparisons in response were made with the control group (no antioxidants). The concentrations of malondialdehyde (MDA), as indices of lipid peroxidation in the sperm samples, glutathion peroxidase (GPX), catalase (CAT) and Superoxide dismutase (SOD) activity and also total antioxidant capacity were measured using commercial colorimetric assay kits.
Results: Extenders supplemented with 5 µg/mL Vit E or 5 µg/mL VitE and 1% Nano-Se had significantly lower malondialdehyde (MDA) concentration compared to control extender (1.15 ± 0.32, and 1.29 ± 0.32, respectively). Moreover, extenders containing Nano-Se demonstrated grater glutathione peroxidase (GPx) activity. Catalase (CAT) activity was higher in extender supplemented with 10 µg/mL VitE and 2% Nano-Se. Moreover, higher TAC was observed in extenders supplemented with VitE and Nano-Se.
Conclusion: The combination of low doses of VitE and Nano-Se in rooster extender was more effective on sperm biochemichal parameters compared to VitE or Nano-Se supplementation alone.
Keywords: Oxidative parameters, Vitamin E, Nano Selenium, Rooster Semen, Cryopreservation

P-71: Protective Effects of Two Antioxidants on Sperm Motility and Membrane Integrity in Liquid Storage

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Background: Artificial insemination in goat is currently limited by the poor fertility obtained following insemination of frozen–thawed semen as results of cryoinjuries which occur at cellular and molecular levels during semen cryopreservation. Alternatively, semen can be stored for several days in liquid form at 4-5°C. However excessive production of ROS during liquid storage plays a central role in induction of injuries to sperm cells.
Materials and Methods: This study aimed to evaluate the role of two additives antioxidants given in combination or alone, a Superoxide mimetic agent, MnTE and two concentration of catalase on total motility and membrane integrity of goat sperm. Each ejaculated semen was split into six aliquots and diluted with a commercially extender containing no antioxidant (control), 0.1 µM of MnTE (Mn), 200 IU catalase (CAT 200), 400 IU catalase (CAT 400), 0.1 µM of MnTE plus 200 IU catalase (Mn + CAT 200), 0.1 µM of MnTE plus 200 IU catalase (Mn + CAT 400) and stored at 4-5 oC for 0, 12, 24 and 48 hours.
Results: The results showed that total motility in 0 and 12 hr of storage were not significantly improved in treatment groups compared to control. However in 48 hours of storage, total motility was significantly better in Mn (54.01 ± 1.49), CAT 200 (64.83 ± 1.21), CAT 400 (70.13 ± 1.27), Mn+CAT200 (63.75 ± 1.17) and Mn+CAT400 (75.93 ± 1.33) compared to control (49.82 ± 0.85).The maximum Improvement in motility and membrane integrity (57.96 ± 1.27) was obtained in Mn+CAT400 at 48 h of storage.
Conclusion: Addition of catalase (200 or 400 IU/ml) and 0.1 µM of MnTE in combination or alone to a commercial extender which is probably optimized with antioxidant(s), maintained the motility parameters and membrane integrity in 24 and 48 h of storage at 4-5°C.
Keywords: MnTE, Catalase, Goat Semen Motility, Liquid Storage, HOST

P-72: Effect of Different Cryoprotectants on The Histologic Structure of Bovine Ovarian Tissue. A Stereological Study

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Background: The vitrification of ovarian tissue is a promising new technique for preserving the fertility of women who are at risk of ovarian function failure and conservation of endangered species and economically important breeds. The objective of this study was to evaluate the effect of cryoprotectants (at three concentrations each) on the preservation of bovine ovarian tissue.
Materials and Methods: Bovine ovarian cortex fragments were vitrified using the different concentration of (10%, 20%, 40%) EG, (5%, 10%, 20%) DMSO with and without 1 mol sucrose. In addition, a toxicity test was performed for each cryoprotectant by exposing the ovarian tissue to them without freezing. After which, histological and stereological assessment were carried out. The number of normal and damage follicles was calculated via the physical dissector technique.
Results: Ovarian tissue vitrified in concentration of 40% EG and 20% DMSO with 1 mol sucrose retained a higher percentage of morphologically normal primordial and primary follicles (73–88%) than ovaries tissue vitrified in 10%, 20% EG and 5%, 10% DMSO without 1 mol sucrose. The exposure of ovarian tissue to lower concentration of EG, DMSO significantly reduced the percentage of normal different stage of preantral follicles when compared with fresh ovarian tissue.
Conclusion: 40% EG and 20% DMSO with 1 mol sucrose were the most effective cryoprotectants for vitrification of bovine ovarian tissue, preserving the structural integrity of somatic and follicle within the ovary
Keywords: Ovary, vitrification, Stereology

P-73: Farnesol Ameliorates The Genotoxicity and Oxidative Stress of Cyclophosphamide in Murine

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Background: The aim of the present study was to evaluate the efficacy of farnesol against the testis injury induced by the anticancer drug cyclophosphamide (CP). Farnesol is a sesquiterpene and has antioxidant and chemopreventive properties. Fruits are the most popular source of it.
Materials and Methods: Adult male albino mice were pretreated with two different doses of farnesol (5 and 10mg/kg by body weight (b.w.) via intraperitoneal injection for seven consecutive days followed by single injection with CP (200 mg/kg b.w.) 1 h after the last injection of farnesol on the 7th day. After 24h, mice were euthanized, testes were immediately removed, and biochemical and histological studies were conducted.
Results: The results showed that farnesol ameliorated the toxicity effect of CP. Pretreatments with farnesol significantly alleviate lipid peroxidation, and the level of reduced glutathione content abnormality induced by CP in mice testis. Histologic results also indicated that

CP had a marked damaging effect on testis tissue including degenerative and destructive effects on spermiogenic lineage and interstitial cells, thinner seminiferous epithelium compared to the control group, and vascular congestion. The advanced degree of protection was seen in the testes of mice pretreated with 10 mg/kg farnesol. Most of the seminiferous tubules revealedits normal structure with the presence of all spermatogenic layers. The testicular content of sperm and spermatocyte was relatively the same with the control group.
Conclusion: We have demonstrated that farnesol presents potential chemopreventive agent for minimizing the undesirable effects of the CP on testis.
Keywords: Cyclophosphamide, Farnesol, Oxidative Stress, Testicular Toxicity

P-74: Finding for Supplementation of Rooster Cryopreservation Media with Cysteine and Cysteamine

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Background: The lower fertility rate of cryopreserved poultry semen compare to mammalian is an obstacle to wide application for artificial insemination in commercial herds. This event is mainly related to particular characteristics of poultry sperm which increment their susceptibility to detriment during cryopreservation. To date, a successful protocol for cryopreservation of rooster sperm has not been achieved. Therefore, the aim of this study was to optimize the rooster sperm freezing with determination of optimum levels of cysteine and cysteamine in extenders during cryopreservation.
Materials and Methods: Sperm samples were collected twice a week from four mature Ross rooster and then samples were pooled to eliminate the individual differences. Then, pooled sperm were divided to seven equal part for dilution with extenders containing different concentrations of cysteine and cysteamine as follows: 1) extenders with 1) no additive, 2) 5 mM cysteine, 3) 10 mM cysteine, 4) 5 mM cysteamine 5) 10 mM cysteamine 6) 5 mM cysteine + 5 mM cysteamine, 10 mM cysteine +10 mM cysteamine. After dilution, sperm was aspirated into straws, sealed with polyvinyl alcohol powder and equilibrated at 4ºC for 30 minutes and then cryopreserved. Motion characteristics, viability, membrane integrity and mitochondria activity were assessed after thawing.
Results: The highest significant percentage of motility, viability and mitochondria activity were observed in 5mM cysteine (61.34 ± 1.02, 57.2 ± 1.34 and 49.01 ± 1.2, respectively) compare to other groups. Progressive motility of frozen-thawed sperm was not affected by cysteine or cysteamine. For cysteamine treatments, there was not significant different between 5 and 10 mM cysteamine and control group. Combination of cysteamine and cysteine and cysteamine in all concentrations had negative effects on sperm motility, viability and membrane integrity.
Conclusion: Cysteine is suitable additive to be more investigate for cryobiology of rooster sperm. It may enhance or preserve the quality of rooster sperm during freezing-thawing.
Keywords: Freezing, Mitochondria, Rooster, Fertility

P-75: Permissibility or Impermissibility of Cloning

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Background: Nowadays, progress of Science and Technology opens new doors to a world of boundless legal system. Including developments in medicine and genetics clonig that its tests and initial experience on plants and animals skirts with challenges, but the question in this regard is that what will happen if the normal process cloning is tested in humans? Due to the relation of human cloning to various aspects of , there are many questions about it and contemporary jurists has been facing various branches of it.
Materials and Methods: This research is based on a study library, as well as some related articles about the prepared Words of the research
Results: Shia scholars have different opinions about it. In relation to permit or impermit of cloning, major Shie scholars are allowed to permit which was written in Esala Alabahh , and only the case of physical cell bank that its owner is not indicated is forbidden, because of the mixing Lineage . The groups who believe in impermissibility of cloning have no valid reason for his fatwa and due to worry about the consequences, they believe in impermissibility of cloning.
Conclusion: The mission of each legal system is to evolve the legal system, avoid stagnation and accept new achievements to the extent that it is possible and comply with domestic laws and obstruction of the investigation and progress of science cannot be done with unfounded reasons. So, the adoption of this institution with national and international control should prevent of misuse in this institution.
Keywords: Coling, Permissibility, Jurisprudence

P-76: Liquid Storage of Rooster Semen; How long Sperm Can Survive in soybean Based Medium

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Background: Fresh storage of sperm at 4oC has detrimental effect on sperm function, but create a condition to maintain and store sperm for relatively suitable time. This helps to technicians to transfer the sperm without freezing to near farms for artificial insemination. During chilled storage of sperm, plasma membrane is more susceptible to damage, therefore using an extracellular protectant against reactive oxygen species would be efficient. The purpose of this study was to evaluate motility and viability of rooster sperm during fresh storage at the times of 0, 6, 12, 24 and 48 h of incubation at 4oc in extender containing soybean lecithin.
Materials and Methods: Sperm samples were collected twice a week using from three mature rooster. Then, sperm samples were pooled to eliminate the individual different and subsequently divided into two parts and diluted with 2 extenders consist of a hand-made extender containing soybean lecithin and a control extender. The extender was based on 1% lecithin and the ratio of dilution was 1:10 for sperm and extender. Total and progressive motility, VAP, VCL, VSL, ALH, BCF, LIN, STR, WOB, Hyperactive, viability and membrane integrity of sperm were assessed at 4oc in different times of incubation (0, 6, 12, 24 and 48 h after dilution). Data were analyzed by Proc GLM of SAS 9.1 and Tukey test was used to compare the means.
Results: Our results showed that over passing time, motion characteristics, viability and membrane integrity are gradually reduced. However, In group containing soybean lecithin, the rate of this reduction was slower. Although the was not significant different in case of total motility and viability at the times of 0 and 6 h of incubation, but

these parameter were higher in group of sperm diluted with soybean lecithin compare to control.
Conclusion: It seems that soybean lecithin could be an efficient protectant for preservation of rooster sperm. More details may be cleared in the future.
Keywords: Liquid State, Rooster Sperm, Viability

P-77: Mitochondria-Targeted Antioxidants as A New Approach in Improvement Post-Thaw Sperm Quality

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Background: Mitochondria participate in many crucial processes in sperm cell. Changes in mitochondrial integrity/functionality, as well as low mitochondrial membrane potential or altered oxygen consumption have been correlated with loss of sperm motility. Recent advances in oxidative stress suggested that ROS intervention should be targeted to mitochondria directly for effective antioxidant activities. Strategies to decrease oxidative damage to sperm potential and the selective targeting of antioxidants to mitochondria have considerable therapeutic potential. The aim of our study was to explore mitochondrial-targeted approaches for ROS reduction to see if these approaches can improve post-thaw quality of sperm cryopreservation.
Materials and Methods: A search of the scientific literature available in the PubMed and Google Scholar databases was conducted for studies on sperm Mitochondria by the use of relevant Key words: spermatozoa, Mitochondria-targeted antioxidants, mitochondrial activity, ROS. The search covered the years 2000 to September 2015 and included English articles and other language publications with English abstracts.
Results: ATP synthesis and ROS production are the most discussed aspects of mitochondrial function. Mitochondria and ROS are thus a nexus of multiple pathways that determine the response of cells to disruptions in cellular homeostasis. The results provided three methods of Mitochondria-targeted Antioxidants that can be studied individually or simultaneously.
Conclusion: The optimization of sperm quality Both chemical and physical properties and the development of appropriate delivery antioxidant systems can provide in the next future a way out to attain effective ways to minimize lethal and sub-lethal effect of ROS in sperm cryopreservation.
Keywords: Sperm, Mitochondria-Targeted Antioxidants, ROS

Embryology

P-78: Induction of Early Puberty following Dose Dependent Administration of Monosodium Glutamate in Adolescent Rats: Epididymal Sperm Analysis

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Background: Monosodium glutamate (MSG) is a food additive which acts as preservative or enhancer of palatability. Male infertility, obesity and hypogonadism have been reported following administration of MSG in adult rats. The aim of this study was to evaluate the possible effects of MSG on the puberty in adolescent rats.
Materials and Methods: 25 days old rats were divided into three control, low dose MSG (6 mg/kg/day) and High dose (60 mg/kg/day) MSG groups. MSG was administrated orally for 40 days. At the end of study, the animals were euthanized and epididymal sperm analysis was performed.
Results: High dose of MSG significantly led to increase of total sperm count and sperm motility in comparison to other groups. Low dose of MSG was not affecting the production of spermatozoa.
Conclusion: In this study, the results of sperm analysis indicated that, despite of previous studies shown that, some alterations in spermatogenesis have been observed following use of MSG in adult rats, the administration of monosodium glutamate in adolescent rats during the period before sexual maturity dose dependently can lead to induction of early sperm production.
Keywords: Adolescent Rats, Early Puberty, Monosodium Glutamate, Sperm Analysis

P-79: The Effect of Enriched Serum of Goats Fed Fish Oil and Vitamin E on The *In Vitro* Maturation of Mouse Oocytes

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Background: Although the pivotal roles of serum, such as Fetal Bovine Serum (FBS) on *in vitro* oocytes maturation were shown, limited information exists on enriched serum with several nutrients for cell cultures. The main objective of this survey is to determine the effect of serum contain vitamin E, oil fish as well as combination of them upon egg plantation.
Materials and Methods: In this experimental survey, serum of indigenous goats fed two months by standard diet (CTR), vitamin E (VITE), fish oil (FO) as well as FO+VITE were replace with FBS in *in vitro* maturation of oocytes. Moreover, one group was used as without serum group (W). The oocytes were obtained from NMRI mice during five weeks. Data were analyzed using SPSS.
Results: Finding showed that FBS as well as FO+VITE had the highest (61.10 and 63.90%, respectively) MII percentage, whereas VITE had the moderate (56.80%) and FO (45.10%), CTR (45.60%) and W (46.10%) groups had the lowest rate of MII, respectively ($P<0.05$). The level of GVBD remained the same for all target groups. In addition, FBS and FO+VITE had the lowest number (18.40 and 19.70%, respectively) of GV and CTR showed the highest number (37%) of the same oocytes. The combination number of maturing oocytes (GVBD + MII) in CTR (62.90%) was the mild and in FO+VITE and FBS (80.10 and 81.20%, respectively) showed maximum rate of meiosis resumption.
Conclusion: It seems that enriched serum contained vitamin E and

fish oil can be substituted with FBS in *in vitro* maturation of oocytes. The points that should be accentuated is that the serum fed with standard ration had undesirable impacts.
Keywords: Enriched Serum, Maturation, Oocytes

P-80: Fluoxetine Suppresses Spermatogonial Stem Cell Self-Renewal in Adult Rats

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Background: Treatment withfluoxetine (FLX), a widely prescribed antidepressant, can lead to reduced male fertility and sperm maturation arrest. The current study was designed to explore the effects of FLX on rat spermatogonial stem cells(SSCs) self-renewal through evaluation glial cell line-derived neurotrophic factor family receptor alpha-1 (GFRα1) expression at mRNA level in testicular tissue.
Materials and Methods: Adult male Wistar rats were randomly allocatedinto experimental and control groups. The experimental group subdivided into two groups which received 5mg/kg/day and10mg/kg/dayFLX orally for 48 days. The mRNA expression of GFRα1was analyzed by reverse transcription polymerase chain reaction (RT-PCR).
Results: Fluoxetine at a dose level of 10 mg/kg/day caused a significant reduction in mRNA expression ofGFRα1.
Conclusion: Our findings revealed that FLX induces male reproductive toxicity via disruption of SSCs self-renewal and differentiation.
Keywords: Fluoxetine, Spermatogonial Stem Cell, Glial Cell Line-Derived Neurotrophic Factor, Rat

P-81: Effects of Hydralazine and Doxorubicin on Sperm DNA Integrity in Mice

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Background: Doxorubicin is one of the most popular anticancer drugs widely used for the treatment of a variety of cancers. However, its activity is not specific to cancer cells and may also harm to healthy cells such as spermatogonia. Hydralazine is a vasodilator drug with potent anti-oxidant properties and iron chelator efficiency that protects spermatogenesis in testes from such doxorubicin injury. The aim of this study was to investigate effect of hydralazine and doxorubicin on sperm DNA integrity.
Materials and Methods: Male NMRI mice were divided four treatment including control (normal saline, i.p), doxorubicin (3mg/kg, i.p. on days 7, 14 and 21), hydralazine (5mg/kg, i.p for 21days), hydralazine-doxorubicin (i.p injection hydralazine starting 7 days before the first application of doxorubicin and continued for 21 days and doxorubicin injection on day 7,14,21. Each dose of doxorubicin given 1 hour post hydralazine). Mice were kept for 21 and 64 days after first treatment (group1 and 2, respectively). At the end of the experimental periods, animals were sacrificed by cervical dislocation. Acridine orange staining was used to monitor the effects of drugs on sperm DNA integrity. Data was analyzed using One-Way ANOVA and Duncan test.
Results: In DOX-treated mice percentage of DNA damage in the epididymal sperm significantly increased compared to control. Also in hydralazine treated mice percentage of DNA damage significantly increased compared to control and Dox-treated mice.But in mice that recive hydralazine along with DOX percentage of DNA damage in the epididymal sperms significantly decreased compared to mice that receive only DOX or hydralazine and it is near to control mice.
Conclusion: These results demonstrate that administration of hydralazine along with DOX can reduce significantly percentage of DNA damage in the epididymal sperms compared to mice that receive only DOX or hydralazine and it is near to control mice.
Keywords: Doxorubicin, Hydralazine, Sperm,DNA integrity, Mice

P-82: Investigation of Effect Achillea Mmillefolium Inflorescences Extract on DNA Damage of Sperms in Cyclophosphamide Treated Mice

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Background: Apart from being an immunosuppressant agent, Cyclophosphamide (CYP) has been known to induce oxidative stress, impact on gonadal cells' DNA and reduce the fertilizing potential. Therefore, the present study was aimed to evaluate the effects of hydro-alcoholic extract of Achillea millefolium inflorescences (AMI), as a potential antioxidant on DNA Damage in CYP Treated Mice.
Materials and Methods: Thirty male adult NMRI mice were randomly arranged into 5 groups. Group 1 received normal Saline (0.1 ml/kg), group 2 received CYP alone (5mg/kg), group 3 received CYP (5mg/kg) + hydro-alcoholic extract of AMI (75mg/kg). Group 4 received CYP (5mg/kg) + hydro-alcoholic extract of AMI (150mg/kg) and Group 5 received CYP (5mg/kg) + hydro-alcoholic extract of AMI (300mg/kg). Treatments were continued for 35 days. At the end, after mice euthanization by cervical dislocation, Caudaepididymis were used to collect sperm cells and rate of DNA Damage were examined by Acridine Orange Staining.
Results: In group 2, the DNA Damage significantly increased compared to control group ($P<0.001$). in group 4 this parameter significantly decreased compared to group 2 ($P<0.001$) and in the group 5 rate of DNA Damage significantly increased compared to group 2 ($P<0.05$).
Conclusion: These findings indicated that AMI (low dose) has protective effect against CYP-induced toxicity in CYP Treated Mice probably by decreasing oxidative stresses. But High dose of AMI caused increase toxicity of CYP.
Keywords: Achillea Millefolium, Acridine Orange, Cyclophosphamide, DNA Damage, Mice

P-83: Evaluation of Effects of Hydro-Alcoholic Extract of Achillea Millefolium on *In Vitro* Fertilization (IVF) In Mice

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Background: Achillea millefolium inflorescences (AMI) is one of the oldest and most well-known medicinal plants with potential antioxidant properties. The purpose of this study was aimed to evaluate the effects of three different doses of hydro-alcoholic extract of AMI on in vitro fertilization in mice
Materials and Methods: Twenty four male adult NMRI mice were

randomly arranged into 4 groups. Group 1 received normal Saline (0.1 ml/kg), group 2 received hydro-alcoholic extract of AMI (75 mg/kg). Group 3 received hydro-alcoholic extract of AMI (150 mg/kg). Group 4 received hydro-alcoholic extract of AMI (300 mg/kg). Treatments were continued for 35 days. The oocytes were obtained from 15 mature female mice. Animals were anesthetized to easy draw, after extraction and normal sperm and fertilized oocytes were incubated for 120 hours in presence of HTF + 4 mg BSA. Statistical analyses were performed using ANOVA and Tukey test.

Results: In the groups receiving low and medium doses of AMI, blastocyst formation and fertilization rate was increased, but not significantly compared to the control group. But in the group receiving high dose of extract, blastocyst formation and fertilization rate was significantly decreased compared to the control group ($P<0.01$).

Conclusion: In this study, AMI has dose-dependent manner, so that at low and medium doses did not revealed significant effect, but high-dose of AMI caused a significantly remarkable reduction in in vitro fertilization and embryos growth.

Keywords: Achillea Millefolium, IVF, Cyclophosphamide, Embryo Toxicity, Mice

P-84: Health Assessment of Selenium Nanoparticle: An Experimental *In Vitro* Fertilization Mice Model

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Background: Selenium as an anti-oxidant and a component in selenoprotein structure has a critical role in reproductive system but its safety dosage margin is very low and can storage in tissue for long time that it is the main reason of chronic toxicity of selenium. Selenium nanoparticle (Nano- Se) has a low toxicity compared with selenium elements. The present study was conducted to elucidate the ability of selenium nanoparticles in fertility rate, embryo development and reproductive anti-oxidant capacity in an experimental in vitro fertilization mice model

Material and Methods: In this study, 15 adult male mice were divided into 3 groups. Control group just was administrated PBS (i.p.) and in another groups, selenite sodium (0.5mg/kg) and Nano-Se (0.5mg/kg) was administrated (i.p.) for 7 days. After 30 days, at the end of this study, all blood sample were collected and the mice euthanized in the end. Oocyte fertilization rate and in vitro embryo development were assessed in all animal following IVF. Glutathione peroxidase (GPX), Superoxide dismutase (SOD) , Catalase, Total antioxidant capacity (TAC) and Malondialdehyde (MDA) were evaluated not only in blood sample but also in semen and testis.

Results: Se induce a significant reduction compare to Nano-se and control on mouse oocyte fertilization rate as well as blastocyst and hatching rate ($P < 0.05$). There wasn't significant different between Nano-se and control. Nano-selenium has potent effects in increasing the antioxidant capacity by increasing, GPX, SOD, Catalase, Total antioxidant capacity (TAC) and reduction of MDA.

Conclusion: Nano-se in comparison to Se has more positive effects on mouse oocyte fertilization, sequential embryonic development and anti-oxidant capacity. Use of Nano-Se instead of Se may not only has better effect on reproductive system but has less toxic effect.

Keyword: Selenium Nanoparticle, IVF, Anti-Oxidant Capacity, Mice

P-85: The Relationship between Sperm Quality, Sperm DNA Fragmentation and Recurrent Implantation Failure

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Background: Recurrent implantation failure (RIF) is one of the main problems in the treatment of infertility with assisted reproductive technology (ART). It was assumed that RIF is just related to either maternal factors or embryonic causes. There is increasing evidence that the integrity of sperm DNA can be related to implantation failure. The aim of this study was to find a relationship between semen parameters and sperm DNA fragmentation (SDF) in the male partners of women diagnosed with RIF following ART.

Materials and Methods: Semen samples were collected from partners of 81 women with RIF following ART and 85 fertile men as control group. There was no evidence of risk factors for RIF in the female partners. Volume, sperm concentration, motility, and morphology were determined. SDF was measured by sperm chromatin structure assay (SCSA).

Results: From the mentioned variables, results indicated that the patient group had significantly lower sperm motility than the control group (40.55 ± 23.56 vs. 58.02 ± 9.27, $P<0.001$) and the percentage of sperm with DNA damage was significantly higher in the patient group, as measured by SCSA (26.10 ± 12.28 vs. 17.98 ± 8.09, $P<0.001$). As compared with the control group, men with higher SDF had an increased risk for RIF (Adjusted OR 1.066, 95% CI: 1.01-1.11). As well, the odds of RIF increased with reduction in sperm motility (Adjusted OR 0.896; 95% CI:0.84-0.95).

Conclusion: Standard sperm characteristics are poor predictors of the outcome of ART treatments. On the contrary, DNA fragmentation has an important impact, independent of the classic semen analysis parameters, on both natural and assisted reproduction. These results can support the hypothesis that SDF is an important cause of RIF.

Keywords: : Recurrent Implantation Failure (RIF), Sperm DNA Fragmentation (SDF), Assisted Reproductive Technology (ART), Sperm Characters

P-86: Isolation, Culturing and Differentiation of Human Amniotic Fluid Stem Cells into The Oocyte Like Cells by Using of Cumulus Conditioned Medium and Follicular Fluid

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Background: Amniocentesis amniotic fluid stem cells (AFSCs) express both germ-like stem cells and mesenchymal-specific cell markers. The purpose of this study was to isolate and culture the stem cells retrieved from cesareans and subsequently differentiate in to the oocyte like cells by using of cumulus cells conditioned medium and follicular fluid
Material and Methods: Amniotic fluid samples were obtained from full term cesarean sections delivered in Arash hospital (Tehran, Iran). The amniotic fluid cells were isolated from samples by using of Algrade density gradient and cultured in αMEM + FBS10% + L-glutamine 1%. Morphology of the cells was evaluated under light microscopy. After 3-4 passages, immunofluorescence and flow cytometry assay were applied to detection of specific mesenchymal and germ cells markers. In the same passage, AFSCs were induced to differentiate into oocyte-like cells by adding 10% human follicular fluid (group 1) and 10% cumulus cells conditioned media (group 2) individually as inducer in αMEM for 14 days
Results: Observation of the isolated cells under microscope showed that amniotic fluid contained different round, star and multi dimension cells. From day 15-17 of culture a population of spindle-shaped cells were appeared and two weeks later were homogenized. After 4th passage flow cytometery analyses demonstrated that mesenchymal stem cells markers (CD44, 90 and 105) and germ cells maker (CD117) were expressed on the surface of AFSCs but there is not any detection of hematopoietic cells (CD34 and 45). Five to 7 days after induction, a subpopulation of large round cells (like oocyte) with approximately 35-50 µm in diameter was observed in both groups.
Conclusion: Our data demonstrated that amniotic fluid stem cells derived from cesarean samples could be isolated, cultured and were capable to differentiate morphologically into oocyte and express adult stem cells (CD 44, 90 and 105) and germ cells (CD117) markers.
Keyword: Amniotic Fluid, Follicular Fluid, Cumulus Cells, Conditioned Medium, Oocyte Like Cells

P-87: Open Pulled Straw Vitrification: A Superior Way to Cryopreserve *In Vitro* Produced Sheep Embryos

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Background: The aim of the present study was to evaluate slow freezing and OPS Vitrification protocols using different cryoprotectant concentrations for cryopreservation of Sheep embryos.
Materials and Methods: IVF derived embryos were subjected to three experimental groups; In experiment 1, Day 6 sheep embryos were cryopreserved by a slow freezing protocol using 10% ethylene glycol (EG), 10% dimethyl sulfoxide (DMSO) or a mixture of 5% EG and 5% DMSO. Hatching rates were higher in the 10% EG group than in the 10% DMSO or EG+DMSO groups (30% vs. 18% and 20%, respectively). In experiment 2, embryos were cryopreserved by open pulled straw (OPS) vitrification using either 33% EG, 33% DMSO or a mixture of 16.5% EG+16.5% DMSO. Re-expansion and hatching rates in the EG+DMSO group (79.16% and 52.74%,respectively) were higher than those in the EG group (64.28% and 30.02%, respectively), whereas the outcomes for the DMSO group were the lowest (45.18% and 8.6%, respectively). In experiment 3, embryos were cryopreserved by OPS vitrification using either 40% EG, 40% DMSO or a mixture of 20% EG + 20% DMSO.
Results: Re-expansion and hatching rates were highest in the EG group than in the EG + DMSO and DMSO groups (92.16% vs. 76.30% and 55.84% re-expansion,respectively; and 65.78% vs. 45.55% and 14.46% hatching, respectively)
Conclusion: OPS vitrification is an efficient method to cryopreserve *in vitro* produced Sheep embryos
Keywords: Cryopreservation, IVF, Sheep, Embryos

P-88: Expression of Melatonin Receptors in IVF/Vitrified Two-Cell and Blastocysts Mouse Embryos

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Background: Melatonin (N-acetyl-5-methoxytryptamine) is the most powerful free-radical scavenger which plays an important role in embryo development thank to its antioxidant and anti-apoptotic properties. In addition, its presence in the culture media may affect melatonin receptor expression. Therefore, the aim of this study was to investigate the expression of melatonin receptors in IVF/vitrified mouse embryos in the presence of melatonin.
Materials and Methods: NMRI female mice were superovulated by injection of 10 IU of PMSG, followed by 10 IU of hCG 48 h later. The sperm were collected from the same strain mice and were used for IVF. The IVF two-cell embryos were vitrified and thawed embryos were then cultured in KSOM medium in the presence of different concentrations of melatonin (10−6, 10−9, and 10−12M) and without melatonin. The rate of cleavage and blastulation in all groups was determined. The expression of melatonin receptors (Mtnr1a and Mtnr1b) in mouse 2-cell embryos and blastocysts were evaluated by RT-PCR. Diencephalon was used as a positive control for melatonin receptors.
Results: Interestingly, 10−12M concentration of Melatonin increased the rate of cleavage and blastulation ($P<0.05$). The results of RT-PCR showed that mRNA of Mtnr1a receptors was expressed in 2-cell embryos and blastocysts, whereas Mtnr1b receptors was not.
Conclusion: Melatonin may have a positive effect on development of mouse embryos via Mtnr1a receptor or it can penetrate into the embryonic blastomers through the cell membrane directly due to its hydrophobic property.
Keywords: Melatonin Receptors, IVF, Vitrification, Mouse Embryo

P-89: Follicles Recruitment following Ovary Culture In Presence of N-Acetyl-L-Cystein Inhibited Amh Effects

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Background: Ovary culture is an appropriate strategy for follicular development especially primordial ones. Amh is a key factor to inhibit the growth of follicles. Our aim was evaluation of N-Acetyl-L-Cystein (NAC) effects on the ovary culture condition following vitrification.
Material and Methods: Ovaries of 8 days–old immature NMRI mice were isolated and allocated into non–vitrified control, vitrified control, cultured non-vitrified–NAC, cultured non-vitrified+NAC, cultured vitrified–NAC and cultured vitrified+NAC groups. Vitrification was performed with ethylene glycol (EG) and propanediol (PROH) combination. Non vitrified and vitrified-warmed ovaries were cultured in α-MEM supplemented with FSH (10 mIU/ mL), ITS (%1), BSA (3mg/mL) and Fetuin (1mg/mL) in the presence and absence of NAC (50 mM) for 5 days. Morphological assessment and gene expression evaluation (Gdf-9, Lif، Bmp-6، Amh, Bax and Bcl-2) were performed done by light microscopy and Real Time-PCR.
Results: Histological results indicated well preservation of tissue integrity and reduction significant of primordial follicles population in cultured non-vitrified and cultured vitrified in presence of NAC (14.05%± 0.78 and 6.04%±1.69) compared to non-vitrified and vitrified control groups (59.73% ± 5.08 and 45.70% ± 5.65). Expressions of all mentioned genes were increased in vitrified control group comparing to non–vitrified control group. This increase in cultured groups was higher in presence of NAC. Amh expression was reduced in both cultured groups in presence of NAC in comparison to the both cultured groups in absence of NAC. Apoptotic index (Bax/Bcl-2 ratio) showed the highest rate in cultured non–vitrified and vitrified groups in the absence of NAC.
Conclusion: Well integrity preservation of ovarian tissue was a good proof to show the compatibility of vitrification method. In addition, it seems that NAC antioxidant could act as a successful Amh inhibitor and could be beneficial in ovary culture which large population of mature follicles is expectable.
Keyword: Vitrification, Ovarian Tissue, NAC Antioxidant, Amh, In Vitro Culture

P-90: Chromium Chloride in The Vitrification Medium Improves *In Vitro* Maturation of Oocytes Derived from Vitrified-Warmed Mouse Ovary

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Background: Nowadays researchers are trying to find ways to preserve individual fertilit, inclouding cryopreservation of gametes, emberyo and ovary. Cryopreservation of ovary in comparison to other samples due to high number of follicles in different developmental stage, used in children and less sensitive to cooling and cryogenic changes in immature oocytes, is a more accepted and appropriate method to treat infertility in cancer patient undergoing chemotherapy and radiotherapy. Some of the different strategies for creating favorable conditions and reduce bad effects of cryopreservationon the survival and growth of the vitrified-warmed samples, are adding elements, hormones, vitamins, growth factors and antioxidants to freezing, thawing and culture medium of vitrified-warmed ovarian tissue. Chromium chloride is one of the raremineral elements, which exists in a few amounts in follicular fluid. The aim of this study was to evaluate the effect of chromium chloride in vitrification medium on *in vitro* maturation of follicles derived from vitrified-warmed mouse ovary.
Materials and Methods: In this study, the ovaries of 2-4 week-old NMRI mice dissected and randomly assigned to following groups: V0,V1,V2,V3 (vitrified warmed ovaries with 0, 10,25 and 50 µM of chromium chloride concentration in vitrification solution. Ovaries in the vitrified groups were frozen sequentially by immersion into two vitrification solution. equilibration solution: 7.5% EG, 7.5%DMSO in α-MEM supplemented with 20%FBS for 7 minutes and vitrification solution: 15% EG, 15% DMSO, 0.5 M sucrose in α-MEM including 20%FBS for 3 minutes in room temperature. Ovaries were placed in the 0.25 ml straw with a minimum volume of vitrification medium and then plunged into liquid nitrogen for 1 week. Warming was performed in α-MEM including 20% FBS that supplemented with descending concentrations of sucrose (1, 0.5, 0.25 M) at room temperature for 5 minutes. Immature oocytes were isolated mechanically from ovaries,they were put in maturation medium and evaluated for *in vitro* maturation (IVM) .Oocytes were evaluted using propidum iodide (PI) staining and fluorescent microscope after thawing and maturation. Immature oocytes were isolated mechanically from ovaries,they were put in maturation medium and evaluated for IVM .Oocytes were evaluted using propidum iodide (PI) staining and fluorescent microscope after thawing and maturation
Results: The results showed that the presence of chromium chloride in vitrification solution is effective. In addition viability of oocytes and oocyte maturation was better in group 10µM ammonium metavanadate concentration compared to the other vitrified-warmed groups. (ANOVA, $P<0.05$).
Conclusion: This study demonstrated that chromium chloride supplementation of vitrification medium has positive effects on the viability of oocytes and oocyte maturation rate.
Keywords: Vitrification, Chromium Chloride , Ovary, *In Vitro* Maturation Mouse

P-91: The Effects of Human Follicular Fluid Supplemented with Zinc and Copper on *In Vitro* Maturation and Subsequent Embryo Development

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Background: Follicular fluid (FF) provides a substantial micromilieu for the development of the oocytes. The oocytes maturation (IVM) is a crucial step for oocytes capable of being fertilized and undergoing normal embryonic development into blastocyst after IVF. IVM is a rescue strategy in ART and scientist has been trying to improve its results. Zinc and Cu are two essential trace elements found in small amounts in a variety of tissues and cells. There are many reports to show the positive effects of zinc and Cu to maturation medium. The objective of the current study is to investigate the effects of adding zinc and copper to human FF on *in vitro* maturation of immature oocytes.
Materials and Methods: Follicular fluid was collected from fertile

women and centrifuged at 2500-g for 20 minutes. Zn and Cu level of FF were determined by atomic absorption spectrometry. Oocytes were collected from 6-8 weeks old NMRI mice. The maturation base medium was ὰ- MEM supplemented with 4 mg/ml BSA and 7.5IU HCG. Treatment groups were FF alone, FF with Cu, FF with Zn based on adding 10% FF supplemented with 1 µg/ml Zn or 4 µg/ml Cu to maturation medium. In vitrto fertilization was done (ὰ- MEM supplemented with 16 mg/ml BSA) by matured oocytes and embryos were cultured (ὰ- MEM supplemented with 4 mg/ml BSA) for 48h. Oocyte maturation and embryo cleavage rate were accessed.
Results: The percentage rates of oocytes reaching to MII stage were 44, 55, 61 and 67%, GVBD+MII were 64, 71, 82 and 91, also embryo cleavage rates were 75, 87, 90 and 91% in the control, FF alone, FF with zinc and FF with Cu groups, respectively. Significant maturation and cleavage rate were observed in all three treatment groups compared with the control group.
Conclusion: Our study has demonstrated that maturation medium supplemented with follicular fluid provides an appropriate environment for vitrified oocyte development. The results of oocyte maturation significantly improved while follicular fluid was supplemented with zinc and copper.
Keywords: Follicular Fluid, Zinc, Copper, *In Vitro* Maturation, Vitrification

P-92: Decline in BRCA1 Expression; Significance in Ovarian Aging

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Background: Women are born with large number of oocytes and these oocytes undergo a progressive apoptosis with increasing age. Accepted opinion is that no new oocytes are produced other than those present at the time of birth. Studies in mice and human have shown that DNA repair genes in oocytes decrease with age, and decreased expression of these genes results in increased DNA breaks and oocyte death. In contrast to the ethical problems associated with monitoring the changes in DNA double-strand breaks in oocytes from young and aged humans, it is relatively easy to carry out such a study using an animal model.
Materials and Methods: In this study, the expression of the mRNA levels of DNA repair genes and protein products of some of the genes in the primordial follicles (Oocyte surrounded by a single layer of cuboidal cells) isolated from immature (18–20 days) and aged (400–450 days) female rats as well as adult sheep were monitored. RNA and protein was isolated from the primordial follicles and the levels of expression of selected genes (BRCA1, BRCA2, RAD51, MRE-11, ATM, H2AX and ERCC2) involved in repair of DNA double strand break were monitored by subjecting RNA to RT-PCR. In addition, the protein extract from primordial follicles was subjected to proteomic analysis.
Results: Results revealed a significant decline in mRNA levels of BRAC1, RAD51, ERCC2, and H2AX and phospho-protein levels of BRAC1 and H2AX in primordial follicles of aged rats. Proteomic analysis revealed a significant decrease in FIGNL1 (Fidgetin like protein 1) and increase in BOK (Bcl-2 related ovarian killer protein).
Conclusion: These results suggest that impaired DNA repair can be considered as a mechanism of oocyte ageing.
Keywords: BRCA1, DNA Repair, Ovarian Aging, Primordial Follicles

P-93: Assessment of Periodic Acid Schiff Reaction in Testicular Tubular Basement Membrane Following the Administration of Monosodium Glutamate and Quince Leaf Extract in Adult Rats

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Background: Monosodium glutamate is a food additive which acts as a preservative or enhancer of palatability. Extreme use of monosodium glutamate may induce multiple complications in biological systems such as neurological effects and subsequent tissue and cellular changes. Monosodium glutamate can induce oxidative stress through production of oxygen radicals and hydrogen peroxide which subsequently lead to oxidative DNA damage and cell membrane peroxidation and cellular death. The Quince (cydonia oblonga) leaf has a large amount of phenolic acids and flavonoids as antioxidant compounds. Reaction of flavonoids with free radicals leads to formation of more stable radicals with lower cytotoxicity. According to cytotoxic effects of monosodium glutamate on testicular tissue and the importance of seminiferous tubules basement membrane in regulation of spermatogenesis, the aim of this study was to evaluate the protective effects of quince leaf extract on the reproductive dysfunction induced by monosodium glutamate through histochemical evaluation of basal laminae carbohydrate changes.
Materials and Methods: Monosodium glutamate (30 and 60 mg/kg/day i.p.) and quince leaf extract (500 mg/kg/day p.o.) was administrated separately or in combination form for eight weeks. Tissue sections were performed on formaldehyde fixed testicular tissue samples and stained with" Periodic Acid Schiff" method.
Results: The results revealed that, the administration of monosodium glutamate dose dependently led to increase of basement membrane thickness. The most positive reaction was seen following the administration of combination form of monosodium glutamate (60 mg/kg) and quince leaf extract. The quince leaf extract had no noteworthy effect on thickness of seminiferous tubules.
Conclusion: It has been concluded that, dose dependent administration of monosodium glutamate can induce some changes in thickness of tubular basement membrane of testicular tissue which, subsequently may lead to generation of some alterations in spermatogenesis. Moreover, treatment with quince leaf extract may lead to reduction in alterations of tubular thickness in lower doses of monosodium glutamate.
Keywords: Monosodium Glutamate, Quince Leaf Extract, Carbohydrate, Testicular Tubular Basement Membrane, Rat

P-94: The Effect of Tricostatin A on Preimplantation Embryo Development following Round Spermatid Injection in Mice

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Background: Round spermatid injection (ROSI) has been proposed as an ART approach to treat infertility in males with primary testicular

failure. Nevertheless, the overall fertilization rate and early embryonic development with ROSI are dramatically lower than those following intracytoplasmic sperm injection. Some investigators have documented that the inefficacy of ROSI might be because of abnormal epigenetic modifications. Therefore, this study is aimed to evaluate the effect of Trichostatin A as an epigenetic modifier on preimplantation embryo development in activated ROSI oocytes.
Materials and Methods: *In vivo* matured oocytes were collected from superovulated female BDF1 mice injected with PMSG followed 48hr later by HCG injection. Oocytes were isolated from oviduct ampullae 14 h after injection of the HCG. Testes were placed in HTF medium, somniferous tubule masses were then cut into small pieces to disperse spermatogenic cells. Round spermatids were treated with Tricostatin A and ROSI was performed using Piezo-actuated micromanipulator. Following, the ROSI-fertilized oocytes were place in activation medium containing SrCl2 for 5-6 hours, and cultured in KSOM. Ultimately, embryo development was monitored 96-144 hours following injection.
Results: Fertilization rate following ROSI with Trichostatin was higher (93 ± 3%,) compared to control group (85 ± 10%), but no significant difference was found. The percentage of 4-cell embryos on day 2 and 8-cell embryos on day 3 was similar for both groups. In addition, a significant differences were determined in terms of blastocyst formation between two groups (Trichostatin: 44 ± 1%, control: 34 ± 1%).
Conclusion: These results indicate that Tricostatin would increase the success rate of development to blastocyst stage which leads to higher efficiency of ROSI.
Keywords: Round Spermatid, ROSI, Trichostatin A

P-95: The Effect of Hormonal Induction on BMP-15 Expression in Mice Oocytes

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Background: The expression state of BMP-15 gene product stored in oocytes that can be affected by factors commonly used in assisted reproductive technologies (ART) such as hormonal induction is one of the potential determinants of oocyte quality and could strongly impact the successful rate of ART procedures. The effect of hormonal induction on BMP-15 m-RNA expression in mice oocytes was investigated in this study.
Materials and Methods: Superovulated mature oocytes were collected from females administered a single dose (5 IU) of PMSG followed by the same dosage of hCG after 48 h. Naturally ovulated mature oocytes were collected from females in estrus stage kaged with a vasectomized male in the evening before the day of oocyte collection. The extraction of total RNA from oocytes, complementary DNA (cDNA) synthesis, and q RT-PCR analysis were carried out. Data were analysed with Rest software.
Results: The lower expression of BMP-15 gene was found in oocytes from mice treated with gonadotropins when compared to non-stimulated animals.
Conclusion: Exogenous endocrine stimulation with gonadotropins affected BMP-15 gene transcript levels in oocytes collected from hormonal treated animals suggesting that superovulation compromises oocyte quality and subsequent development. Therefore, ART procedures have the potential to disrupt proper embryo development.
Keywords: Gonadotropins, Superovulation, Oocyte Quality, BMP-15

P-96: Effect of Petroselinum Crispum Extract on Serum Nitric Oxide Level in Male Mice

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Background: Subfertility affects 15% of all couples in the world. Assessment of spermatogenesis plays a central role in the evaluation of the subfertile couple semen analysis and testicular biopsy constitute the most important investigations for the evaluation of male factor infertility. Nitric oxide and cyclic monophosphate signaling pathway 3.5 (cGMP) as an important signaling cascade in many mammalian cells, including Sertoli cells, germ cells are found in the testes. The aim of present study was to determine Parsley effect on serum nitric oxide (NO) levels in mice.
Materials and Methods: Twenty-eight healthy adult male mice were divided into four groups; each group containing seven rats. Hydroalcoholic extract of P. crispum was prepared and administered intraperitoneally (0,100, 150 and 200 mg/kg) to 28 mice, for 14 consequent days. NO concentration in the blood serum was determined with the Greiss method. The Greiss reagent is made up of a 1% solution of sulfanilamide and 0.1% naphthylethylenediamine dihycrochloride. Sample serums were collected and kept at -20° C. The protein the serum was deleted using zinc sulfate (6 mg/400 µl). Sodium nitrite (0.1 M) was used for the standard curve, and increasing concentrations of sodium nitrite (5, 10, 25, 50, 75, and 100 µM) were prepared. sodium nitrite and blood serum and was read by an ELISA reader (stat fax 100. USA) in 540 nm and 630 nm filters.
Results: The results show that the levels of nitric oxide decreased significantly between the groups receiving 100mg / kg extracts of parsley and control exists in other groups receiving 150, 200 mg/kg concentrations of ethanol extracts of parsley, despite there was no significant difference in reduction of nitric oxide.
Conclusion: This study's findings confirm that increasing the nitric oxide level may play an important role in the apoptosis process in germinal cells and spermatogenesis process. It seems that increasing the viability of sperms is due to decreasing the reactive oxygen species (ROS) in the medium via P. crispum extract treatment.
Keywords: Petroselinum Crispum , Nitric Oxide , Male Mice

P-97: Royal Jelly Improves Diabetes-Induced Toxicity in Rat Testis

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Background: Royal jelly (RJ) is a natural compound. Diabetes has destructive effects on male reproductive system. The purpose of this study is to investigate the effect of the RJ on detoxifying diabetes in the testis tissue of adult male rats.
Materials and Methods: The present experimental study was conducted on 32 adult male rats. The rats were randomly divided into 4 groups of 8 each, including the control group, the royal jelly group and the diabetic and RJ-treated diabetic groups. The control group received no drugs, while the RJ and RJ-treated diabetic groups were

given daily dose of 100 mg/kg/b.w of RJ. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) at 50 mg/kg. Their testis tissue sections were then prepared and examined. The antioxidant status was examined by evaluating testicular levels of ferric reducing antioxidant power (FRAP) and catalase (CAT) activity.
Results: The results showed that diabetes could increase the presence of degenerated epithelium and separation of the basement membrane, appearance of giant multinucleated cells, and could decrease number of germ cell layers, spermatogenous arrest seminiferous and tubular atrophy. Notably, RJ administration caused a recovery in above-mentined parameters. RJ treatment markedly increased activity of CAT and FRAP.
Conclusion: RJ improved STZ-induced impairment in testis, probably through its antioxidant effects.
Keywords: Diabetes, Royal Jelly, Streptozotocin, Testis

P-98: The Effects of Rosa Damascena Extraction on Histological Changes of Rat Testis

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Background: According to the historical record, consumption of herbal extractions to leads to improvement male reproductive. The aforesaid studies are being constantly updated. In the present study, we tried to explore the effects of rose extraction on histological characteristics of adult rat testis.
Materials and Methods: In the present research 40 mature male rats (200-220 g) were divided into 4 groups including 1 control group and 3 test groups (n=10). The extraction (Rosa damascena extraction, Barij Essence, Kashan, Iran) was prescribed in different dosages (10, 20, 40 mg / rat / day) for 60 days by oral gavage. The control group received saline by oral gavage. The rats were killed and histological parameters of testes were analyzed after measuring by using digital scale. The data was analyzed by Independent-samples T-Test by using 16 SPSS software and consider significant the level of $P<0.05$.
Results: Gavaging different doses of rose extraction had no side effects on rats. Although there was no significant difference in weight and dimension of testis, the number of sertoli and leydig cells increased significantly in extraction treated groups compared to those of the control group ($P<0.05$). The number of Leydig cells with a dose of 10 mg essence not have significant difference compared to control, but doses of 20 and 40 mg showed significant difference in comparison to that control group ($P<0.05$). Sertoli cells with dose of 40 mg, not 10 and 20 mg of rose extraction revealed significant difference ($P<0.05$) compared to the control cells.
Conclusion: Rosa damascena extraction can be included up to 40 mg/rat/day without affecting health status while increasing Sertoli and Leydig cells.
Keywords: Histology, Spermatogenesis, Rose Extraction, Rats

P-99: The Effects of Rose Essential Oil (Rosa damascene) on Parameters of Semen Quality and Reproductive Hormones in Rats

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Background: High concentrations of long-chain fatty acids in the lipid structure of sperm cells needs to have effective antioxidant system. Herbal extractions are among the richest reservoirs of antioxidants. Despite the antioxidant compounds such as carboxylic acids, linalool, Eugenol and Citronellol in rose, no research has been conducted about the effects of its oral medication on the male productive system. In the present survey the effects of different dosages of Rose Essential Oil oral medication on spermatogenesis and reproductive hormones has been evaluated.
Materials and Methods: In the present study 04 mature male rats (044-004 g) were divided into 0 groups including control group and 3 test groups (n=04). The extraction (Rosa damascena extraction, Barij Essence, Kashan, Iran) was taken in different dosages (04, 04 and 04 mg / rat / day) for 04 days by oral gavage. The control group received saline by oral gavage, too. The rats were killed and qualitative criteria of caudal epididymis sperms as well as concentrations of testosterone, LH and FSH were evaluated by cardiac puncture. The data was analyzed by Independent-samples t test by using 00 SPSS considering significant at $P\leq4040$.
Results: Progressive motility of spermatozoa were 00, 03, 00% in ESS04, ESS04 and ESS04 groups respectively, compared to those of the control group (00.0%) and the first group shows a significant increase ($P<4040$). Sperm viability of ESS04 (00%) and ESS04 (05%) group increased significantly compared to those of control (00.0%) ($P<4040$). Only in the group of ESS04, Count, motility and morphology of normal sperms were significantly higher than those of control group. Although there were no significant changes for the several dosages comparison FSH to control group, LH and Testosterone concentrations were significantly affected by rose oil.
Conclusion: The present results demonstrate the beneficial effects of dietary supplementation with high dose of Rose Essential Oil to improve the sperm quality of mature rat, which warrants further studies.
Keywords: Spermatogenesis, Reproductive Hormones, Rose Extraction, Rat

P-100: Effect of Sevofluranon on Testicular Molecular Cellular Function and Spermatogenesis Quality in Neonatal Male Mice

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Background: Sevofluranon potential effects on male and female fertility have not been adequately investigated. So, this study investigated Sevofluranon effect on spermatogenesis process in male neonatal mice.

Materials and Methods: 24 neonatal NMRI male mice were classified in 3 groups randomly. Experimental group 1 received 2 litter MAC/30min Sevofluranon combined 2 litter/min oxygene per day in a single dose. Experimental group 2 received 1 litter MAC/30min Sevofluranon combined 2 litter/min oxygen during 7 days in a sequential dose. Control group did not receive any treatment. All groups were scarified after treatment. Testicular tissue was evaluated for cellular and molecular evaluation. Histological assessment, immunohistochemistry and apoptosis process was done by H&E staining and TUNNEL assay. PLZF, Vimentine and Oct4 markers was used in immunohistochemistry. Bax and Bcl2 expression profile was evaluated in testicular tissue by real time PCR. Data was analyzed by ANOVA and Tukey post hoc test.
Results: Our results showed that integrity of testiculat tissue preserved in all experimental groups. Count of spermatogonial and sertoli cells did not any significant differences in all groups. The result of apoptosis assay showed 11% ± 2 and 8%±1 apoptosis in spermatogonial cells in the group 2 and 1, respectively. Also, Bax/Bcl2 was 3.224, 2.631 and 8.318699 in control, experimental group 1 and 2, respectively. This result was significant (P≤0.05) between groups 2 with other groups.
Conclusion: A single 30 min exposure of 1 litter MAC Sevoflurane in the presence of 2 litter/min oxygen preserve integrity of testicular tissue and lower apoptotic cells in neonatal mice testis. Ratio of Bax/Bcl2, apoptotic and germ cell count during 7 days exposure was significant in comparison to one day exposure. This observation produced by Sevoflurane is related to dose and duration of exposure.
Keywords: Spermatogenesis, Sevofluranon, Apoptosis, Gene expression.

P-101: Culturing Mouse Peritoneum Mesothelial Stem Cells

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Background: Peritoneum mesothelial stem cells have been reported to reside in the monolayer of mesothelium and the peritoneal cavity as free floating cells. Recently, anterior abdominal wall peritoneum mesothelial stem cells (APMSCs) can give rise to differentiated cells. The purpose of this study was to investigate the viability, stem cell surface markers and differentiation capacity of APMSCs.
Materials and Methods: Peritoneum mesothelial cells were isolated from the cultured mouse anterior abdominal wall peritoneum in DMEM F12 medium supplemented 10% (vol/vol) FBS. The morphology was observed by using of a light microscopy at and viability of cells was validated by MTS assay. After 2-3 passages, APMSCs were processed for immunoflourescence assay in order to specific markers. Also, specific differentiation medium were defined for stem cells differentiation to osteocyte and adipocyte. APMSCs at the 4th passage were cultured in DMEM F12 medium containing 10% human follicular fluid for 24 days.
Results: Following 2 or 3 passages, the number of cells assumed homogeneous morphology and there was enhanced cell viability during culture *in vitro*. After second passage, immunoflourescence assay demonstrated expression of the surface antigens, stem cells and epithelial cells markers $CD90^+$, $CD44^+$ and Cytokeratin19, alternatively. In addition, differentiation of APMSCs into osteocytes and adipocytes has been observed when specific medium was used. Finally, during the period induction with human follicular fluid, a subpopulation of the cultured cells appeared that had a morphological resemblance to oocyte-like cells and blastocyst-like complex.
Conclusion: Our data demonstrated that mesothelial stem cells could be isolated from anterior abdominal wall peritoneum and they were capable to differentiate into blastocyst-like complex and oocyte-like cells.
Keywords: APMSCs, Differentiation, Blastocyst-Like Complex, Oocyte-Like Cells

P-102: Curcumin Protects the Testis inChronic variable Stress-Treated Rats withor Without Recovery Period; A Stereological and Histochemical Study

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Background: Chronic variable stress (CVS) can jeopardize reproductive organ including testis. Curcumin (CUR) as an anti-oxidant and anti- apoptosis component is the key element of the turmeric. The objective was designed to evaluate the possible protective effect of CUR on negative effect of CVS with or without a recovery period on the testis structure and function.
Materials and Methods: Sprague-Dawley rats were assigned to seven groups: control, distilled water, CUR (100mg/kg/day CUR in0.5mL of olive oil), olive oil, CVS, CUR+stress and olive oil+stress. Half of the animals were sacrificed after 15 days and the second half was allowed to recover for 50 days. Testosterone and cortisol serum levels, semen parameters, spontaneous acrosome- reaction spermatozoa (SARS), acrosome-intact spermatozoa (AIS) and testis stereological structure were evaluated.
Results: Significant changes occurred in testosterone and cortisol serum levels, semen parameters, the percentage of the SARS and AIS in CVS and CVS+recovery groups compare to the control rats (P<0.01). The tubules length, epithelial volume, number of Sertoli cells, Leydig cells, spermatogonia type A and B, spermatocytes, elongated and round spermatids were reduced 40-63% in CVS animals in comparison with the control ones (P<0.01). During the CVS+recovery period the aforementioned parameters were reduced 20-50%. The parameters in CUR+stress animals changed in a lesser extent as compared with CVS rats with or without a recovery period (P<0.01).
Conclusion: Exposure of rats to 15 days of stress can alter testicular structure and function even after 50 days of recovery period. Curcumin can protect the testis in the stress-exposed rats.
Keywords: Stereology, Histochemistry, Stress, Curcumin, Testis

P-103: Maturation and Fertilization Rates of Human Oocytes Near Granulosa Cells

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Background: Culture conditions used for *in vitro* maturation (IVM) of oocytes can significantly influence the maturation rates and embryo development. A particularly crucial component for natural cytoplasmic oocytes maturation is induced by the normal presence of granu-

losa cells (GCs) or cumulus cells (CCs) surrounding oocytes. During performance of micromanipulation techniques, Such as intracytoplasmic sperm injection (ICSI) or *in vitro* fertilization (IVF), human oocyte is denuded (stripped of its surrounding CC). In this regard, to increase the number of mature oocytes appropriate for ICSI procedure, we aimed to investigate the role of GCs co-culture on human oocyte maturation fertilization rate, and embryo development *in vitro*.
Materials and Methods: 133 immature oocytes retrieved and were randomly divided into two groups; oocytes that were cultured with GCs (Group A) and oocytes cultured without GCs (Group B). Only oocytes that reached metaphase II (MII) stage after IVM were used for ICSI procedure. Maturation rate, fertilization rate, and embryo development were compared the statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) for Windows.
Results: The mean age, basal FSH, and number of oocytes recovered for the patients were not different between the two groups. The number of oocytes that reached M II (mature oocytes) was 59(84.28%) in the GC-co cultured group compared to 41(65.07%) in group B (P = 0.011). No significant difference between fertilization rates was found between the two study groups (P = 0.702). The number of embryos that were developed in the GC- co cultured group was 33(75%) compared to 12(42.85%) in group B (P = 0.006). The top quality embryos were significantly lower in group B compared to group A (P = 0.003). Also, the rate of blastocyst formation in group B was markedly lower than that in group A (P = 0.000).
Conclusion: Findings of the current study revealed that culturing immature human oocytes with GCs prior to ICSI procedure improves the maturation rate and embryo development.
Keywords: Fertilization, Granulosa Cells, Human Oocytes

P-104: Mouse Primary Follicles Culture on Intact and Decellularized Human Amniotic Membrane

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Background: *In vitro* culture of ovarian follicles represents one of the most important tools in the field of assisted reproduction. Since folliculogenesis requires well cell-matrix interaction, the natural scaffolds seem to be useful in follicular culture. Human amniotic membrane has many advantages for using in ovarian follicular culture *in vitro*, as a biological layer and supportive structure because of its easy obtaining and rich ECM.
Materials and Methods: Amniotic membranes (AM) were removed from the placenta, separated from the chorion and decellularized with trypsin and EDTA. DNA quantitative and histological assays were performed to determine whether the treatment successfully eliminated genomic components. The small parts of IAM and DAM were individually coated on the floor of 96-well microplate and each well was filled with 150 µl of base medium (BM): MEM-α + 1% FSH, 1% ITS and 5% FBS. Then, isolated mouse primary follicles (90-110 µm in size) were individually put in each well. The follicles cultured only in BM were considered as control (C) group and those cultured on IAM and DAM were named experimental groups (E1 and E2). Nine days after culture, size, morphology, viability and estradiol production of the follicles were evaluated.
Results: At the end of culture, the primary follicles cultured on IAM had a better growth and development into the well large preantral follicles, whereas the same follicles in the other groups showed a lower growth speed and rate. In addition, subjected to experimental groups, the viability rate and estradiol production was significantly higher than the control one.
Conclusion: Amniotic membrane as a rich nutritional matrix can provide an appropriate supportive layer for mouse primary follicles and can be considered as a natural system improving the outcome of isolated follicular *in vitro* culture.
Keywords: Human Amniotic Membrane, Ovarian Follicular Culture, Decellularization, Natural Scaffolds

P-105: PPARγ Protein Level in Sperm of Astenozoospermic and Normospermic Men

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Background: Many studies have been shown that several functional sperm mRNAs and proteins are delivered into the oocyte after fertilization,it seems that This event plays an important role in fertility.Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear fatty acid receptor that has been implicated in energy homeostasis and it modulates lipid metabolism. However, to our knowledge, the question of whether PPAR gama protein level are different in several sperm quality has not been addressed.The current study was designed to determine and compare PPARγ protein expression in sperm of normozoospermia and astenozoospermia men.
Materials and Methods: Ejaculated sperm have been collected from normozoospermia and astenozoospermia men (n=30) referred to Royan Institue. Exclusion criteria consisted of the presence of urogenital infections, systematic and chronic diseases (e.g., renal and liver disease, type 2 diabetes), osteometabolic disorders, malignancy and malabsorption. The semen parameters were measured by CASA . The sperm parameters of normospermic men were motility >40%, morphology >4%, concentration >15 million/ml. PPARγ protein expression determined by Flow cytometry.The anti-PPARγ antibody (Thermo scientific) was used as primery antibody.Adipose cell and RBC cell were used Respectively as positive and negative control. Data were analyzed using the MIXED procedure of SPSS2 Program.
Results: PPARγ protein expression was determined by Flow cytometry in sperm of both groups. The mean percentage of expression was 2.1300 for astenozoospermia and 1.6429 for normozoospermia men. The protein expression was unaltered by grouping (normozoospermia and astenozoospermia) (P>0.05).
Conclusion: Our data suggest that PPARγ protein is expressed in human sperm, however in this limited observation, no significant differences were seen for PPARγ protein between normozoospermia and astenozoospermia men .
Keywords: PPARγ, Sperm, Flow Cytometry

P-106: Fennel Causes Developmental Retardation of Preimplantation Mouse Embryos

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Background: Fennel (foeniculum vulgare), a well-known medicinal plant, is used extensively due to its pharmacological properties. This study was carried out in mice in order to examine if fennel exposure could disrupt the early embryonic development.
Materials and Methods: Adult male mice were randomly categorized into experimental and control groups. The experimental group subdivided into three groups, which received 0.37 mg/kg/day, 0.75 mg/kg/day and 1.5 mg/kg/day fennel oral drop orally for 35 days. Epididymal sperm fertilizing potentials were assessed in four groups following *in vitro* fertilization.
Results: Fennel treatment resulted in defective preimplantation embryogenesis in a dose-dependent manner as evidenced by significant reductions in fertilization and blastocysts rates as well as elevated amounts of embryo arrests.
Conclusion: These data suggest that fennel may impact the reproductive functions of male mice resulting in abnormal early embryonic development. It is clear that the necessity of more researches investigating the mechanisms of fennel effects on the male reproductive system is inevitable.
Keywords: Fennel, Early Embryonic Development, Fertilization, Mice

P-107: The First Report on Laying Hen Ovarian Tissue Vitrification by Using Different Cryoprotectants

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Background: Although successful cryopreservation techniques of avian gonads can be an important step towards the preservation of several species, little information has been reported on cryopreservation of avian ovaries. The aim of the present research was to evaluate of vitrification effect by several cryoprotectants on laying hens ovarian tissue.
Materials and Methods: To vitrification, laying hens ovarian tissues after fragmentation were divided into six groups including: C (control), EG (ethylene glycol), DMSO (dimethyl sulfoxide), PROH (propanediol), DMSO+EG and PROH+EG. Morphologic evaluation of follicles in ovarian tissues of vitrified and control groups were carried out. The number of intact follicles was estimated. The composition of vitrification solutions were as follows: EG group: V1=7.5% EG and V2=15% EG+0.5 M sucrose, DMSO group: V1=7.5% DMSO and V2=15% DMSO+0.5 M sucrose, PROH group: V1=7.5% PROH and V2=15% PROH+0.5 M sucrose, DMSO+EG group: V1=7.5% DMSO+7.5% EG and V2=15% EG+15% DMSO+0.5 M sucrose and PROH+EG group: V1=7.5% EG+7.5% PROH and V2= 15% EG+15% PROH+0.5 M sucrose. Each group Ovaries were dehydrated with V1 and V2 solutions and picked up with the cryopin. The samples were exposed with each solution for 1 minute in 4º C. Also imaging of intact and degenerate follicles was performed in control and vitrified tissues.
Results: EG+DMSO had the highest primordial follicles (57%) between vitrification groups, whereas the EG and DMSO groups (mean: 34 %) in the middle and PROH and EG+PROH groups were the lowest (mean: 18.5%) ($P<0.05$) compare with control (94%). primary follicles were similarly affected by EG (28%), DMSO (25%), EG+PROH (25%), but EG+DMSO (61%) and PROH (11%) have the highest and lowest one ($P<0.05$) compare with control (91%).
Conclusion: Vitrification of laying hen ovaries using DMSO+EG can provide more protection of primordial and primary follicles. Therefore, it could be suggested to save the storage of gonadal tissues especially in endangered species.
Keywords: Vitrification, Ovary, Laying Hen

P-108: Evaluation of Igf2r and Igf2 Expersion in Liver and Brain of Fetuses of Superovulated and Natural Cycle Mice

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Background: Ovarian stimulation effects on oocyte and embryo quality and also fetal growth and development. Igf2 and Igf2r are imprinting genes which express only in paternal and maternal alleles respectively. These are involved in embryo and fetal growth. In this study the expression level of these genes in liver and brain of fetuses of superovulated and natural cycle mice were evaluated.
Materials and Methods: Igf2 and Igf2r genes expression in liver and brain of 19 day fetuses in natural cycle (as control group) and superovulated (as experimental group) mice were evaluated by Real-Time PCR. In order to analyze the data, descriptive and inferential statistics were used. Wilcoxon Signed Rank Test was used to compare gene expression between groups. Data software SPSS version 16 was used and the values of $P<0.05$ was considered significant.
Results: The results illustrate that the expression level of observed imprinting gene Igf2 was increased in superovulated group compared to control in liver significantly but in brain there was no significant difference in Igf2 expression between the two experimental groups. Expression level of Igf2r was increased in superovulated group compared to natural group in brain significantly. But in liver there was no significant difference between the two experimental groups.
Conclusion: According to our results, ovarian stimulation causes higher expression of Igf2 gene in liver and Igf2r gene in brain of superovulated mice compared with natural cycle mice fetuses. This Can be a result of gene over expression or biallelic expression of these imprinting genes which occurs in abnormal conditions.
Keywords: Ovarian Stimulation, Mouse Fetuses, Igf2, Igf2r

P-109: Comparison of Phospholipase C Zeta Protein Level in Asthenozoospermic Patients and Normal Healthy Donors

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Background: Although the few studies have conducted on fertilization rate of asthenozoospermic patients, their outcomes have indicated poor fertilization rate even by using assisted reproductive techniques as compared to normal individuals. Sperm oocyte activating factor (SOAF) deficiency may be associated with low fertilization rate. PLCζ (phospholipase C zeta) as putative factor for SOAF could evaluate in poor fertilization rate patients. To our knowledge, level of PLCζ protein in impaired sperm motility men has not been reported. Thus, the purpose of this study was to compare the expression profile of PLCζ protein in the spermatozoa of asthenozoospermic and normozoospermic men.
Materials and Methods: Semen samples from 10 normozoospermic and 10 asthenozoospermic men who referred to Royan institute were collected by masturbation after 3 days of sexual abstinence. Post liquefaction, sperm count, motility and morphology were assessed according to WHO 2010 criteria. Then PLCζ protein expression was studied using Western Blotting and immunofluorescence techniques. Images were analyzed by "Image J" software.
Results: The level of PLCζ protein that assessed by western blotting and immunofluorescence techniques was not significantly different in spermatozoa of asthenozoospermic men as compared to normozoospermic controls. PLCζ deficiency was not revealed in impaired sperm motility patients.
Conclusion: In this limited observation, no significant differences were seen for PLCζ protein expression between asthenozoospermic and control group. Therefore, measurement of PLCζ protein level may not be used as a predictor marker for fertilization rate in asthenozoospermic patients. It will be necessary to conduct large scale studies to confirm our data.
Keywords: Phospholipase C Zeta, Asthenozoospermic, Fertilization Rate, Male Infertility

P-110: Evaluation of Phospholipase C Zeta Protein Expression in Unexplained Infertile Men

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Background: Assisted reproductive techniques (ART) have an effective role in treatment of infertile couples. Despite the continuously expanding applications of these techniques, it is estimated that 1–5% of intracytoplasmic sperm injection cycles still fail because of the oocyte activation failure. One of the reasons have been proposed for this failure is using improper sperm based on the SOAF (sperm oocyte activating factor). A number of proteins have been suggested as the candidate of SOAF. However, based on a large body of evidence, PLCζ (phospholipase C zeta) has known as the strongest candidate. PLCζ plays an important role in Ca^{2+} oscillations generation and eventually oocyte activation. Therefore, the objective of this study was to determine the expression of PLCζ protein in sperm of unexplained infertile individuals.
Materials and Methods: Semen samples collected from 10 fertile men (as control group) and 10 male patients following one or more failed ART cycles and unexplained infertility, whereas female infertility problems were not diagnosed in their partners. Standard semen analysis was performed to determine normal sperm parameters in samples. Subsequently, the expression of PLCζ protein was assessed by Western blot and immunofluorescence. Images were analyzed by "Image J" software.
Results: PLCζ expression levels as determined by western blotting and immunofluorescence in unexplained infertile men were not significantly different from fertile group. No deficiency of PLCζ was demonstrated in this group of patients.
Conclusion: Our data suggest that in unexplained infertile men with normal semen analysis parameters but with failed ART cycles, PLCζ deficiency might not be considered as an important cause for the ART failure. Further studies on larger population of patients are required to obtain our data.
Keywords: Phospholipase C Zeta, Unexplained Male Infertility, Oocyte Activation

P-111: Appropriate Dose of Nano-Curcumin for Enhancing Embryo Development; An Experimental Study

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Background: Considering wide range usage of Nano-curcumin (NCMN) as supplementary substrate in various fields of medication, the possible side effects of NCMN seems to have high importance. Therefore, present study was done in order to uncover the possible beneficial and/or detrimental effect of NCMN on *in vitro* embryo development.
Materials and Methods: To follow-up current study, 24 mature female Wistar rats were assigned into control and test groups. The animals in test group were divided into 7.5 mg/kg b.w-1, 15 mg/kg b.w-1 and 30 mg/kg b.w-1 NC-MN-received groups. The NCMN was administrated orally by gavage. After 45 days, the oocytes were picked-up by inducing superovulation (15 IU PMSG and 15 IU HCG). The sperms were collected from healthy male rats and (1×10^6 per mL) added into fertilization drop.
Results: As preliminary data, the animals in 7.5 mg/kg b.w-1- received group exhibited higher zygote percentage versus control and other test groups. Moreover, the NCMN at dose level of 7.5 mg/kg b.w-1 significantly ($P<0.05$) decreased arrested embryos and enhanced 2-cell, blastocyst and hatched embryos percentages in comparison to control group. However, the animals in higher doses (15 mg/kg b.w-1 and 30 mg/kg b.w-1)-received groups revealed remarkably ($P<0.05$) lower zygote percentage as well as embryo development.
Conclusion: Our data showed that, NCMN at dose level of 7.5 mg/kg b.w-1 could be used as a beneficial supplementary substrate to provoke *in vitro* embryo development. Moreover, we showed that administrating 15 mg/kg b.w-1 and 30 mg/kg b.w-1 of NCMN negatively affects the embryo development.
Keywords: Nano-Curcumin, Zygote, 2-Cell, Blastocyst, Hatched

Embryos

P-112: Protective Effect of Curcumin agains Zinc Oxide Nanoparticles on Sperm Parameters in Adult Rat

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Background: Zinc oxide nanoparticles are one of the most widely used nanoparticles in fields of industry, medicine, pharmaceutical sciences, cosmetics, and nutrition. Zinc oxide nanoparticles has cytotoxic effects on testis and could induce oxidative stress. Curcumin is an antioxidant compound anti-inflammatory and anti-tumor promoting activities. The aim of this study is to investigate the possible protective effect of curcumin against Zinc oxide nanoparticles in male rats.
Materials and Methods: Adult male wistar rats (150-200 gr) were divided into 4 groups , control and experimental group1(ZnO nanoparticles) , experimental group 2 (curcumin), experimental group 3 (ZnO nanoparticles + curcumin). Control group received only distilled water. The experimental group 1 were treated with ZnO nanoparticles 500 mg/kg/day. The experimental group 2 treatment with curcumin 100 mg/ml /daily and the experimental group 3 treatment with ZnO nanoparticles 500 mg/kg + curcumin 100 mg/ml/daily (IP) single injection for 30 days. At the end of the experimental period the mean body weight growth and the ratio between body and testis weight were calculated and compared with the control groups, Spermatogenesis Parameters such as, progressive motility,Sperm viability, normal morphology, SDFA and The total count of sperm and the level of Malondialehyde (MDA) were evaluated. Sperm chromatin quality was assessed by nuclear staining using acridine orange and aniline blue. The data were analyzed using Danken and one way variance test and the P≤ 0.05 were considered significant.
Results: A significant decrease in the number, viability , motility and percentage of abnormality the sperm was found in experimental group 1 (ZnO nanoparticles). This decrease was significantly compensated by curcumin in experimental group 3 (ZnO nanoparticles + curcumin) compared to experimental group 1. Administration of curcumin alone could significantly increase sperm viability and motility as comared with the control group. The level of MDA was increased in the experimental group 1. However, administration of curcumin with ZnO nanoparticles reduced the level of MDA.
Conclusion: These results suggest curcumin have antioxidant properties that make it able to protect sperm parameters against ZnO nanoparticles induced oxidative stress.
Keywords: Curcumin,Zinc Oxide, Nanoparticle,Sperm,Rat

P-113: Comparison of DNA Methyltransferase Expression in Fresh and Vitrified Mouse Ovarian Tissue Derived Pre-antral Follicles

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Background: Cryopreservation of ovarian tissue is a good option in females with ovarian failure. DNA methyltransferases (Dnmts) have crucial role in normal folliculogenesis and could be affected by cryopreservation. Our aim was evaluation of Dnmts (Dnmt1, Dnmt2, Dnmt3a, Dnmt3b, Dnmt3l) genes in fresh and vitrified-warmed mouse ovarian tissue derived pre-antral follicles.
Materials and Methods: Ovaries of 13-day old NMRI mice were isolated and randomly allocated into two groups: fresh and vitrification groups. Vitrification was performed by ethylene glycol (EG) and dimethyl sulfoxide (DMSO) combination in 2 steps and warming was done in descending concentration of sucrose (1,0.5,0.25 M). Afterwards, middle-sized pre- antral follicles were isolated in both groups and cultured in α-MEM supplemented with (FSH 1%, ITS 1%, FBS 5%) for 1 day. The expression of Dnmts genes were evaluated with Real-Time PCR method.
Results: Although there was no significant difference between fresh and vitrified groups in Dnmt3a, Dnmt3b, Dnmt2 expression, there was a significant difference (P<0.05) in Dnmt1 and Dnmt3l after 1 day culture of pre-antral follicles that derived from fresh and vitrified ovaries.
Conclusion: Our finding implies association between vitrification and alteration of DNA methylation level in ovary derived cultured pre-antral follicles.
Keywords: Pre-Antral Follicle, Dnmt, Vitrification

P-114: Effects of Aqueous Extract Vaccinium Arctostaphylos Fruit on Balb/C Mouse Embryos 7Th to 10Th Days of Pregnancy

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Background: Aqueous extract of vaccinium arctostaphylos fruit in addition to a powerful antioxidant property,used as a drug for blood pleasure and diabetes. But it is effects on during pregnancy has not been reviewed. Therefore in study effects of aqueous extract of vaccinium arctostaphylos on Balb/C mouse embryos 7Th to 10Th days of pregnancy.
Materials and Methods: In this study 50 female Balb/C mice were randomly divided into 6 equal groups acontrol group (non- injection) and witess (injection of saline) and4 experimental groups.a LD50 was determined in condition of09/11 mg/kg.bw *in vivo* and selected dose for injection 3mg/kg.bw. Injection was done by enema. For security of results above experiences was repeated 2times.Data was checked with SPSS17 software and ANOVA test subjest to P<0.001, P<0.05.
Results: Injected Vaccinium arctostaphylos is caused reduces the weight of the placenta and Embryos, Crown–Rump Length and estradiol levels and Increased levels of the progesterone. Abnormalities such as Exohepatic,C-shaped body, bleeding under the skin and Asymmetry in the development of fingers and toes body were seen copared to the control group.
Conclusion: In according to this observations can be concluded aqueous extract of vaccinium arctostaphylos is caused tratogenic effects and reduced growth in the embryonic period, using that is dangerous during pregnancy and may be use as a contraceptive pill in future.
Keywords: Vaccinium Arctostaphylos, Estrogen,Progesterone,Ano malies, Mouse Embryo

P-115: Cleavage Rate of *In Vitro* Matured Ovine

Oocytes Vitrified in Different Concentrations of Trehalose

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Background: The ability to efficiently cryopreserve domestic oocytes has many potential applications in livestock industry. This study was designed to determine the optimum concentration of trehalose in vitrification solution of *in vitro* matured ovine oocytes.
Materials and Methods: After *in vitro* maturation, oocytes were randomly distributed in 4 experimental (vitrified) and 1 control (fresh) groups with 5 identical replicates for each. Each replicate contained at least 20 oocytes per treatment. Experimental groups were designed according to different concentrations (0, 0.25, 0.5 and 1 M) of trehalose in vitrification solution. After warming, survived oocytes were subjected to *in vitro* fertilization. Cleavage rate was evaluated at day 5 post insemination. In addition, some vitrified-warmed and fresh oocytes were used to test the zona pellucida (ZP) solubility in 0.25% pronase. All percentage data were analyzed by one-way ANOVA. The level of statistical significance was set at $P < 0.05$.
Results: Oocyte survival after vitrification was higher in 0.5 and 0.25 M trehalose (92.9 ± 2.2%, 93.2 ± 2.2% respectively) than the others vitrification groups (44.4 ± 2.1%, 53.9 ± 2.4% in 0 M and 1M respectively; $P<0.05$). Also, cleavage rate was higher in 0.5 M (65.5 ± 4.7 %) compared to other vitrified groups (61.1 ± 4.0, 27.9 ± 16.3% and 43.2 ± 7.3% in 0.25, 0 and 1 M respectively). On the other hand, there was no significant difference between 0.5 M and control group in total cleavage rate (65.5 ± 4.7 % versus 71.8 ± 3.5%; $P<0.05$). Moreover, zona pellucida digestion time was longer in 0M than other vitrification and control groups ($P<0.05$).
Conclusion: *In vitro* matured ovine oocytes vitrified at 0.5 M trehalose, fertilized and developed to morula at higher rate than 0.25 and 1 M. Therefore, 0.5 M may be the optimum concentration of trehalose in vitrification solution of *in vitro* matured ovine oocytes. However, high concentration of trehalose reduces the cleavage rate in vitrified oocytes.
Keywords: Ovine, Oocyte, Vitrification, Trehalose Concentration, Zona Pellucida

P-116: The Effect of Culture Medium Containing Fetal Calf, Bovine Albumin and Autologous Sera on Expression of Developmental Genes and Fragmentation Rate in Mouse Embryos

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Background: Research studies on reproductive mechanism of laboratory animals are essential for further advancement of assisted reproductive techniques (ART). One of these studies includes the assessment of the effect of types of sera in culture medium on development of pre-implantation embryos. This study, for the first time the effect of different protein supplements (BSA or FCS), and autologous serum (mouse serum) on the expression level of Oct4 and Cdx-2 developmental genes and fragmentation in mouse blastocysts was examined.
Materials and Methods: Two pronucleuse stage embryos (2pn) from *in vivo* were collected. The 2pn's were randomly divided into three culture medium (4% bovine albumin serum (BSA)- 5% fetal calf serum (FCS)- 10% autologous serum (AS)). These embryos were cultured to the blastocyst stage. Quantitative expression of two developmental genes, namely Oct4 and Cdx-2, were performed in these groups, using RNA purification and Real-time RT-PCR. Then the rate of blastocysts fragmentation were measured.
Results: Quantitative PCR analysis showed that the expression level of both genes, Oct4 and Cdx-2 was not significant between 3 groups, but the fragmentation rate in embryo culture containing FCS were increased significantly ($P<0.05$).
Conclusion: In embryo culture, medium supplemented with BSA and FCS are commonly used and considerable efforts have been directed toward searching for possible FCS and BSA alternatives, in this study autologous serum (mouse serum) have been identified as promising substitutes, which have a similar effect on embryo culture compared with FCS and BSA and simultaneously are free from ethical concerns and infection.
Keywords: Autologous Serum, Bovine Albumin Serum, Fetal Calf Serum, Developmental Genes, Fragmentation

P-117: Effects of Lavendula Officinalis Queous Extract on Reproductive System in Balb/C Mouse

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Background: Lavendula officinalis aqueous extract in addition to the sedative properties treatment,effects on diabetes and depression.But it is effects on reproductive system has not been reviewed .Therefore in study Investigation of Lavendula officinalis aqueous extract on reproductive system in Balb/C mouse.
Materials and Methods: At the first get a herb aqueous extract and determined LD50 and when observed lack of experience symptoms of lethal, experiment was continued with selected dose 6(group1), 12(group2), 18(group3), mg/kg.bw. Injection was done on the 12 days by enema on 65 mouse. At the same time with the experimental groups,a control group(non-injection) and witess (injection of saline) for comparison of results was held,too.for security of results above experiences was repeated 3 times. Data was checked with SPSS-20software and Duncan test subjest to ($P<0.001$).
Results: In experimental groups1 and 2, showed a significant decrease in large and small diameters of ovaries and uteri ($P<0.05$). In experimental group3 showed a significant increase numbers of Primary follicles ($P<0.05$). In all experimental groups showed a significant decrease Body Weight ($P<0.05$) and a significant decrease Growing and Graafian follicles,Corpus Luteum and uterine glands and decrease thickness of endometrium, myometrium and perimetrium, Increased embryo abnormalities in groups 2 and 3 ($P<0.001$), Increased duration of fertility and the number of live births in group 2 ($P<0.05$).
Conclusion: In according to this observations can be concluded Lavendula officinalis herb aqueous extract has damaging effect on female reproductive system and may be use as a contraceptive pill in future.
Keywords: Lavendula Officinalis, Reproductive System, Mouse

P-118: Effect of Silver Nanoparticles on Oxidant/Antioxidant Markers of Mouse Ovarian Granulosa Cells

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Background: Exposure of human to silver nanoparticles (SNP) has been increased during the past decade, but there is no enough information about the adverse effect of those on normal cells of various tissues such as reproductive tissues. In the present study the effect of different concentrations of SNP on antioxidant enzyme activities, superoxide anion and malondialdehyde production in mouse granulosa cells was studied.
Materials and Methods: Granulosa cells were isolated from ovary of adult mice. Cells were cultured in DMEM medium and exposed to 10-15 nm SNP at various concentrations (100-500 µM) for 24. After that cells were homogenized in RIPA buffer and the activities of superoxide dismutase, catalase, glutathione proxidase were determined using commercial kits. Superoxide anion production and malondialdehyde levels were measured using NBT and thiobarbituric acid assays, respectively.
Results: Our results demonstrated that 24 exposure of granulosa cells to SNP caused a dose dependent elevation of superoxide anion production in concomitant with increased level of malondialdehyde and depleted SOD and GPX activities. SNP at dose of 300 µM caused the highest oxidative stress in granulosa cells from ovary of adult mice.
Conclusion: Our findings demonstrated that SNP in a dose dependent manner could induce oxidative stress in mouse granulosa cells, supporting the adverse effect of SNP on function and vitality of ovarian cells.
Keywords: Silver Nanoparticles, Oxidative Stress, Granulosa Cells, Mouse

P-119: The Effect of Ceratonia Siliqua L Hydroalcoholic Extract on Sperm Parameters (Motility) in Mice

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Background: Male fertility issues are usually not related to having an unusually low sperm count, but to having sperm with low motility. That is, they don't get around very well. Antioxidants are essential for sperm motility. Ceratonia siliqua L extract possesses the important antioxidants quercetin and selenium. This study investigates the effects of Ceratonia siliqua L extract on sperm parameters in testes of male mice.
Materials and Methods: In this experimental study, male mice were divided into control and 3 experimental groups Ceratonia siliqua L extract at doses of 50,100,150,200 mg/kg was administered in traperitoneally for 10 days. Also weight of testes, motility rate and number of sperms were assessed. Data analysis was performed using one-way ANOVA followed by Tukey test.
Results: Our results showed that after Ceratonia siliqua treatment, there were improved sperm parameters in the 150 mg/kg-treated group compared to the other groups ($P \leq 0.05$).
Conclusion: Ceratonia siliqua L extract increases the number, motility of sperm because of antioxidant components of Ceratonia siliqua L for example selenium and quercetin.
Keywords: Ceratonia Siliqua L Extract, Sperm, Male Mice

P-120: Development of Mouse Preantral Follicle after *In Vitro* Culture in Chicken Embryo Extract Containing Medium

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Background: *In vitro* culture of isolated ovarian follicles is one of the solutions for fertility restoration in cancer patients after. Many growth factors have been used for since chick embryo extract (CEE) contains many undefined factors that stimulate cell growth, it was hypothesized that addition of CEE to standard medium might enhance the growth rate and develop of the preantral follicle. In order to elucidate this, we investigate the effect of CEE supplementation in culture medium development of preantral follicles.
Materials and Methods: In this study, preantral follicles (90 -110 µm) were isolated from prepubertal mouse ovaries and cultured individually in droplets with 0 and 5% CEE. After 14 days' culture, ovulation was induced by using epidermal growth factor and human chorionic gonadotropin. The survival rate of follicles and nuclear maturation of ovulated oocytes were evaluated.
Results: After 2 days' culture, the number of surviving follicles were not significantly altered with and without supplementation of CEE ($P>0.05$). While the rate of antrum formation was increased by addition of CEE in culture medium (29% CEE vs. 25% control). Furthermore, the number of MII oocytes were higher with addition of CEE ($P<0.05$).
Conclusion: The main purpose of the present study was to determine the effects of various concentrations of CEE on the developmental competence mouse cultured isolated pre-antral follicles. supplementation of CEE enhanced antrum formation and more mature oocytes; hence, future studies are needed to explore undefined factors existing in CEE that stimulate cell growth .
Keywords: Chick,Embryo,Extract,Preanteral,Follicle

P-121: Short-Term Exposure to Different Concentrations of Hydrogen Peroxide Significantly Affects Ovine *In Vitro* Embryonic Development

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Background: Despite widespread application of ART, current rate of pregnancy remains unsatisfactory. Stressful condition of in vitro embryo culture could not completely mimic the in vivo situation that can be itself a probable reason for these undesirable results. Although,

a growing body of literature suggests that a short-term exposure of oocytes to a stressor such as hydrostatic pressure or osmotic stress might induce stress tolerance in embryos and improves embryo development. So, in this study we aimed to investigate effect of short-term exposure of cumulus–oocyte complexes (COCs) to hydrogen peroxide (H2O2) during in vitro maturation on subsequent embryo development.

Material and Methods: Aspirated COCs were matured for 24 h, at the last hour of maturation; oocytes were subjected to different concentrations 10 (10-H2O2), 100 (100-H2O2) and 1000 (1000-H2O2) μM of H2O2. Two cohorts of oocytes were also assigned as control groups; a group without H2O2 (Control) and the second one without H2O2 and sodium pyrovate (Negative control). After maturation, oocytes from each group were evaluated for nuclear maturation. A group of oocytes were subjected to parthenogenetic activation followed by in vitro embryo culture for assessing their developmental parameters.

Results: Short-term exposure to H2O2 had no effect (P>0.05) on nuclear maturation (65% (control), 64% (Negative control), 68% (10-H2O2), 64% (100-H2O2), and 69% (1000-H2O2). Although, the blastocyst formation rate increased (P<0.05) from 35.27% in control group and 42.37% in Negative control group to 50.26% in 10-H2O2 and then decreased (P<0.05) into 0.0% in 100-H2O2 and 1000-H2O2 groups.

Conclusion: Collectively, our findings indicated that short-term exposure of oocytes to different concentrations of H2O2 could deeply affect oocyte developmental competence positively or negatively depending on its concentration in culture medium.

Keyword: Oocyte, Hydrogen Peroxide, Development, Tension

P-122: Fluoxatine-Induced Apoptosis in Ovarian Tissue; An Experimental Study for p53, Bcl-2 and Caspase-3

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Background: Fluoxetine (FLX), as a main drug considered for inhibiting serotonin reuptake. FLX is commonly used for treating mood disorders, obesity, cataplexy and alcohol dependency. Serious sleeping problems, loss of appetite, dry mouth and rash are reported as side effects for FLX. Thus, present study was done in order analyze the dose and time dependent effects of FLX on follicular growth.

Materials and Methods: Thirty five, mature female wistar rats (weighting 200-210 gr), were assigned into control and test groups. Animals in test group subdivided into; 5 mg/kg, 10 mg/kg, 20mg/kg FLX-received groups. Control group received saline normal 0.1 ml/day. All chemicals were administrated orally by gavage for 21 and 42 days. The protein expression of p53, Bcl-2 and caspase-3 was evaluated immunohistochemically.

Results: Observations showed that FLX, in dose and time dependent manner, enhanced p53 and caspase-3 expression. Meanwhile, no significant differences were observed for Bcl-2 expression after 21 and 42 days. However, it was significantly changed depending on administrated dose. Accordingly, higher doses of FLX (10 mg/kg and 20 mg/kg) significantly (P<0.05) diminished Bcl-2 expression.

Conclusion: Our data showed that FLX, by up-regulating apoptosis promotes follicular atresia.

Keywords: Fluoxetine, Female, p53, Bcl-2, caspase-3

P-123: Stereological Assessment of The Effect of Supplementation Maternal Diet by Fish Oil on The Testicular Tissue of Offspring

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Background: Although major part of the assessment studies on fatty acid supplements were on maternal diet and positive effects on the brain and eyes, confirmation of these effects on children have not been tested for reproductive organs. Stereological study aimed to investigate the effect of gavaged fish oil (FO) for mothers on the testicles of male offsprings.

Materials and Methods: Eighteen mature female NMRI mice were divided in to 3 Groups (n=6): I) mothers fed Control diet (CTR; Standard diet prenatal and postnatal period); II) mothers gavages 0.01 ml/d/mice Fish Oil (FO) + CTR diet during prenatal period III) mothers gavages 0.01 ml/d/ FO + CTR diet during postnatal period till weaning of offspring. Male offspring were sacrificed and their right testis was fixed, processed, stained with haematoxylin and eosin. The diameter, High Epithelium and Thickness of seminiferous tubules were estimated using stereological methods. Data were analyzed using SPSS.

Results: The diameter of seminiferous tubules was the highest in FO postnatal period. The diameter of seminiferous tubules dramatically affects by treatments (194, 182 and 205mm3 for CTR, FO prenatal and FO postnatal, respectively (P < 0.05). The High Epithelium of seminiferous tubules in FO postnatal (85μm) was higher than other groups (64.5 and 71 μm) for FO Prenatal and CTR respectively (P < 0.05). The Thickness of seminiferous tubules was not affected by maternal nutrition.

Conclusion: This study showed that fish oil supplementation for mother during postnatal may improve the testicular tissue in adult mouse which warrants further studies alongside with antioxidant.

Keywords: Omega-3, Testes, Maternal Nutrition, Offspring

P-124: Protective Effect of Hydro-alcoholic Extract of Cornusmas F on Methotrexate-Induced Damages in Mice Testicular Tissue

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Background: Methotrexate (MTX is widely used as a chemotherapeutic agent in malignancies. However, it is reported that MTX affects the reproductive tissue by enhancing oxidative stress. Cornus

mas fruit extract (CMFE) as antioxidant chemical was administrated in order to evaluate its protective effect against MTX-induced metabolic alterations as well as oxidative stress.
Materials and Methods: Frothy eight mature male mice were divided in 6 groups as control (normal saline 0.1ml/day, i.p), MTX alone-treated (20 mg/kg/week), CMSE+MTX-treated groups (250, 500 and 1000 mg/kg/day, po) and CMFE alone-treated group. All animals received chemicals for 35 days. Intra-cytoplasmic carbohydrate and unsaturated fatty acid (UFA) were assessed by using special histochemical analyses. The serum level of testosterone were evaluated
Results: Observations demonstrated that MTX alone administration reduced cytoplasmic ratio of carbohydrate in first three layers of germinal epithelium and up-regulated the UFA levels. In contrast, CMFE reversed the condition and enhanced the carbohydrate ratio and inhibited UFA storage approximately close to control group. Moreover, the CMFE promoted the serum level of testosterone and up-regulated the MTX-reduced TAC level. Conclusion: Our data suggested that CMFE by controlling the energy substrate based on carbohydrates decreased cellular atrophy and diminished inflammation.
Conclusion: Therefore, by provoking the testicular antioxidant status enhanced cellular survival.
Keywords: Cornusmas, Methotrexate, Testicular, Mice

P-125: Wharton's Jelly Mesenchymal Stem Cells Derived from Human Umbilical Cord Could Be Isolated, Cultured and Differentiated Morphologically into The Oocyte Like Cells

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Background: The umbilical cord because of having younger adult stem cells may be an excellent source of mesenchymal cells against other tissues. The purpose of present study is to isolate human umbilical cord mesenchymal stem cells (HUCMSCs) and then investigate their differentiation capacity to oocyte like cells.
Materials and Methods: The umbilical cords were obtained from newborns delivered in Arash hospital (Tehran, Iran). Then Wharton's jelly was cut into 5 mm2 explants and cultured in DMEM-low glucose supplemented with 15% fetal bovine serum. After 2 weeks, tissue explants were removed from the plate and a few isolated cells adhered on plate and reached to 80 till 90% confluence by another 2 weeks later. Then in 3rd passage, the cultured cells were assessed to examine the expression of mesenchymal markers. In the same passage, HUCMSCs were induced to differentiate into oocyte-like cells by adding 10% human follicular fluid (group 1) and 10% cumulus cells conditioned media (group 2) individually as inducer in AMEM for 21 days.
Results: The isolated cells from Wharton's jelly showed fibroblast-like phenotype and colony formation up to 4th passage. Flow cytometry analysis indicated that CD34 and CD45 (hematopoietic cells markers) are not expressed and CD73, CD90 and CD105 (mesenchymal stem cells markers) are expressed on the surface of HUCMSCs. Four days after induction, a subpopulation of large round cells (like oocyte cells) with approximately 50 μm in diameter was observed in group 1 and one or two days later in group 2.
Conclusion: The present study demonstrate that HUCMSCs could be isolated from Wharton's jelly using explant method and could display morphologically oocyte-like cells under the influence of human follicular fluid and cumulus cells conditioned medium. Therefore, human umbilical cord mesenchymal stem cells may be considered as an alternative cell source for preserving the fertility.
Keywords: HUCMSCs, Follicular Fluid, Cumulus Cells, Conditioned Medium, Oocyte-Like Cells

P-126: The effect of 2 and 3 Dimensional *In Vitro* Culture of Mouse Ovarian Follicle on The Mitochondrial Distribution and ATP Content of Matured Oocytes

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Background: The mitochondria play important role in maturation of oocyte and embryo development. Therefore the objective of this study was to investigate the effect of 2 and 3 Dimensional *in vitro* culture of preantral follicle on the mitochondrial distribution and ATP content of matured oocyte.
Materials and Methods: Isolated Preantral ovarian follicle were cultured in two dimensional and three dimensional culture system for 12 day then the ovulation were induced. At the end of culture the ATP content of the matured oocytes was measured by luciferin-luciferase reaction. Distribution of oocyte mitochondria was studied using Mito Tracker Green staining under fluorescent microscope and compared with *in vivo* matured oocyte as control group.
Results: The ATP content of MII oocytes derived from *in vivo* and *in vitro* condition was significantly different in *in vitro* culture and *in vivo* matured oocytes samples but there was not significant different between two and three culture system groups. The pattern of mitochondrial distribution in MII oocytes derived from in two and three culture system was similar but it was different between MII oocytes collected from *in vivo* and in vitro matured MII oocytes. However, the florescent intensity of mitochondrial staining was different in all the groups in the study.
Conclusion: *In vitro* 2 and 3D culture of preantral ovarian follicle culture not affect the matured oocyte ATP content and distribution of mitochondria but some alteration was seen in mitochondria distribution of in vitro matured oocytes in comparison to their controls.
Keywords: ATP Content, Mitochondria, Oocytes

P-127: Correlation between Hormonal Changes and Sperm Chromatin Integrity in Infertile Men

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Background: Since hormones both initiate and maintain spermatogenesis, they may serve as surrogates of semen quality in epidemio-

logic studies. For this reason, in the present study, we evaluated the influence and predictive ability of reproductive hormones on sperm chromatin integrity, and condensation among men who were partners in an infertile couple.
Materials and Methods: In this research, 219 infertile men undergoing assisted reproductive treatment were evaluated with hormone levels including follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, between 2012 to 2014. Sperm chromatin structure and condensation were assessed with toluidine blue (TB) and aniline blue (AB) tests and the percentage of abnormal sperm chromatin structure and condensation was compared in men with different hormone levels. Statistical analysis was performed using multinomial logistic regression and $P<0.05$ was considered to be statistically significant.
Results: There are significant differences in abnormal sperm chromatin condensation, in men with low levels of FSH (95% CI: OR=2.1), LH (95% CI: OR=1.6), and testosterone (95% CI: OR=2.99). For sperms with damaged chromatin, there was a positive relationship between the high levels of FSH, LH and increasing percentage of damaged sperm chromatin.
Conclusion: The tests for sperm chromatin condensation showed a significant association with hormonal changes. It has also been shown that the abnormal sperm chromatin parameters could be result of hormonal alterations in IVF-ET cycles.
Keywords: ART, Infertile Men, Hormonal Changes, Chromatin Integrity

P-128: The Effects of Human Follicular Fluid Supplemented with Zinc and Copper on Vitrified/ Warmed Mouse Embryo Development

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Background: Follicular fluid (FF) provides a very important microenvironment for the development of oocytes that includes many substances which may increase embryo quality. According to the analysis of FF, the chemical constituents of FF have been grouped in the following categories: hormones; growth factors, interleukins; reactive oxygen species (ROS); anti-apoptotic factors; proteins and amino acids; sugars and trace elements (Zn, Cu and Fe). Human FF supplemented IVF medium has been presented to improve *in vitro* growth to the morula and blastocyst stages. Embryo cryopreservation is an essential part of assisted reproduction. With regard to importance of embryo vitrification and positive effects of FF, we decided to evaluate the effects of human FF supplemented with zinc and copper on embryo quality and blastocyst formation from vitrified/ warmed morula stage mouse embryos.
Materials and Methods: Morula embryos were obtained from 6-8 weeks NMRI female mice after hormonal stimulation by PMSG and HCG by mating with male mouse then embryos were transferred to culture medium supplemented with 4 mg/ml BSA. FF were collected from fertile women and centrifuged at 2500 g for 20 minutes. Cu and Zn level of FF were determined by atomic absorption spectrometry. The embryos were vitrified and warmed according to Kitazato protocol by using the closed pull straws and VIT and THAW Kit, then randomly were divided in four groups, including: control, FF alone, FF with Cu and FF with Zn. Embryos were cultured in culture medium containing 10% FF supplemented with 4µg/ml Cu or 1µg/ml Zn at 37ºC for 72 hours. The quality and hatching rate of vitrified/ warmed embryos were assessed.
Results: The embryo quality was assessed based on embryo grading. The percentage rate of grade A embryos was 47, 61, 65 and 68%, also, hatching rate was 44, 57, 64 and 70% in the control, FF alone, FF with Cu and FF with Zn groups, respectively. We observed significant hatching rates and percentage rates of grade A embryos in the FF alone, FF with Cu and FF with Zn groups compared with the control group.
Conclusion: Our study indicated that follicular fluid supplemented with zinc or copper compensates damages caused by freezing/ thawing process and improves embryo development.
Keywords: Embryo Vitrification, Follicular Fluid, Zinc, Copper

P-129: Survey of Expression of Marker Genes in Spermiogenesis (Include, Acrosin Genes) in Induced Human Spermatogonial Stem Cells for Differentiation into Sperm Cells

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Background: In recent years, human adult stem cells have been used to treat various diseases, one of the diseases that azoospermia description as lack of sperm in the semen after centrifugation of the sample analysis, causing infertility in the men. One of the best sources of human adult stem cells is testis that have stem cells including pluripotent stem cells (Spermatogonial stem cells)this cells can be used to treat azoospermia. The aim of this study was to evaluate the spermatogonial cell differentiation into male gametes.
Materials and Methods: Spermatogonial cells after separating from testicular biopsy were cultured in T25flasks. In the third passage cells in four different groups for 1to 4 weeks under the effect of a medium containing extracts of sheep testicular and then Acrosin expression was investigated using Western blot techniques.
Results: After induction of spermatogonial cells by the extracts of sheep testicular these cells change shape(sperm like)and also Acrosin protein expression was observed.
Conclusion: We now know that Acrosin is the major proteinase present in the acrosome of mature spermatozoa. Study induced cells showed that Acrosin is expressed, so we can conclude these cells completed spermatogenesis stage and started spermiogenesis stage.
Keywords: Azoospermia, Stem Cells, Spermatogonial Stem Cells, Acrosin, Western Blotting

P-130: Impact of Oocyte Morphology on Blastocyst Formation

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Background: Oocyte quality has been considered as a variable that influences the implantation potential of derived embryos. In this Study the objective is expression between oocyte dysmorphisms and blastocyst developmental competence.
Materials and Methods: In this review article,electronic searches were undertaken in PubMed, Scholar Google.
Results: The development of intracytoplasmic and extracytoplas-

mic anomalies during the maturation process of oocyte may lead to fertilization failure chromosome aneuploidy, and developmental impairment of the embryo despite normal fertilization. Large PVS has possible negative influence on the blastocysts degree of expansion and hatching status, ICM quality, and TE cell quality. Smooth endoplasmic reticulum aggregates (SERa) arise by dilatation and fusion of smooth endoplasmic reticulum saccules during the gamete's maturational process. It has also been suggested that ERC arise as a consequence of ovary hyperstimulation. These dysmorphic oocytes present a high aneuploidy rate (18–37%). One particular dysmorphism that definitely affects oocyte structure is a shape anomaly. Shape anomaly was correlated with decreased blastocyst development. After fertilization, ZP assists the oviductal transport of the embryo and plays a role in protecting the integrity of the developing embryo .A spherical shape of the ZP ensures maximal contact between the blastomeres;in this scenario, the embryo will cleave as expected, giving a crosswise arrangement of four cells with intense contact that will facilitate compaction owing to the larger number of tight junctions available. Thus, it is expected that cleaving embryos derived from ovoid oocytes face delayed compaction and blastocyst formation.
Conclusion: The cause for oocyte morphologic abnormalities is probably multifactorial. Ovarian stimulation and hormonal environment changes may result in the maturation of abnormal oocytes Morphologic.evaluation oocyte may be a prognostic tool for blastocyst development and quality. The options available to women with specific oocyte defects cleavage-stage ET would be a better approach. In these cases there is not benefit from postponing ET until day 5 of development.
Keywords: Oocyte, Morphology, blastocyst, Embryo

Ethics and Reproductive Healths

P-131: The Role of EMSs Parenting Origins and Coping Styles in Individuals Diagnosed with Infertility and Their Spouse: A Case Control Study

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Background: To compare Early Maladaptive Schema (EMSs) parenting origins and copying style in two groups of individuals with infertility problem and their spouse who were referred to Royan Institute.
Materials and Methods: This cross-sectional study included 244 individuals with at least one failure in assisted reproductive technology (ART). The infertile individuals were recruited as case and their spouse as control (124 case (female=64 male=60) and 120 control (female=60 male=60)). Two scales were administered. Measurement scales of material necessities, EMSs parenting origins, copying styles, and their subscales.
Results: A significant difference was observed in defectiveness/shame in father parenting origins in case group but there was no significant difference observed among their mothers' EMSs parenting origins. Also, a strong significant difference in distancing item of copying style was observed among case group.
Conclusion: Important information about a patient's psychological status and disease risk can be obtained from family history. As children are the product of parenting styles, EMSs parenting origins probably play an important role in providing the setting of infertility. Based on the current study, an infertile individual feeling of self-worth is highly dependent on his/her father's defectiveness/shame schema. The stigma of infertility which infertile individuals carry with themselves can cause them to feel less worthy, defective and shamed and to avoid criticism and distress perceived, they choose to distance from the social and psychological source of infertility.
Keywords: Infertility, EMS Parenting Origins, Coping Styles

P-132: The Influence of Islamic Life on Fertility Health

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Background: Life style means a special style of life of a person, group, or society, creating an individual and social identity for mankind. Islam as an enduring religion takes a comprehensive look at all human's existential aspects, presenting an especial and unique style of life. Since one of the important measuring factors of religion is its usage in human life's management in society, all Islamic teaching for human, in addition to the happiness in the Hereafter, ensure the salvation, health and successfulness of man in his individual and social life (see: Quran: 4/134). Because Islam introduces health as a basic necessity and the guarantee for the survival of generations, it makes many solutions at hand in order to maintain and meet this need. In religious teachings we confront dozens of recommendations concerning feeding, sexual relationship, pregnancy, and parenting, i.e. those details which consist of mental and spiritual health of family and society. In Islam sexual instinct is something innate and divine gift put in mankind by God Almighty (Quran: 7/189). Also Islam believes that marriage is the best way to control this instinct (Quran: 25/ 67-8), comparing to material schools of thought around the world and pondering these verses we conclude that the Islamic teachings are complete and comprehensive.
Materials and Methods: The present article, based on Quran studies and hadith teachings, aims to clarify the relations between religious teachings and health of fertility.
Results: In Islamic teachings sexual relations and love not only do not contradict with spirituality but belong to human manners and characteristics. Pregnancy and breast feeding is so important in Islam that the holy Prophet (pbuh) states:"when a woman is pregnant she looks like a fasting wakeful person who fights by her body and wealth for the sake of Allah" ('Amali of Saduq, p. 496). There are also many orders in the holy Quran on lawful and clean food, behaviors of breast feeding (al-Kafi, vol. 6, p. 40, h. 2), emphasis on mother's milk (ibid, h. 1), and the time of breast feeding (Quran: 2/223). It seems Quran and hadith teachings are as the best and effective example on sexual health and fertility and the insurance of survival of mothers and babies.
Conclusion: In Islamic teachings sexual relations and love not only do not contradict with spirituality but belong to human manners and characteristics. Pregnancy and breast feeding is so important in Islam that the holy Prophet (pbuh) states:"when a woman is pregnant she looks like a fasting wakeful person who fights by her body and wealth for the sake of Allah" ('Amali of Saduq, p. 496). There are also many orders in the holy Quran on lawful and clean food, behaviors of breast feeding (al-Kafi, vol. 6, p. 40, h. 2), emphasis on mother's milk (ibid, h. 1), and the time of breast feeding (Quran: 2/223). It seems Quran and hadith teachings are as the best and effective example on sexual health and fertility and the insurance of survival of mothers and babies.

Keywords: Life Style, Sexual Health, Fertility, Religious Life

P-133: Prevalence and Risk Factors Associated with Congenital Malformations in Ardabil, Iran

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Background: Congenital anomalies are amongst the most common causes of disability in developed and developing countries.The objective of present study was to determine prevalence and risk factors associated with congenital malformations in Ardabil, Iran.
Materials and Methods: A case–control study was conductedfrom Nov 2010 to July 2011 in three maternal hospitals located in Ardabil. All the live newborns were examined during the first 24 hours of life. Out of 6868 live births during the study period, 57 neonates with congenital malformations were selected as cases and 180 normal neonates as a control group. Data were collected using a self-designed questionnaire from review of prenatal and hospital delivery records. Data analysis was performed by chi-square, univariate, and multivariate logistic regression using SPSS version 1.
Results: The prevalence of congenital malformations was 0.82%. Musculoskeletal system malformation was the most common congenital abnormality (35.1%) followed bycentral nervous system (22.8%), digestive system (17.5%) urogenital system (15.8%), chromosomal anomalies (8.8%). Polyhydramnios (P=0.001, OR=14.4, CI: 3.07-68.0), oligohydramnios (P=0.009, OR=13.09, CI: 1.9-89.0), preeclampcia (P=0.000, OR=11.37, CI: 2.99-43.14), unwanted pregnancy (P=0.000, OR=4.9, CI:2.0-13.0)), urinary tract infection in weeks 10-6 of pregnancy (P=0.045, OR=2.88, CI: 1.0-18.11), consanguineous (P=0.038, OR=2.23, CI: 1.0-4.78) were determined as risk factors for congenital malformations.
Conclusion: Improving the quality of family planning services and pre-pregnancy care, women education on importance ofsupplements useinpregnancy, ultrasonographicevaluation of high risk womenforearly detection ofcongenital malformations,advisingblood relative coupleson importance of genetic consultation andearlydiagnosis ofcongenital abnormalitiescanbean appropriate policytoward theprevention ofcongenital anomalies.
Keywords: Congenital Malformations, Risk Factors, Ardebil

P-134: Frequency of Postpartum Depression among Mothers Conceived by Assisted Reproductive Techniques (ART) in Compare to Naturally Conceived Mothers Using Edinburgh Postnatal Depression Scale (EPDS)

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Background: The aim of the present study was to determine the frequency of postpartum depression among mothers conceived by assisted reproductive techniques (ART) in compare to naturally conceived mothers using Edinburgh Postnatal Depression Scale (EPDS).
Materials and Methods: In this historical cohort study, 406 mothers with infants from 3 to 9 months old were investigated. Three hundred eight women with normal pregnancies as control group were selected among mothers referred to Tehran healthcare centers for child vaccination. Ninety eight women who get pregnant using ART at Royan institute were enrolled as ART group. General questionnaire including age, education, occupation, age of infants, the history of twin pregnancy, the number of children, delivery method, history of infant hospital admission, lactation status, and history of depression and cause of infertility for ART group was completed for each mother. The valid questionnaire of EPDS was used to measure depressive symptoms. Data analysis was performed using SPSS software version 20 and Levene, One-way ANOVA, t test and linear regression, Tukey and Dennett's statistical tests. P value less than 0.05 was considered as significant level.
Results: The mean age of studied mothers was 28.87 years old with standard deviation (SD) of 5.18. The mean age of infants was 5.18 months with SD of 1.3. The frequency of postpartum depression in the total mothers, control and ART groups was 24.6, 26, and 20.4%, respectively. The difference in rate of depression was not statistically significant between control and ART groups (P =0.26). Linear regression analysis showed that education level, history of infant hospitalization, number of children, and history of depression are influencing factors on mother's depression.
Conclusion: It seems that post partum depression in mothers conceived by ART is similar to those naturally conceived.
Keywords: Postpartum Depression, Assisted Reproductive Technology, Natural Pregnancy, Edinburgh Postnatal Depression Scale (EPDS)

P-135: Difficulties in Emotion Regulation and Fertility Related Quality of Life in Women Undergoing Assisted Reproductive Techniques: Possible Role of Anxiety and Depression

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Background: The purpose of this paper is to find out any relation between difficulties in emotion regulation (DERS) and fertility related quality of life (FertiQol) in infertile women undergoing assisted reproductive techniques (ART). Also, as there may be some effects of depression and anxiety on quality of life, we evaluated the possible mediator role of these mood disorders between DERS and FertiQol.
Materials and Methods: This study was a cross sectional study done in Royan Institute which is a referral infertility clinic in Tehran. The study population was infertile women who referred to the clinic for ART. The sampling was convenience and the proposed sample size was 225. We used 4 questionnaires: a. DERS, b. Ferti Qol, c. hospital anxiety and depression scale (HADS) and a demographic questionnaire. This study was approved by Royan Institute Ethical Committee and data were analyzed using descriptive methods plus path analysis and logistic regression.
Results: We could not find any direct relationbetween DERS and FertiQol but a mediator role for anxiety and depression was shown. There were relation between anxiety and depression with FertiQol

and DERS (P<0.01). Among 6 sub scales of DERS, only "Limited access to emotion regulation strategies" showed relation with Fertiqol (P<0.01).
Conclusion: The effect of DERS on quality of life of the infertile women is mediated by anxiety and depression.
Keywords: Anxiety, Depression , Difficulties in Emotion Regulation , Infertile Women

P-136: Fetus Genetic Manipulation, Ethical Challenges and Considerations

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Background: Genetic manipulation of the embryo is a field of human knowledge, where that genetic engineering can provide an acceptable treatment for some disorders. This science which is in growing had important successes in the treatment of some disorders. However, the science causes numerous challenges in the fields of medicine, biology, ethics, law, philosophy, religion and economy. The recognition and attention to these challenges will prevent of errors, abuses and future problems.
Materials and Methods: We conducted a comprehensive search in the literature of medical ethics and genetics that were published in 1990 to 2015; and in the SID, PubMed, Scopus and Google Scholar databases by using the key words such as ethical challenges and legal problems.
Results: It showed that there are some problems such as the occurrence of viral infections, 'eugenics' and 'children Order' phenomena and high financial costs. These problems can causes ethical challenges such as monopoly, discrimination against persons with disabilities, aggression to fetus freedom and the conflict between private and public interests.
Conclusion: Fetus genetic manipulation can causes some ethical challenges. Thus, it is required to be controlling in the genetic manipulation, especially by considering ethics and law.
Keywords: Genetic Manipulation, Fetus, Law, Bioethics, Challenges

P-137: Pregnancy Related Complications in Patients with Systemic Lupus Erythematosus

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Background: One of the most important of autoimmune diseases that affects women in their childbearing age is Lupus. There are some important matters to create a dilemma for both the physician and the patient for making a decision about pregnancy. Hormones level alteration during the pregnancy and its relation to the disease enhances the abortion rate and foetus complications. The aim of this study is to determine the distribution Poster of pregnancy complications in women with Systemic Lupus Erythematous (SLE).
Materials and Methods: This cross-sectional study was designed on 60 SLE patients with the ages of 18-35 years old. The cases were selected from Rasoul-e-Akram hospital using purposive sampling. The inclusion criteria were being affected by SLE patients based on clinical and laboratory signs without other diseases.
Results: According to the findings, the complications were included: 45% miscarriage, 41.7% Antiphospholipid Ab, 20% gestational HTN, 3.3% eclampsia, 13.3% pre-eclampsia, 41.7% proteinuria, 23.3% preterm labour and 5% required haemodialysis. There was a significant association between the number of miscarriages and antiphospholipid Ab (p= 0.003). Moreover, the number of preterm labours were significantly related to proteinuria (p=0.021).
Conclusion: This study showed that the prevalence of miscarriage had a meaningful association with antiphospholipid Ab. However, proteinuria, the use of prednisolone, hydroxychloroquine and azathioprine had no significant relation to miscarriage. The prevalence of preterm labour had a significant association with proteinuria.
Keywords: Lupus, Pregnancy, Pregnancy complication

P-138: Early Maladaptive Schemas and Coping Styles in Individuals Diagnosed with Infertility and Their Spouse: A Case Control Study

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Background: To compare early maladaptive schemas and coping style in two groups of individuals with infertility problem and their spouse who were referred to Royan Institute.
Materials and Methods: A case-control study was employed. A total of 262 individuals with at least one failure in ART participated; infertile individuals as case and their spouse as control. (140 case, female=71 male=69) and 122 control (female=60 male=62)). Young's Early Maladaptive Schema Questionnaire (YSQ-SF) and Ways of Copying (WOCQ) were used and to analyze data independent t-test was employed.
Results: The results showed no significant difference in early maladaptive schemas between case and control. But there was a significant difference observed in subjugation schema in women diagnosed with infertility. Plus there was a significant difference (P= 0.026) in ways of coping in distancing strategy particularly among men (0.010).
Conclusion: Based on the current study, cognitive structures such as early maladaptive schemas play no particular role in infertility except for women's. But Infertility can cause some psychological disturbances. The social stigma caused by infertility, can cause individuals to avoid criticism and perceived distress. Men specially choose to distance from the social and psychological source of infertility. Therefore it matters that psychological help accompanies medical care in this process.
Keywords: Infertility, Early Maladaptive Schema, Coping Styles

P-139: The Relation of Social Support and Quality of Life in Women Undergoing Assisted Reproductive Technology

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Background: The aim of our study was to evaluate the relation between social support in women undergoing assisted reproductive technology (ART) and their fertility related quality of life (FertiQol)
Material and Methods: This was a cross sectional study performed in Royan Institute, a referral infertility clinic in Tehran, capital of Iran. 350 infertile women were recruited by convenient sampling. Cases were referred to the institute for the first time and had no history of ART failure. Social support was evaluated using the 12 item Multidimensional Scale of Perceived Social Support (MSPSS) questionnaire. The quality of life was checked by Fertility Quality of Life (FertiQol) with 34 items. This study was approved by Royan Institute Ethical Committee and data were analyzed using logistic regression.
Results: Our data showed that social support by others is the most important predictor for quality (P<0.001) of life followed by family support (P=0.026) then support by friends (P=0.033).
Conclusion: There were positive relations between all dimensions of social support with quality of life.
Keyword: Quality of Life, Infertile Women, Assisted Reproduction, Social Support

P-140: The Effect of Depression and Anxiety on Cognitive Performance in Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS), the most common and important endocrine abnormalities in women of childbearing age, has a prevalence of 2.2 to 26 percent. The prevalence of depression and anxiety has been reported differently in these patients. The objective of the present study was to investigate levels of anxiety and depression in this group of women and its association with cognitive function compared to healthy women.
Materials and Methods: In these cross-sectional study, 45 patients with polycystic ovary syndrome, diagnosed with Rotterdam criteria, and 45 healthy women as control group were selected. Anxious and depressed mood subjects were evaluated by Beck 2 Depression and Anxiety questionnaire. Also, cognitive function were assessed using the Montreal Cognitive assessment (MoCA).
Results: The average Beck 2 score in patients was 20.22 ± 9.73 and in control group was14.62±8.16, p=0.004, which was statistically significant. Also, between the average Beck Anxiety scores in patients (17.17 ± 10.76) and control group (12.97 ± 9.90) was found statistically significant difference (P=0.05). Frequency of severe depressed and anxious mood, based on Beck criteria, in the patient group were 28.8% and 26.6%, and in the control group were 4% and 13%, respectively, which was found statistically significant difference, (P=0.007, 0.02). Also, it wasn't found significant relationship between cognitive function and levels of depression (r=-0.03, P=0.81) and anxiety (r=-0.14, P=0.35).
Conclusion: The prevalence of depression in patients with PCOS is more than normal women, but despite of many universal reports, any relationship was not observed between depression and anxiety levels with cognitive function Cognition in our PCOS study group.
Keywords: Polycystic Ovary Syndrome, Cognitive Function, Depression and Anxiety

P-141: Intimate Partner Violence and Unintended Pregnancy

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Background: Domestic violence and unintended pregnancy is a serious personal, social and global public health concern. In this study it is our aim to explore the relationship between Domestic violence against wives and unwanted pregnancy
Materials and Methods: This cross-sectional study was done on pregnant women 16–40 years of age living in Jahrom south of Iran between August 2014 and December 2015. This research was implemented through questionnaires including the demographic characteristic. The form of partner violence including emotional abuse, physical violence and sexual violence was assessed with a validated questionnaire. Logistic regression was used to estimate multivariable adjusted odds ratios and 95% confidence intervals.
Results: The pregnancy was reported to be unintended by 226 (42.8%) of the study population. Compared with non-abused women, abused women had a 4.03-fold increased risk for unintended pregnancy. The regression analyses yielded significantly (P<0.05) increased risk of unwanted pregnancy for physical violence (OR=0.9, 95% CI=0.53-1.74) and for sexual violence (OR=1.88, 95% CI=1.314-2.715), emotional violence (OR=1.491, 95% CI=1.055-2.109) than women who were never abused. Age, Educational level, Number of living children to women are also important risk factor of unwanted pregnancy.
Conclusion: The results of this study showed that the frequency of unwanted pregnancy and violence against women is high and violence has an important effect on the unwanted pregnancy. So, healthcare systems should screen violence abused women before and during pregnancy to recognize exposed women and prevent unwanted pregnancy
Keywords: Intimate Partner Violence, Pregnancy, Unwanted Pregnancy

P-142: The Study of Self-Concept And Self-Discrepancy among Women Receiving Oocyte who Referred to Royan Center And Fertile Women Who Referred to Health Center in Tehran 2014

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Background: A technology for fertility assistance is the use of donated oocyte. Although the data about medical aspects of this process is available, the knowledge of psychological affairs related to this therapy is limited. Self-concept and self discrepancy are able to

investigate the psychological aspects of every person and therefore, we decided to study these measures on women who receive oocyte and fertile women.
Materials and Methods: In this cross-sectional investigation, carried out in 2014, 53 women were divided randomly into two groups including donors and receivers. The data were collected by a three part questionnaire including demographic questions, self-concept scale and self discrepancy scale questions (consisting of "ideals" and "have to be" self discrepancy data). The data were analyzed through SPSS19 and by use of statistical tests including Kruscal-wallis and ANOVA.
Results: The results showed that self-concept and self-discrepancy of fertile woman was better than infertile women who receive and donate oocyte. And this difference was statistically significant(P=0.045)
Conclusion: According to the results of this study can be said infertile women that for the treatment needs to get an oocyte from another woman are vulnerable to mental health. Therefore, these findings could help the health authorities and health service providers, to adopt the necessary measures for psychological support required by this group of women and the implementation of appropriate programs to prevent the complications of treatment with donors.
Keywords: Self-Concept; Self Discrepancy, Oocyte Recipient Women, Fertile Women

P-143: The Effect of Acceptance and Commitment Therapy with Hypnosis (ACHT) in The Permanent Cognitive Changes for Reducing Cognitive-Behavioral Physical Anxiety in Infertile Women

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Background: Anxiety Disorders are called as the most common contemporary disease that is associated with the dysfunction, Low quality of life and increasing the costs of hygiene and health and worry has been defined as a chain of thoughts, images, negative emotions and relatively uncontrollabl (macgoven, 2014). On the other hand studies suggest that hypnosis enables participants to respond successfully to certain task suggestions such as those aimed at altering cognition or perception (macgoven, 2014).
Materials and Methods: Among the 200 referred to a psychiatric and Gynecological clinic in 2014 in Babol with the cognitive problem and anxiety disorder toward pregnancy and delivery, 45 patients that were compatible with research conditions (Age 23-35, at least Associate Degree,without experience pregnancy, without special physical and mental disorders), 14 patients were divided in two equal groups (n = 7) and pre-test and post-test were performed by the,Cognitive-behavioral physical Anxiety Inventory(for review the level of anxiety and state of Thinking), Locke-Wallace of Marital Adjustment Scale (For better recognition and status of client), individual interviews (at the beginning and end of the study), 10 sessions of group therapy with the content of cognitive-learning and Self-hypnosis (with bringing up the stories for each individual based on the schema) and the Hypnosis in a group ,while the control group only received individual counseling and education.
Results: According to the results of this study by using self-hypnosis with plot of the story based on the childhood and belief of person along with Acceptance and Commitment Therapy in group therapy with content of cognitive and hypnosis resulted in reduction (41%) of anxiety and improvement of schema (34.2%) compared to the control group. In addition Individual clinical interview After three and six months of treatment was performed, The experimental group had more cognition to solve conflicts in life.
Conclusion: According results of this research if a better understanding of the content of the story of hypnosis of any person with Acceptance and Commitment Therapy) is present, led to a more conscious and more durable acceptance in treating many disorders including anxiety concerning pregnancy . Although this research needs to be expanded but can be help with assimilating the specific hypnosis text to each individual based on his beliefs with Acceptance and Commitment Therapy and ultimately by more recognition and readiness of people to deal with personal and social anxiety in conditions of resolving the crisis in form of adaptive and thus help to mental health.
Keywords: ACHT, Anxiety,Infertile Women

P-144: The Role of EMSs Parenting Origins and Coping Styles in Individuals Diagnosed with Infertility and Their Spouse: A Case Control Study

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Background: To compare Early Maladaptive Schema (EMSs) parenting origins and copying style in two groups of individuals with infertility problem and their spouse who were referred to Royan Institute.
Materials and Methods: This cross-sectional study included 244 individuals with at least one failure in assisted reproductive technology (ART). The infertile individuals were recruited as case and their spouse as control (124 case (female=64, male=60) and 120 control (female=60, male=60)). Two scales were administered. Measurement scales of material necessities, EMSs parenting origins, copying styles, and their subscales.
Results: A significant difference was observed in defectiveness/shame in father parenting origins in case group but there was no significant difference observed among their mothers' EMSs parenting origins. Also, a strong significant difference in distancing item of copying style was observed among case group.
Conclusion: Important information about a patient's psychological status and disease risk can be obtained from family history. As Children are the product of parenting styles, EMSs parenting origins probably play an important role in providing the setting of infertility. Based on the current study, an infertile individual feeling of self-worth is highly dependent on his/her father's defectiveness/shame schema. The stigma of infertility which infertile individuals carry with themselves can cause them to feel less worthy, defective and shamed and to avoid criticism and distress perceived, they choose to distance from the social and psychological source of infertility.
Keywords: Infertility, EMS Parenting Origins, Coping Styles

P-145: Coping Strategies and Assisted Reproductive Techniques Outcomes

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Background: The purpose of this study is to evaluate the relation between coping strategies used by infertile women undergoing assisted reproductive techniques (ART) with the outcome of this treatment.
Materials and Methods: This cross sectional study has been done in Royan Institute, a referral infertility clinic in Tehran. The study population was 101 infertile women who referred for the first time to the clinic for ART. The sampling was convenience. Lazarus and Fulkman questionnaire was used to evaluate the coping strategies among infertile women. Clinical pregnancy defines as visualization of gestational sac by ultrasound. This study was approved by Royan Institute Ethical Committee and data were analyzed using independent t-test logistic regression.
Results: There were significant negative relation between "emotion-focused" sub scale of coping strategies and the outcome of treatment. Although "problem focused" had positive relation with outcome, but it was not statistically significant.
Conclusion: "Emotion focused" sub scale of coping strategy is negatively related to the outcome of ART in infertile women.
Keywords: Coping Strategies, Infertile Women, Assisted Reproduction, Outcome

P-146: The Study of Self-Concept and Self-Discrepancy among Women Receiving Oocyte who Referred to Royan Center and Fertile Women who Referred to Health Center In Tehran 2014

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Background: A technology for fertility assistance is the use of donated oocyte. Although the data about medical aspects of this process is available, the knowledge of psychological affairs related to this therapy is limited. Self-concept and self discrepancy are able to investigate the psychological aspects of every person and therefore, we decided to study these measures on women who receive oocyte and fertile women.
Materials and Methods: In this cross-sectional investigation, carried out in 2014, 53 women were divided randomly into two groups including donors and receivers. The data were collected by a three part questionnaire including demographic questions, self-concept scale and self discrepancy scale questions (consisting of "ideals" and "have to be" self discrepancy data). The data were analyzed through SPSS19 and by use of statistical tests including Kruscal-wallis and ANOVA.
Results: The results showed that self-concept and self-discrepancy of fertile woman was better than infertile women who receive and donate oocyte. And this difference was statistically significant (P=0.045)
Conclusion: According to the results of this study can be said infertile women that for the treatment needs to get an oocyte from another woman are vulnerable to mental health. Therefore, these findings could help the health authorities and health service providers, to adopt the necessary measures for psychological support required by this group of women and the implementation of appropriate programs to prevent the complications of treatment with donors.

P-147: Incidence of Preterm Labor and its Relevant Risk Factors in Mothers Referring to Health Centers in Ahvaz

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Background: A preterm labor is when a baby is born before 37 completed weeks of pregnancy which is one of the most common causes of infant mortality. The aim of this study is to determine the incidence of some risk factors cause preterm labor.
Materials and Methods: In the first phase, in a cross-sectional study, 843 files about mothers who had delivery in the health houses in the Sugarcane refining area in Ahvaz during from 2011 to 2013 were studied. In the second phase, a case-control study was designed in which 80 mothers who had preterm labor were entered in the study as the cases and 240 mothers who had term labor as controls. Possible risk factors including maternal age, history of preterm labor in previous pregnancies, high blood pressure, urinary tract infection and lack of proper weight gain during pregnancy were evaluated in two groups using a researcher-made questionnaire. In descriptive statistics, frequency and percentage frequency and in analytic statistics, Chi-square test and logistic regression were analyzed.
Results: In this study, the incidence of preterm labor was 10.53%. In terms of risk factors of preterm labor including mother's improper weight gain (P<0.001), anemia (P<0.001), high blood pressure (P<0.001), urinary tract infection (P=0.002), and previous preterm labor (P=0.003), no statistical significant difference was observed between two groups (P>0.05).
Conclusion: Based on the results of this study, some factors such as improving the quality of prenatal care and follow-up and proper treatment of mothers, and especial cares including controlling the blood pressure, urinary tract infections and maternal weight loss can reduce the incidence of preterm labor.
Keywords: Preterm Labor, Risk Factors, Incidence, Ahvaz

P-148: Intimate Male Partner Violence and Women's Sexual dysfunction

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Background: Sexuality is an integral part of a woman's life. A variety of factors, such as physical disorders, social-religious beliefs, age, psychological factors, depression, mental tension, disbelief, an unfulfilling relationship with one's spouse and emotional and physical violence, can affect a woman's sexuality. Aims: The present study aims to explore the rate of domestic violence against women and its impact on women's sexuality.
Materials and Methods: This cross-sectional study was done on 813 women referring to genecology clinics of Jahrom, Iran from April to October 2015. Sampling conducted in Convenience method. The study data were collected by demographic, violence questionnaire and Female Sexual Function Index. The data were analyzed using Student's t test and logistic regression.
Results: The prevalence of violence was 43.2%. Also there are significant relationships between violence and age)OR=1.33 95%. CI=2.22-7.95). The rate of violence become 1.1 time by increasing the length of marriage (OR=3.15 95%.CI=1.42-4.12). Moreover, domestic violence was significantly associated with womens education

level (OR=11.75 95%CI=2.15-64.12 P=0.002), husband's education level(P=0.02). The results showed that the mean score of NON-Abused women and Abused women's sexual function was 17.74 ± 8.82and 14.59 ± 10.63, respectively. However, a significant difference was found between the two groups regarding the domains of sexual function (P>0.05).
Conclusion: This study indicated that the frequency of domestic violence is rather high that increased the risk of sexual dysfunction. Thus, routine screening for violence and sexual dysfunction is warranted to aid early detection of violence and sexual dysfunction.
Keywords: Violence, sexual function, Female Sexual Function Index, Women

P-149: Assessing of Sexual Function in Women who Underwent Colpoperineorrhaphy Referred to Peymanieh Hospital of Jahrom

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Introduction: One of the effective factors on the female sexual activity is surgeries. Even if female surgeries are small and non significant but they can be effective on patient's mood and her thought of herself. With Regards to FSD and spread of elective colpoperineorrhaphy, The purpose of the study was to explore the prevalence of sexual problems in post- colpoperineorrhaphy.
Materials and Methods: This comparative descriptive-analytical study was conducted on the women who underwent colpoperineorrhaphy, and who did not undergo the surgery referred to Peymanieh hospital from March 2013 to February 2015. The participants were 145 of whom 46 patients underwent colpoperineorrhaphy, and 99 did not undergo surgery. Information gathering tool and method was a two parts questionnaire: 1) demographic variables 2) FSFI questionnaire.
Results: The average total FSFI Score in surgical group was 20.08 ± 7.33, and control group was 23.12 ± 5.05 (according to our study, 87/3% of women who underwent the surgery had a sexual dysfunction. The most of the dysfunction were related to pain during intercourse. The results of this study show that 52.2% of women reported that they have very often pain during intercourse. Comparing the groups, desire, Arousal, ,orgasm, , satisfaction was not significant change. but lubrication ,pain was significant.
Conclusion: Six months after colpoperineorrhaphy,it was found that the studied patients' sexual problems were increased as compared to the control group. This is indicates that the elective colpoperineorrhaphy doesn't have any positive effects on the female sexual functions. Therefore, it is recommended that the people and authorities in charge of health problems should pay more attention to female sexual problems, and a ward can be created at the health centers for sexual counseling before colpoperineorrhaphy.
Keywords: Sexual Function, Colpoperineorrhaphy, surgery

P-150: The Evaluation of Complications of Pregnancy above 35 Years in Pregnant Women Referred to Jahrom's Pymanieh Hospital

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Introduction: Maternal age is one of the factors that affect on pregnancy outcomes. The objective of this study, evaluation of Complications of pregnancy above 35 years in pregnant women referred to Maternity ward of Jahrom's Pymanieh hospital.
Materials and Methods: This research was performed as descriptive analytic method. Patient's information was obtained from their files. Variables assessment include: age, parity, gestational age, abortion, disease during pregnancy, birth weight, neonatal problems and congenital anomaly. Data were analyzed by SPSS software.
Results: File of 2049 pregnant women was studied that 9.56% were above 35 years. Pregnancy complications include: 9.2% preterm labor, 15.3% post term delivery 3.1% bleeding in pregnancy, 3.1% bleeding in pregnancy, , 29.6% abortion. The frequency of different types of diseases includes: Anemia 14.8%, Hypertension 7. 7%, diabetes mellitus 6.7%, Gastrointestinal problems 3.1%, Pyelonephritis 1%, Previous history of disease 8.2%. Delivery method was 53.1% normal vaginal delivery, 46.9% cesarean section.
The causes of cesarean section include: Repeat cesarean section 43 (21.9%), Causes of Obstetrics 18(9.2%), elective caesarean section 15(7.7%), High blood pressure at 7(3.6%), meconium 6 (3.1%), breech 3(1.5%).53 patients (27%) had a history of previous cesarean and 39 patients (19.9%) were first cesarean.
Conclusions: Pregnancy at inappropriate age and Suffering from some diseases, especially hypertension, diabetes, anemia, increased rates of cesarean section were the most important health problems of pregnant women. Health status during pregnancy depends on before pregnancy of health care so, prior pregnancy Care must be basic prenatal care.
Keywords: High Risk Pregnancy, Fetal Complications, Maternal Health

P-151: The Fertility Quality of Life (FertiQoL) Instrument: A Validation Study in Iranian Infertile Women

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Background: Infertility and its treatment can have a considerable effect on a person's quality of life. The Fertility Quality of Life (FertiQoL) instrument is currently the most frequently used instrument to measure QoL in people experiencing fertility problem. The objective of this study was to examine the psychometric properties of the FertiQoL in Iranian infertile women.
Materials and Methods: This cross-sectional study included 155 women with fertility problems undergoing IVF in Royan Institute, Tehran, Iran from January to March 2014. A battery of instrument was used, including the FertiQoL, the Satisfaction with Life Scale (SWLS), the Hospital Anxiety and Depression Scale (HADS), and a demographic questionnaire. The psychometric properties of the FertiQoL were examined: construct validity using confirmatory factor analysis (CFA), reliability using Cronbach's alpha and convergent validity by examining the relationship with SWLS and HADS.
Results: The results of the CFA generally supported the four-factor model of Core FertiQoL and two-factor model of Treatment FertiQoL. Both module of FertiQoL and their subscales revealed acceptable internal consistency ranging from 0.643 to 0.911. However, the FertiQoL may be improved if Q15 and T2 items are removed from the scale. These items had low loadings on Relational and Environment

factor and decreased their internal consistency. The FertiQoL and their subscales significantly correlated with both SWLS and HADS, indicating and acceptable convergent validity.
Conclusion: The Persian version of FertiQoL demonstrated adequate psychometric properties for assessing QoL in infertile women.
Keywords: Infertility, Quality of Life, Validity, Reliability

Female Infertility

P-152: Expression Analyses of Leukemia Inhibitory Factor Receptor in Women with Unexplained Infertility

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Background: Infertility is a complex medical problem for several reasons. It is very frequent, since about 15% of couples worldwide fail to conceive, the women factor being involved in roughly 50% of cases. Infertility is commonly defined as the failure of conception after at least 12 months of unprotected intercourse. Unexplained infertility is a common cause of infertility in which defect in the implantation can cause this type of failure infertility. Several genes, including LIF and LIFR involve in infertility which defect in each of them can lead to a defect in the implantation. LIF receptor is a heterodimer that is contained by two transmembrane proteins included LIFR and gp130. The aim of this study is investigation of correlation between LIFR gene expression and female infertility.
Materials and Methods: 46 uterine endometrium Tissue samples belonged to women with unexplained infertility were taken by agynecologist and also 30 healthy women as controls were used. After RNA extraction and cDNA synthesis LIFR gene expression was evaluated using Real-time RT-PCR.
Results: The results of this study show significant differences in levels of LIFR gene expression between the control and patient groups. Regardless of whether this difference in expression can be general or special for this receptor, a high variance was found among patient women against time of the terms which had undergone the biopsy.
Conclusion: Natures of Receptor LIFR can dependence of dosage expression. The results of this study show that this gene plays an important role in implantation and there is a significant relationship between LIFR and molecules involved in the implantation.
Keywords: Unexplainedinfertility, Expression Analysis, LIFR

P-153: Role of Nutrition as The Backbone of Normal Fertility in The Persian Traditional Medicine

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Background: Being fertile needs normal function of multiple organs in human body. One of important issues in having n normal fertility is nutrition. Obesity and extreme weight loss can impair fertility. Healthy nutrition plays a significant role in maintaining normal uterus function before and after conception. Healthy nutrition also contributes to normal pregnancy and breast feeding.
Materials and Methods: This notion of significant role of nutrition in fertility has been recognized for quite a long time in the Persian traditional medicine and it has been widely discussed a long period medical textbooks such as Canon, Zakhireh, Eksir, Teb akbari, Alhavi, and Kholaseh alhekmat. Those reference books have also pointed out the negative effect of obesity and extreme weight loss on fertility.
Results: There is evidence that obesity can prone humans to develop deposition of adipose tissue around semen ducts and ovulation paths. It can damage the ducts' microenvironment, too. Obesity can increase tissue moisture; so semen and ovule production can be damaged. Also obesity can disrupt the normal process of intercourse.
Conclusion: Extreme weight loss is associated with weakness, and it can be due to indigestion or malnutrition. Extreme weight loss can result in general weakness and hepatic dysfunction; it has also has detrimental effects on ovum and sperm production cycles and also implantation. Here we show evidence that scholars of the Persian traditional medicine has had a special attention to the role of nutrition in normal fertility.
Keywords: Traditional Persian Medicine, Nutrition, Obesity, Extreme Weight Loss, Fertility

P-154: Effect of Mentha Spicata Essential Oil on LH, FSH and Testosterone Level of Polycystic Ovarian Syndrome Rat Model

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Background: Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting one in 15 women worldwide. High level of androgens are considered the main culprit of PCOS. According to antiandrogenic effects of mentha spicata in this study we evaluated the effects of this herbal plant on sexual hormones of PCOS rats.
Materials and Methods: Female wistar rats were allocated into one control and five experimental groups. PCO induction was carried out by treatment of rats daily with letrozole (1 mg/kg) orally for 28 days. Groups treated according to the following: Control (distilled water), Group I: Letrozol, Group II: Letrozol+spearmint oil (150 mg/kg), Group III: Letrozol+spearmint oil (300 mg/kg). In all experimental groups treatment with essential oil was carried out for 20 days after PCOS induction. At the end of the treatment, animals were killed and blood samples were collected for evaluation of FSH, LH and testosterone. Statistical analysis was performed using SPSS 16 software. One-way ANOVA with post-hoc Tukey was done for comparison of values. $P<0.05$ were considered statistically significant.
Results: The level of testosterone in PCOS-induced rats groups which received mentha spicata was significantly lower than PCOS-induced groups without any treatment. There was no significant change in the level of LH and FSH between all groups.
Conclusion: Mentha spicata essential oil can be used as an alternative medicine for reducing the level of testosterone in PCOS.
Keywords: PCOS, Testosterone, Rat

P-155: The Role of Transcript Expression Level of Mitochondrial Nuclear -Encoded NRF1 Gene in Single Human Oocyte Maturation in Patient Women With Polycystic Ovary Syn-

drome (PCOS)

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine system disorder among women of reproductive age. Oogenesis in the PCOS ovary is often disrupted, leading to suboptimal oocyte competence for fertilization.The impaired mitochondrial function in human oocytes are still unknown.It has been shown that a mitochondrial deficit could couse a failure of oocyte maturation. mtDNA is specific with a maternal transmission. Nuclear respiratory factor-1 (NRF1) is a transcription factor that acts on nuclear genes encoding respiratory subunits and components of the mitochondrial transcription and replication machinery. NRF-1 also causes mitochondrial function and their biogenesis. The Aim of This Study was to Determine the role of transcript expresstion level of mitochondrial nuclear -encoded NRF1 gene in single human oocyte maturation in patient women with PCOS.
Materials and Methods: Oocytes at the various stages of oocyte maturation were donated after consenting infertile healthy women due to male factor and patient women with PCOS ; 20–35 year-old, undergoing ICSI protocol.Mitochondrial transcript expresstion of NRF1 gene analyzed using single –cell TaqMan real time PCR-based assay
Results: It was the first report transcript expression level in single human oocyte using taqman qPCR.The transcript expression level of the NRF1 gene was low at the germinal vesicle (GV) stage in both groups ($P>0.05$).Significant difference was observed in metaphase I (MI) and metaphase II (MII) stages of single human oocyte maturation between healthy women and patient women with PCOS ($P<0.05$). Transcript expression levels of NRF1gene decreased during oocyte maturatin in patient women with PCOS ($P<0.05$).
Conclusion: These findings indicate that reduction in the transcript expression level of mitochondrial related (NRF1) gene lead to impair in human oocyte maturation as well as poor oocyte quality in patient women with PCOS, thus, our results may be new approach into the pathogenesis of infertility in PCOS.
Keywords: Oocyte Maturation, PCOS, Single Cell, Taqman Real-time-PCR

P-156: The Role of miRNAs from Follicular Fluid in Human Oocyte Quality

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Background: Follicular fluid(FF) includes various hormones, proteins, metabolites, and regulatory molecules which play important roles in the development and maturation of oocytes.Although assisted reproduction techniques (ART) is increasing every year, , the average rate of pregnancy obtained with ART is still low at 25%. The low efficacy may be due to poor oocyte quality. The problem related to ART requires tobe solved .miRNAs are small post-transcriptional modulatory molecules which function by binding to their specific mrna targets. miRNAs play important roles in many processes of physiology and have been implicated in numerous diseases.therefore the purpose of the current study was to determine miRNAs roles in the follicular fluid.
Materials and Methods: This review, we briefly discuss about The role of miRNAs from fluicular fluid in human oocyte quality.
Results: Our finding revealed that the existence of mirnas in human follicular fluid have important roles in steroidogenesis and also have a role in pathogenesis of polycystic ovary syndrome (PCOS). Moreover,it was identified that there is a differentially expressed of mirnas called as hsa-mir-424 which exist in higher proportions in ff from patients with advanced age women. Additionally,there was found 13 differentially expressed mirnas at the metaphasei and metaphaseii stage of oocyte maturation.
Conclusion: The current finding may aid to novel understanding of the role miRNAs as biomarkers for oocyte quality and subsequently improve ART
Keywords: Oocyte Quality, Follicular Fluid, MiRNAs

P-157: A Rare Case of Pericentric Inversion 21: Case Report

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Background: Structural chromosome abnormalities are estimated to occur in around 0.5% of newborn infants. Pericentric inversions are among the most frequent chromosomal rearrangements with a frequency of 1–2%. Miscarriages, infertility and chromosomally unbalanced offspring can be observed in carriers of a pericentric inversion. Since then at least 15 cases of this inversion have been reported. One couple with the first-cousin marriage referred to genetic center that had one infant with congenital anomalies and miscarriage history. The proband was an 5 month old infant with mongoloid face, mental retardation, congenital heart disease, left hand simian crease, increased distance between 1st and 2nd toes as clinical manifestations.
Materials and Methods: Cytogenetic examination was performed on the proband and her parents by GTG banding technique with a resolution of ~450, metaphase spreads prepared from PHA-stimulated peripheral blood lymphocytes. The karyotype was described in accordance with the ISCN, 2014.
Results: Analysis of 25 metaphase cells in parents showed a pericentric inversion of one chromosome 21, inv21(p12q22.3) with normal phenotype. Chromosomal study in the proband reveals 46,xx,der(21;21) (q10;q21),+21 .
Conclusion: The abnormal karyotype of the investigated couple could be acknowledged as a reason of miscarriage and chromosomally unbalanced offspring. Therefore, amniocenthesis for finding the chromosomal abnormality as a prenatal diagnosis are proposed for the patient if further pregnancy does not lead to miscarriage.
Keywords: Pericentric Inversion 21,Miscarriage,PND

P-158: Study on Effects of Iron Oxide Nano Particles on The Number of Ovarian Follicles of Adult NMRI Mouse Strain

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Background: Nanoparticles have very specific chemical and physical characteristics in their size, shape and high proportion of surface to volume. These characteristics have made them appropriate to be used in many medical and biological applications. Therefore, The aim of this paper is study on the effects of iron oxide nanoparticles on number of ovarian follicles in adult NMRI mouse Strain *in vivo*.
Materials and Methods: In this research, 30 female adult mice (NMRI) divided to 3 groups: control, sham and experimental doses of 50, 100 and 150 mg/kg. With starting of sexual cycle (pro-estrus), intraperitoneal injections in four consecutive days of estrous cycles (pro-estrus, estrus, estrus and di-estrus) was performed. After resting in two cycles, mice were killed in pro-estrus cycle.
Results: In this study, Number of primordial follicles in the experimental group 1 and Primary follicles in experimental groups 1 and 3 showed significantly increase compared with control group. Secondary follicles in the experimental group 2 showed significantly decrease compared with control group. Graafian follicles and atresia in experimental group 2 and 3 showed significantly decrease compared with control group. Corpus luteum showed no significant changes in the ovaries.
Conclusion: Generally the results of this study are revealed dual potential of nano iron oxide in the toxicity and activation oogenesis *in vivo*. Appropriate amounts of iron oxide nanoparticles can stimulate the process of cell division and are considered as a stimulus to activate factor required oogenesis. While increase in iron oxide nanoparticles enhances its accumulation in the cells, finally it causes loss the regulation of cell division and cell toxicity.
Keywords: Iron Oxide nanoparticles, Ovarian Tissue, Follicles, *In Vivo*, NMRI Mouse Strain

P-159: Leukemia Inhibitory Factor Expression in Endometrium of Infertile Women

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Background: Infertility is a critical component of reproductive health. the most common 'cause'of infertility is 'unexplained' .Infertility is a multifactorial and heterogenic complication. Implantation failure is one of the most important causes of unexplained infertility. LIF protein as a cytokine has important role in implantation. Leukemia inhibitory factor (LIF), plays a pivotal role in regulating uterine receptivity. LIF over expressed in the secretory middle phase of the menstrual course during the implantation time and any defect in the expression of it can leading to a lack of readiness of the uterus result in implantation failure. In this study, we analyzed the expression of LIF gene in uterine of women affected by asymptomatic infertility.
Materials and Methods: Aimed to this order, 46 women with unexplained infertility who were registered to Hajar Hospital of Shahrekord, from October2014 to Febrivery 2016were included. The median age of all patients at diagnosis was 32 years old (range from 20–40 years old). Total RNA was extracted and after cDNA synthesis, LIF gene expression was evaluated using Real-time RT-PCR.
Results: A significant variance at levels of LIF gene expression between the control and patient groups was observed. LIF has been a target for a nonhormonal contraception.
Conclusion: Association between patient's ages and life diet can affect the expression of LIF gene. On the other hand, in these types of studies we should consider stress factor. Cross function of LIF protein with the other hormone factors can be interfere with our results. The description of this gene can provide new ways in treatment of implantation deficiencies.
Keywords: Infertility, Implantation, LIF

P-160: Evaluation of N-acetyl Cysteine Effect in Ovulation Induction: Response of Patients Who Are Candidates for Intra Uterine Insemination

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Background: In this study, we aimed to evaluate the effect of N-acetyl cysteine (NAC) in polycystic ovary syndrome (PCOS) among patients who are candidates for intra uterine insemination (IUI), in order to achieve the best outcomes to increase the pregnancy rate.
Materials and Methods: This interventional study included PCOS patients less than 38 years old. Participants were randomly divided into two groups; experimental and control groups. The experimental group was administered 1.2 gr NAC + 100 mg clomiphene citrate (CC) + 5mg letrozole daily of the 3rd day of menstruation cycle for 5 days. The control group had the same drug regimen but without NAC. In order to induce maturation, follicles GONAL-f was injected on days 5, 7, and 9 of menstrual cycles in all participants. Followed by, transvaginal ultrasound was performed to follow up follicles size and endometrial thickness. When the follicle size was 18mm or more, HCG (5000 IU) was injected intramuscular. Patients underwent IUI 24 to 36 hours after HCG injection.
Results: There was no significant difference between study groups regarding FSH level (P=0.66) and LH level (P= 0.67). Also, it was not observed significant difference between two groups concerning the mean endometrial thickness (P= 0.14) and the mean number of mature follicles (P= 0.20).Moreover, there was no difference between the two treatment groups about pregnancy occurrence (P=0.09).
Conclusion: Although no significant difference was observed between two study groups, but administration of NAC in CC-resistant PCOS patients is recommended to study with larger sample size and assessing the biomedical profile to conceive accurate results that should be considered as an adjuvant therapy in selected patients.
Keywords: N-acetyl Cysteine, Polycystic Ovary Syndrome, Intrauterine Insemination

P-161: A Mice Model for Endometriosis

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Background: Presence of endometrial glands and stromal outside the uterine cavity is called endometriosis (EMS). EMS is a complex estrogen dependent disease which is a chronic and common gynecological disorder and affects about 10 to 15% of premenopausal women. It has been reported that over 40% of infertile women and one-third who undergo laparoscopy for chronic pelvic pain suffer from EMS. There is also limited understanding about the etiology and pathophysiology of EMS. So the aim of this study was to establish a mouse model for endometriosis to gain more knowledge about this disease.
Materials and Methods: Twenty female adult BALB/c mice of 6-8 weeks old that were housed in the absence of male mice, were used to induce endometriosis. 17β-Estradiol administrated at day 0 and continues weekly. At day zero a donor mice was sacrificed and obtained its uterine horns under sterile condition, endometrium was chopped and transferred to peritoneum cavity of recipient mice. After 21 days the recipient mice were sacrificed and the peritoneum cavity were seek to find any sign for endometriosis. For evaluating obtained tissue H&E staining was used.
Results: Endometriosis like tissues were observed in the peritoneum of recipient mice and histological studies proved the features of endometriosis tissues.
Conclusion: In this study we successfully induce endometriosis in BALB/c mice and histological studies via H&E staining prove the endometriosis characteristics in obtained tissues.
Keywords: Endometriosis, Premenopausal, H&E Staining

P-162: The Frequency of Staphylococcus Aureus Isolated from Endocervix Infertile Women in Northwest Iran

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Background: Infertility is one of the major important social issues. Because of the asymptomatic cervical infection, it has caused the problem to remain silent in the majority of patients without diagnosis and treatment. The present study was intended to assess the frequency of Staphylococcus aureus isolated from infertile women's endocervix in northwest Iran.
Materials and Methods: All specimens were collected during vagina examination by use of a sterile speculum and swabbing, were characterized by standard microbiological analysis. After determination of susceptibility against important antibiotics, polymerase chain reaction (PCR) was used to identify mecA and tst genes.
Results: Twenty six (26%) and 9 (9%) women's urogenital tracts were colonized by S. aureus and Candida spp., respectively, of which three (11.5%) patients were infected with fungi and S. aureus, simultaneously. Antibiotic susceptibility results showed high activity of vancomycin and co-trimoxazole on isolates. Regarding PCR results, mecA sequences were detected in 7 (26.9%) strains, whilst the tst gene encoding TSST-1 was not detected in any of clinical strains.
Conclusion: The prevalence of S. aureus was very high in infertile women. Therefore, it demands that the all the patients attending in infertility treatment centers be investigated thoroughly.
Keywords: Infertility, Staphylococcus Aureus, Endocervix, MecA

P-163: Endometriosis: Its Relation to Stem Cells and Cancer

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Background: Endometriosis is a common chronic disorder outside the endometrium and myometrium causing major problems including infertility. It is characterized by the presence and proliferation of functional endometrial glands and stroma outside the uterine cavity. Endometrial stem cells (EnSCs) are adult stem cells isolated from the endometrial tissue. EnSCs comprise of a population of epithelial stem cells, mesenchymal stem cells, and side population stem cells. In this paper, the recent advances in endometriosis and its relationship to cancer stem cells is discussed.
Materials and Methods: A search that was conducted to articles of electronic and scientific literature database such as Science Direct, PubMed, Scopus, Medline and ISI Web of Science was performed using key words Endometriosis, stem cells and rcancer' published from 1990 to 2016.
Results: Malignant tumors derived from endometriosis are rare. Recent evidence has shown that the presence of endometriosis, in addition to the increased risk of cancer at the site of deployment, the risk of non-Hodgkin's lymphoma, malignant melanoma and breast cancer also increases. Endometriosis as well cancer to invasive cell, uncontrolled growth, formation of new blood vessels and decrease the rate of apoptosis is determined. It seems that mutations in effective enzyme gene in metabolism and detoxification such as GALT, GUST could be involved in the pathogenesis of endometriosis into ovarian carcinoma. PTEN is a tumor suppressor gene and had the most common (50%) mutations in endometrial carcinoma. Endometrioid ovarian carcinomas have mutations of this gene in the mutant but not in other types of ovarian cancer.
Conclusion: The basic studies on stem cells, new insights into the pathophysiology associated with gynecologic disorders associated with abnormal proliferation of endometrial such as endometrial hyperplasia, endometriosis and adenomyosis will create. Although endometriosis is a benign disease, recent studies show that these patients can be considered as a neoplastic process. Epidemiological evidence from studies cohort has shown that endometriosis is an independent risk factor for ovarian cancer. In addition, the common risk factors of ovarian cancer patients are also another confirmation of this fact.
Keywords: Stem Cell,Cancer,Endometriosis

P-164: Reproductive Effects of Berberis Integerrima Root in Female Rats

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Background: During the last few decades there has been an increase in the study of medicinal plants and their traditional use in different parts of the world. Berberis integerrima is a medicinal shrub used in conventional therapy for a number of diseases. The reproductive effects of Berberis in female rats were investigated in the present study.
Materials and Methods: Sperm-positive adult female rats were orally administered (P.O.) the aqueous extract of Berberis integerrima root (100 and 200 mg/kg), distilled water (10 ml/kg) for seven days. On day 10 of pregnancy, the implantation sites were recorded. In the fertility study, adult female rats received the same test substances for 14 days and, the fertility index and litter size determined. In the uterotrophic test, normal and ovariectomized immature rats were treated

for seven days with the dry extract of Berberis integerrima root (100 and 200 mg/kg) in the absence and presence of 17â-estradiol benzoate 1µg/animal/day, s.c. On day 8, the uterine growth index was measured.
Results: Results of the study showed a significant increase (P<0.05) in the implantation sites and litter size of animals receiving 100 mg/kg of the extract. In the estrogenic assay, normal immature rats were sensitive to the treatment with Berberis integerrima root than the ovariectomized ones.
Conclusion: Our results give added scientific support to the popular use of Berberis integerrima root in the treatment of some cases of women's sterility/infertility related problems.
Keywords: Berberis Integerrima Root, Implantation, Fertility, Uterotrophic, Rat

P-165: Reactive Oxygen Species and Doxorubicin Induced Ovarian Damage

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Background: Doxorubicin (DOX) is one of the most potent anticancer drugs. Ovarian induced toxicity by doxorubicin has been demonstrated before. DOX-induced cardiotoxicity is known to be caused mainly by (ROS) reactive oxygen species. Therefore In this research ROS production due to the effect of DOX has been evaluated.
Materials and Methods: DOX was administered at a dosage of 2.5 mg/kg of body weight, i.p. totally 10 mg/kg of body weight was injected for 4 times, every 3 days, during the 12 days to 10 female mice as DOX group (6-8 week days old). Follicular population, apoptosis and ROS production in DOX group were compared with control group (no injection). Ovarian tissue sections were stained using (H&E) Hematoxylin and Eosin. The apoptosis incidence was carried out using imunohistochemical evaluation of via Caspase3 expression in sections of ovarian tissue. ROS production was measured with (MDA) malondialdehyde assay in blood serum samples.
Results: In DOX treated group the number of antral follicles was significantly decreased when compared to control group (184.6 ± 10.21 vs. 224.8±6.63) (P<0.05). Visual incidence of apoptosis via Caspase3 expression showed higher incidence of apoptosis in selected slides from DOX group compared to control group. MDA level was significantly higher in DOX group compared to control group (7.69 ± 0.12 Vs 3.71 ± 0.13) (P<0.05).
Conclusion: DOX has changed normal ration of follicular population, increased apoptosis and ROS production. Therefore here the adverse effects of DOX on ovarian function are demonstrated. Furthermore DOX induced ovarian damage is seems to be mainly due to ROS production by DOX in body.
Keywords: Apoptosis, Doxorubicin, Follicle, Reactive Oxygen Species (ROS), Ovary

P-166: Polycystic Ovary Syndrome Had No Effect on Hypothalamic Arcuate Nucleus Expression of Kisspeptin mRNA in Rats

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Background: Polycystic ovary syndrome (PCOS) can be accompanied by disturbances in frequency and amplitude of gonadotropin releasing hormone (GnRH). On the other hand, expression of kisspeptin in arcuate nucleus of hypothalamus has a stimulatory effect on gonadotropin releasing hormone (GnRH) release. Therefore, the present study assessed mRNA expression of Kiss1 mRNA in the arcuate nucleus after induction of PCOS in rats.
Materials and Methods: Female Sprague-Dawley rats were divided into control and PCOS groups (n=12). PCOS was induced by exposure to continuous light for 90 days. Six adult ovariectomized female rats were used as control in real-time PCR test. The relative gene expression of Kiss1 was assessed using real-time PCR. Furthermore, alterations of ovary were histologically compared between groups. The data were analyzed by one-way ANOVA and LSD post hoc test (P≤0.05, SPSS 22).
Results: Number of secondary follicles and corpus luteum in the PCOS group were less than the control; but, the number of tertiary and atretic follicles in the PCOS group was more than the control (P<0.05). The mRNA expressions of Kiss1 in the PCOS group and control were not different (P>0.05).
Conclusion: Constant light induction of PCOS in rats did not alter kisspeptin gene expression in the arcuate nucleus of hypothalamus. This finding shows that kisspeptin-GnRH pathway may not have role in pathogenesis of PCOS.
Keywords: Polycystic Ovary Syndrome, Kisspeptin, Hypothalamus, Rat

P-167: Protective Role of Crocin against Nicotine-induced on Level of FSH, LH and Estrogen in Female Mice

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Background: Nicotine is a major toxic component of cigarette smoke and it is a major risk factor in the development of functional disorder of several organ systems. Nicotine from tobacco products is absorbed into the blood through the lungs, and across nasal and buccal mucosa. Saffron is widely used a food flavor and has well known medicinal effects. Recent studies have revealed that main components of saffron including carotenoids: crocin, crocetin,picrocrocin and safranal have a large number of physiological effects on different biological systems. We decided to assess the possible effect crocin and nicotine on ovary Injuries , ovarian follicles diameter and serum nitric oxide, LH, FSH and Estrogen levels in mice.
Materials and Methods: In this study, 56 male rats were divided

in to 8 groups:control, nicotine–treated group (1 mg/kg/day); crocin -treated groups (25, 50, 100 mg/kg./day); and nicotine and crocin treated group interperitoneal administration for successive 28 days. After 24 hours animal were killed , the ovary was sampled:tissue sections were prepared and examined by light microscope. weight ovary, and serum nitric oxide, FSH, LH and Estrogen levels were analyzed (one-way ANOVA). Then data were $P<0.05$ was considered significant.

Results: The results indicated that nicotine administration significantly decreased ovary weight (0.53%) , ovarian follicles diameter (primordial 1.74%, primary 4.75%, secondary 11.5% and antral 11.5%) and ovary hormones level (LH 21.5%, FSH 8.2%, Estrogen 3.09%) and blood serum nitric oxide level (42%) compared to saline group ($P<0.05$). However, crocin and crocin plus nicotine administration significantly boosted ovary weight (1.58%), ovarian follicles diameter (primordial 2.35%,primary 5.31%, secondary 14.38% and antral 22.9%) and ovary hormones level (LH 29.8%, FSH 18%, Estrogen 4.55%) and blood serum nitric oxide level (2.53%) in all groups compared to nicotine group(percentage represent the maximum dose) ($P<0.05$).

Conclusion: This study showed that the crocin canthe maximum dose (100 mg/kg) improve ovarian changes and ovarian hormones dependent after nicotine administration.

Keywords: Crocin, Ovary, LH, FSH, Estrogen

P-168: A Survey into Zearalenone Effects on Fertility Disorders

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Background: Zearalenone is a non-steroidal estrogenic mycotoxin which is produced by Fusarium species on a number of important human food resources such as wheat, barley and maize and can poison them. This study aims at showing the great effects of Zearalenone on sexual organs and reproductive disorders.

Materials and Methods: The researchers studied and analyzed data collected by data bases such as ISI, Science direct, pubmed and magiran.

Results: Results of laboratory tests indicate that Zearalenone causes steratogenic syndrome in domestic animals. Further studies show that this dangerous mycotoxin might result in telarche, genycomastia, early puberty, cervical and endometrial cancer, reduced fertility, some changes in genital anatomy and changes in estrogen and estradiol hormones.

Conclusion: Due to the numerous disorders caused by Zearalenone as the result of long-term consumption of grains, the necessity of improvement and continuous monitoring of grain storage situation, to prevent further complications, is undeniably felt more than ever.

Keywords: Zearalenone, Fertility, Mycotoxin, Grains

P-169: Ameliorative Effect of Nigella Sativa Hydro-Alcoholic Extract on PCOS-Reduced Embryo Development

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Background: The poly cystic ovarian syndrome (PCOS) is a wide range clinical and morphological disorder in women with an endocrine abnormality, specifically for androgen biosynthesis and metabolism. Nigella sativa (NS) is an annual herb, which is native in Mediterranean region and Asia. NS-extract has been known for prominent antioxidant property. The aim of this study was to analyze the ameliorative effect of Hydro-alcoholic extract of Nigella sativa on PCOS-reduced *in vitro* embryo development ratio.

Materials and Methods: Twenty mature female rats were randomly divided into 4 groups as Control, PCOS-induced (received 4mg/kg/B.W-1 estradiol valerate, IM), PCOS+ NS-extract (200 mg/kg B.W-1)-treated and PCOS+NS-extract (600 mg/kg B.W-1)-treated. Animals received NS orally by gavages. Following 63 days, hormone pregnant mare serum gonadotropin, (PMSG, 25 IU, IP) was injected. Then GV stage oocyts were collected form the ovary. GV stage oocytes transferred to TCM medium (26- 28 hours). The sperms (1×10^6/ml HTF medium) for *in vitro* fertilization (IVF) were obtained from healthy mature male Wistar rats.

Results: Observations revealed that, the PCOS significantly ($P<0.05$) reduced 2-cell, blastocyst and hatched embryos development versus control group. Meanwhile, administration of 200 mg/kg and 600 mg/kg from NS-extract enhanced 2-cell, blastocyst and hatched embryos development compared to non-treated PCOS-induced group. Taking together, NS-extract at dose level of 600 mg/kg induced significantly better embryo development ratio.

Conclusion: Our data showed that, the 600 mg/kg NS-extract exerted better *in vitro* embryo development. Increased *in vitro* fetal development in NS-treated animals may be attributed to NS-induced antioxidant substrates.

Keywords: Nigella sativa, PCOS, Rat, Infertility, Fetal Development

P-170: The Study of Success Rate of Intra Uterine Insemination in Infertile Couple

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Background: The high prevalence of infertility in society has become one of the major social problems. Infertility is seen in 10-15% of couples. Intra Uterine Insemination (IUI) is a simple primary method which is effective on the treatment of infertility with ovulation disorders, unexplained infertility, cervical factor and some other male factors. . This study aimed to evaluate The success rate of IUI in infertile women in Kermanshah.

Materials and Methods: In this retrospective study during 2012-2013, 253 infertile couples were evaluated, using a questionnaire containing demographic data, duration of infertility and pregnancy outcomes. The results were statistically analyzed by SPSS.

Results: Pregnancy rate in this study was 23.3% (59 cases), With 17 twin pregnancy . The best results were obtained in couples with unexplained infertility (39% of pregnancies). There wasn't a significant difference between pregnancy rate and duration of infertility in this study, and the rate of pregnancy was higher in younger women (25-35 years old).

Conclusion: IUI is one of the low-cost and primary methods of infertility treatment . Patients Age is an important factor in infertility so that patients 35-40 years of age did not have a good prognosis in our study. More studies needs to be done for semen analysis and more infertility factors and compare the results of the pregnancy outcomes and rates.

Keywords: Infertility, Male Factors, Female Factor, IUI

P-171: Pregnancy Rate following Fresh and Frozen Embryo Transfer Cycle in Women with Endometriosis

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Background: Controlled ovarian stimulation using exogenous gonadotropins may affect endometrial development and receptivity in IVF cycles. This study was designed to compare the pregnancy rates following fresh and frozen embryo transfer cycle in women with endometriosis
Materials and Methods: In this randomized controlled trial study, a total of 58 eligible patients with endometriosis were included during 2013-2015. Patients were randomly assigned to one of two groups of fresh or frozen-thawed embryo transfer. The patients with endometriosis under 35 years of age, at least one endometrioma and with a history of IVF failure were included the study. Exclusion criteria were the patients with: severe male infertility, underlying factors for implantation failure and undesirable endometrium. Clinical pregnancy rate was primary outcome measure.
Results: Both groups were comparable in regards to mean age, body mass index , type of infertility, infertility duration, history of surgery, fertilization rate, number of total embryos, number of transferred embryos and grade of transferred embryos. There were no statistically significant differences between the groups with respect to clinical pregnancy rate and implantation rate.
Conclusion: Women with endometriosis undergoing ART have the similar chance of achieving clinical pregnancy following fresh or frozen-thawed embryo transfer cycles. It seems that these women should not delay the reproductive treatment on behalf of intervention for endometriosis or frozen-thawed embryo transfer cycle. However, more studies are suggested, particularly those evaluating large cohorts.
Keywords: Endometriosis, Embryo Transfer Cycle, Frozen-Thawed Embryo, Outcome, Pregnancy Rate

P-172: The Effect of Zolpidem on Oogenesis and Uterus Tissue and Sexual Hormones of Adult NMRI Mouse Strain

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Background: Zolpidem (with the brand name of Ambien) is a non-benzodiazepine hypnotic wich binds to the benzodiazepine binding site on the GABA-A receptors .The aim of this study was the effect of zolpidem on reproductive system of female adult NMRI mouse.
Materials and Methods: In this experimental study thirty adult female mouse NMRI strain at a mean weight of 30±26 grams were divided into five groups. Zolpidem solution was prepared in distilled water at 5, 10, and 20 (mg/kg of body mass) doses, and 0.5 cc injections were done intraperitoneally every day for 14 days. The control group received no injection. The sham group received distilled water (as solvent of zolpidem) and treatment groups of 1, 2, and 3 received doses of 5, 10, and 20 mg/kg. The treatment groups were sacrificed one day after the last injection, and their hearts were dissected and blood samples were obtained. The concentrations of the hormones were measured by the ELISA test,and the texture of their right and left ovaries and uterus tissue was separated and examined after the process of alcohol supply, molding, shredding as well as Hematoxylin and eosin painting and The results were evaluated via the Tukey-test, ANOVA by SPSS program.
Results: The results showed a significant decrease in the mean serum FSH, LH, Estradiol in the experimental groups compared to the sham and controlhistological studies of sections showed significant decrease in the oogenesis in the three experimental groups ($P<0.05$). The degeneration of ovary was observed in experimental groups and showed degeneration of uterus gland in experimental groups.
Conclusion: Injection zolpidem significantly be effective on oogenesis ,uterus tissue and concentration of FSH, LH, Estradiol hormones.
Keywords: Zolpidem ,Oogenesis, Uterus ,Sexual Hormone

P-173: Augmenting Reproduction: Kisspeptin, A New Kid behind The Wheel

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Background: GnRH or its analogue is a drug of choice to treat reproductive disorder, a global problem causing huge economic loss. However GnRH, being a glycoprotein, its repeated administration results in development of anti-GnRH antibodies making its application ineffective. As an alternate kisspeptin (KP), KiSS1 gene product and potent GnRH secretagogue, has recently been reported. Till today, no study on either blood concentrations of kisspeptin or placental expression patterns of KiSS1/KiSS1R genes during different phases of pregnancy has been conducted in bovine species. We, therefore, proposed to study kisspeptin-KiSS1R system during entire estrous cycle both in spontaneous and kisspeptin induced estrus, the effects of kisspeptin-10 on the follicular development and subsequent ovulation and blood concentrations of kisspeptin & placental expression patterns of KiSS1/KiSS1R genes during different phases of pregnancy in bovine species.
Materials and Methods: A highly sensitive enzyme immunoassay for determination of kisspeptin/metastin in different body fluids/tissues using biotin-streptavidin amplification system and second antibody technique was developed. For characterization of the kisspeptin-KiSS1/KiSS1R system during entire reproductive cycle, blood kisspeptin and transcripts encoding kiss1 and kiss1r genes were measured during different days of the cycle. Effects of kisspeptin-10 on ovarian activity and gonadotropin/kisspeptin secretory response were studied through color Doppler ultrasonongraphy and hormone estimation, respectively. The kisspeptin-KiSS1/KiSS1R system during different stages of bovine pregnancy was established. A new method of ovulation synchronization based on kisspeptin protocol for getting all calves together as per our wish was developed.
Results: For the first time, we have developed a highly sensitive (0.1ng/ml) and cheapest enzyme immunoassay (71 times cheaper than commercially available human kisspeptin ELISA kits) for determination of kisspeptin/metastin in different body fluids and tissues using second antibody format and streptavidin-bioin amplification system. We found that exogenous kisspeptin enhances follicular growth and helps the dominant follicle to ovulate. Based on the follicular dynamics, we developed a kisspeptin-based estrous/ovulation synchronization protocol for timed-artificial insemination thereby getting all calves of the farm together for better mangement and economics. We found that up-regulation of the transcripts ending kiss1 and kiss1r is important for successful maintenance of pregnancy. Our results revealed that blood kisspeptin concentrations increased more than 4.5 times and 8 times higher during mid and late than early stage of pregnancy, respectively. Hence, kisspeptin may be reliable marker

of detection of early pregnancy. Similarly, abundances of transcripts for kiss1/kiss1r has been increased a pregnancy advanced.
Conclusion: For the first time, we developed a 71-times cheaper kisspeptin enzyme immunoassay than commercially available kits. We developed a new method of estrus synchronization based on kisspeptin. For the first time, we showed that blood kisspeptin may be used as a biomarker for detection of early pregnancy. As both the cattle cow and woman are mono-ovulatory, cow may be followed as a model of human and therefore kisspeptin hold promise to ameliorate the problems of infertility in women.
Keywords: Kisspeptin, Pregnancy, Kiss1, Infertility, Kiss1r

P-174: Gestational Diabetes Affects Kcnj11 Gene Expression in Pancreatic Islets of Rat Offspring

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Background: A large number of epidemiologic studies demonstrated that infants of diabetic mothers have an increased risk for type 2diabetes throughout life. Also, in animal models of Gestational diabetes, it has shown that offspring overtly develop diabetes during childhood and adulthood. Insulin-producing β-cells in the endocrine pancreas, plays a pivotal role in maintaining glucose homeostasis. Kcnj11 channels play a critical role in the regulation of insulin secretion in the pancreatic beta cells. So, this study was conducted to determine the effect of gestational diabetes on Kcnj11 expression in offspring's pancreatic β-cells.
Materials and Methods: Adult Wistar rats aged 10-12 weeks were randomly allocated in control and diabetic group. The diabetic group received 40 mg/kg/bw of streptozotocin on day zero of gestation. After delivery, diabetic offspring of GDM mothers and control dams, at the age of 15 week were randomly scarified and pancreases harvested. Langerhans islets of diabetic and control groups were digested by collagenase digestion technique. After RNA extraction, we investigated the expressions of the Kcnj11 gene by quantitative real-time PCR.
Results: Fasting blood glucose concentration was significantly increased in offspring rats By 15 weeks of age, about 60% of the IDMs developed mild hyperglycemia. Furthermore, real-time PCR result showed that GDM significantly reduces the expression of Kcnj11 in Langerhans islets cells of offspring (**P < 0.01).
Conclusion: Our data showed that downregulation of Kcnj11 gene is related with development of diabetes in offspring of gestational diabetic rats.
Keywords: Gestational Diabetes Mellitus, Langerhans Islets, Gene Expression, Rat Offspring

P-175: Comparative Study of The Effect of different Levels of Glutamine on Chinese Hamster Ovary Cells Producing Recombinant Human Follicle Stimulating Hormone

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Background: Follicle stimulating hormone (FSH) is one of the most common and effective gonadotropin hormones used in infertility treatment. Ammonia is one of the side product of metabolism glutamine at medium culture. This side product is toxic for cells and decreases recombinant protein production. Glutamine is known as an essential and critical component of medium culture and omitting glutamine is not possible. At this study tried to obtain the optimum level of glutamine in medium culture that prepares an adequate amount of glutamine and preventing ammonia accommodation.
Materials and Methods: The cells were growth in serum-free medium culture Lonza glutamine-free antibiotic free by adding 3 mM glutamine supplement. Then cells were culture spontaneously in medium containing primary different levels of glutamine 0 mM, 2 mM, 4 mM, 6 mM of glutamine. For determination of glutamine and ammonia concentration the L-Glutamine ammonia assay kit (SKU= K-GLNAM, megazyme) were used Trypan blue staining method was applied for cell counting and measuring Cells Viability. In continuous Elisa were used for quantification assays.
Results: The obtained results in this study showed that, the most productivity of the cell at 2 mM glutamine at medium culture. Also, Increase in cell viability and longevity was indicated at 2 mM glutamine. Quantification assays demonstrated a reduction in the expression of FSH following the increase of the concentration of glutamine.
Conclusion: The maximum expression was seen at medium culture with 2mM Glutamine. Cells viability and longevity increased in medium containing 2 mM, also omitting the glutamine from the medium culture have a negative effect on cell growth, cells proliferation, and FSH production.
Keywords: Follicle Stimulating Hormone, Glutamine, Infertility

P-176: Determination The Optimum Condition for Producing Follicle Stimulating Hormone

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Background: Follicle stimulating hormone (FSH) produced in recombinant Chinese hamster ovary (CHO) cells used for infertility treatment. Maximum qualified production of recombinant protein with minimum cost in large scale makes this production commercially justified.In this case, in this study try to investigate the effect of fetal bovine serum as the most expensive component of medium culture on the expression of FSH titer. Temperature and pH were two other environmental parameters considered for this experiment.The optimum pH in the range of (6/7-7/6) and the optimum temperature in the range of (28ºC-37ºC) was determined.
Materials and Methods: Taguchi assay used as a statistical method for designing the experiments. Consider range for pH , temperature, and FBS concentration were 6/7-7/6, 28ºC-37ºC and 3%-10%, re-

spectively. a Trypan blue staining method was applied for cell counting and measuring Cells Viability.Cell growth and morphology were monitored by an optical microscope. Elisa method and qRT-PCR for determination amount of FSH production were applied.
Results: The obtained results in this study showed the maximum cell viability and growth achieved in medium with 10% FBS, pH=7.0. In comparison with the control condition (pH=7.3, 37ºC and 10% FBS) 14 fold more expression were observed.
Conclusion: Using this optimum condition helps to produce 14 fold more FSH in CHO cells with no more spending money. The most effective parameter was temperature then pH and concentration of FBS at medium culture respectively.
Keywords: Follicle Stimulating Hormone, Environmental Condition, Infertility

P-177: Histomorphometric Attributes of Ovaries after Constant Light Induction of Polycystic Ovarian Syndrome Model in Rat

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Background: Several methods have been developed for induction of polycystic ovarian syndrome (PCOS) in rats such as exogenous chemical and hormonal changes. In the present study histomorphometric alterations of changing environmental factor of light on induction of PCOS was evaluated in rats. Furthermore, the effect of parity on occurrence of PCOS and on this histomorphometric indices were investigated.
Materials and Methods: Female Sprague-Dawley rats which were consist of two groups of primiparous and nulliparous were subdivided into two subgroups of PCOS and control (n=6). Both PCOS groups were exposed to constant light for 90 days. Ovaries were sectioned after 90 days and stained with hematoxylin-eosin stain. The number of secondary, tertiary (antral), and atretic follicles and corpora lutea were counted. The diameter of granulosa and theca layer, total diameter of follicles and their antrum and total diameter of corpora lutea were measured. The data were analyzed by one-way ANOVA and LSD post-hoc test ($P \leq 0.05$, SPSS 22).
Results: Number of antral and atretic follicles in the PCOS groups were more than the controls ($P<0.05$), but the number of secondary follicles in the PCOS groups was less than the controls ($P<0.05$). There was no corpus luteum in the PCOS groups ($P<0.05$). In addition, total diameter and antrum diameter of antral follicles in the both PCOS groups were greater than the controls; while granulosa layer diameter of PCOS groups was less than the controls ($P<0.05$). Furthermore, however the severity of alterations of histomorphometric indices in nulliparous rats were more than primiparous ones, but parity had no effect on the induction of PCOS ($P>0.05$).
Conclusion: Constant light induction of PCOS in rats reflects ovarian features of polycystic ovaries similar to PCOS women in rats, and therefore because of removing of exogenous chemical manipulations, this model is appropriate for nervous system studies on PCOS.
Keywords: Constant Light, Polycystic Ovarian Syndrome, Parity, Follicle, Rats

P-178: Effects of Administering Letrozol, Tamoxifen, Vit E and Estradiol Versus Letrozol,Tamoxifen in an Ovulatory Disorders in Treatment of Infertile Women; A Randomized Clinical Trial

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Background: This study was conducted to evaluate efficacy of administering Letrozol, Tamoxifen, vit E and Estradiol as a treatment in an ovulatory disorders compared with Letrozole, Tamoxifen.
Materials and Methods: In this randomized clinical trial study 202 PCOS female patients were participated. After initial infertility work up, participants allocated into two groups of Letrozole, Tamoxifen and drugs (Letrozole, Tamoxifen, vit E and Estradiol).Efficacy of two regimens were evaluated by analyzing endometrial quality and thickening, follicular size, pregnancy rate, abortion rate and frequency of OHSS occurrence.
Results: The results of this study clarify that 0.07 percent of the patients taking medication suffered from OHSS and 38 percent had successful pregnancy .None of the patients had abortion .Mean of endometrial thickness is considerably significant in pregnant women ($P<0.001$). Also mean of follicular size in pregnant women was considerably significant.($P<0.001$).
Conclusion: In PCOS patients, administering Letrozol, Tamoxifen, vit E and Estradiol compared with Letrozole, Tamoxifen was more efficient in treatment of infertility in women with an ovulation.
Keywords: Letrozol, Tamoxifen, Vit E, Estradiol

P-179: Comparison of Efficacy and Outcome of Suprefact Ampule (Buserelin) versus HCG Ampule as Trigger of Ovulation Release in Infertile Women with Poly Cystic Ovarian Syndrome": A Randomized Clinical Trial

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Background: Infertility, due to endocrinologic disorder of PCOS has represented a rising pattern among women in fertility ages. This syndrome affects patients with undesirable gynecological and psychosocial problems. In this study, we aimed to assess the efficacy and side effects of Suprefact (Buserelin) versus HCG in ovulation release in infertile women with PCOS.
Materials and Methods: In this randomized clinical trial(parallel) study, 70 infertile women with diagnosis of PCOS who referred to infertility clinic in jahrom were allocated into two groups, receiving

either hCG or Suprefact for ovulation release. Patients in both groups were initially administered 2 doses of Tamoxifen 10 mg and Letrozole 2.5 mg. Thereafter, if follicles were less than 14 and their sizes were between 18 to 25 millimeters in diameter, HCG or Suprefact ampules were administered in this stage. Dosing was based on individual evaluations of each patient; 500 to 10000 units hCG and 1 to 10 units Suprefact. Ovulation was evaluated based on ultrasonography results. Analysis of data was performed using SPSS software version 19 and Maneva and t-test. P>0.5 was considered statistically significant.
Results: Patients were studied in regards of successful pregnancy and multiple pregnancy rates, as well as frequency and severity of ovarian hyperstimulation syndrome (OHSS). Pregnancy rate was significantly higher in Suprefact group; 37.1% vs. 5.7% (P value=0.0027), and no cases of multiple pregnancy occurred among patients. Frequency of OHSS was also higher in HCG group with a higher severity(P value>.05).
Conclusion: Suprefact can be used safely with an acceptably high rate of success for ovulation release in infertile women with PCOS.
Keywords: Buserelin, Ovulation, , HCG, Infertility

P-180: Molecular Analysis of VEGF in Susceptibility to Endometriosis in Iranian Women

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Background: Endometriosis is a chronic gynecological disease with an unclear pathophysiology characterized by the presence of the endometrium outside the uterine cavity. Genetic, endocrine, immunological, and environmental factors have been suggested in its pathogenesis. It is a multifactorial and polygenic disease in which angiogenesis may be implicated. Angiogenesis is under the control of numerous inducers, including vascular endothelial growth factor (VEGF). Vascular endothelial growth factor (VEGF) is an endothelial cell-specific angiogenic protein suspected to be involved in the pathogenesis of endometriosis by establishing a new blood supply to the human exfoliated endometrium. The aim of the present study was to assess the role of the vascular endothelial growth factor (VEGF)-2549 insertion/deletion (I/D) polymorphism in susceptibility to endometriosis.
Materials and Methods: This study is comprised 100 Iranian women with endometriosis and 200 healthy woman with out endometriosis recruited as control VEGF-2549 I/D polymorphism was determined using polymerase chain reaction (PCR).Genotyping for the - 2549 I/D polymorphism was performed using the forward 5' - GCTGAGGATGGGGCTGACTAGGTA – 3' and reverse 5' - GTTTCTGACCTGGCTATTTCCAGG – 3' primers.
Results: The frequency of the II, ID, and DD genotype was 14 versus 17.5%, 52 versus 50%, and 34 versus 32.5%, in patients and controls, respectively. A statistically significant difference was not observed for genotype distribution among the patients and controls (P=0.3).
Conclusion: The VEGF - 2549 I/D polymorphism has not a role in the susceptibility to endometriosis in the population of Iran.
Keywords: VEGF, Endometriosis, Polymorphism, Gen, Susceptibility

P-181: Evaluation of Serum Anti-Mullerian Hormone Level in Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine system disorder among women of reproductive age. This syndrome is identified by chronic anovulation and hyperandrogenism, but there is not a precise agreement on this disorder explanation and its experimental criteria. so finding a good experimental marker in order to diagnosis can be useful. recently Anti-mullerian Hormone (AMH) which is secreted by small follicular granulosa cells to regulate growth of primary levels of follicle, is presented as a marker for PCOS diagnosis. Unfortunately, in Iran few studies in this field has been done. therefore by this study we will find if this hormone or other sexual hormones can be regarded as a differential marker or not.
Materials and Methods: This case-control study consists of 51 patients referred to the highly specialized jihad daneshgahi infertility treatment center. 25 patients were PCOS and the other was control group. PCOS were diagnosed by Rotterdam's criteria. statistical analysis of obtained data was done by SPSS software
Results: According to statistical analysis in women with PCOS , level of AMH independent of other factors ,is higher. The levels of LH and LH to FSH is higher too (P<0.001). There is no relationship between AMH and other hormones in women with PCOS.
Conclusion: Evaluation of serum AMH can be diagnosis test in case of PCOS.
Keywords: Anti-Mullerian Hormone, Polycystic Ovary Syndrome, Follicle-Stimulating Hormone, Luteal Hormone

P-182: Platelet-rich Plasma (PRP) Auto-implantation on Mesovarium Ameliorated PCOS-reduced ovulation; Evidence for C-myc Expression and Redox System Status

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Background: Polycystic ovarian syndrome (PCOS) is a heterogeneous disorder, which is reported in 5-10% of women of reproductive age. The PCOS affects the reproductive, endocrine, antioxidant and metabolic functions leading to chronic anovulation as well as infertility. Thus, present study was done in order to analyze the ameliorative/therapeutic effect of platelet-rich-plasma (PRP) on oncogene C-myc expression, ovarian redox system potential and PCOS-induced atresia.
Materials and Methods: Mature female rats were randomly were divided into 5 groups as control (underwent simple laparotomy and sampled following 15 and 30 days) and PCOS-induced (by administrating 4 mg/rat estradiol valerate.IM) groups. The test groups were divided to control-PCOS (sampled 15 and 30 days post-PCOS-induction), PRP-treated PCOS-induced (sampled 15 and 30 days post-PCOS-induction). The blood samples from each animal were taken and PRP (8×106 cells) was collected. Then, the PRP was implanted in blood scaffold in mesovarium. Following 15 and 30 days, the ovarian samples were dissected out. The protein and mRNA expression of C-myc, tissue glutathione peroxidase (GSH-px), total antioxidant capacity (TAC) as well as total thiol molecules (TTM) level were analyzed.
Results: Auto-implantation of PRP resulted in a significant (P<0.05)

reduction in C-myc expression and up-regulated the ovarian TAC level, significantly (P<0.05) enhanced the GSH-px and TTM.
Conclusion: Our data showed that auto-implantation of PRP significantly down-regulates the PCOS-increased C-myc expression, which in turn results in improved follicular growth. Moreover, PRP by ameliorating the ovarian GSH-px and TTM enhances the ovarian antioxidant status.
Keywords: PCOS, C-myc, GSH-px, TTM

P-183: Omega-3 Ameliorates Diabetes-Reduced *In Vitro* Fertilization Ratio; Evidence for 2-Cell Embryo Development

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Background: It has been previously shown that, diabetes significantly reduces the fertilization ratio in both female and male genders. Thus, present study was performed to clarify the ameliorative therapeutic effect of omega-3 at two dose levels.
Materials and Methods: For this purpose 32 mature female Wistar rats were assigned into four groups as; Control (received no chemical) and test groups. The animals in test groups were subdivided into 3 groups as; non-treated diabetes-induced (STZ, 50 mg/kg, ip), 300 mg/kg omega-3 and 600 mg/kg omega-3-treated groups. Following 45 days hormone pregnant mare serum gonadotropin, (PMSG, 25 IU, IP) was injected.Then GV stage oocyts were collected form the ovary. GV stage oocytes transferd to TCM medium (26- 28 hours). The sperms (1×10^6/mlHTF medium) for *in vitro* fertilization (IVF) were obtained from healthy mature male Wistar rats.
Results: As preliminary data, the animals in non-treated diabetes-induced group exhibited significantly (P<0.05) lower oocyte versus treated animals. More analyses showed that, omega-3 at both dose levels enhanced the 2-cell embryo percentage in comparison to non-treated animals. Generally, all animals in test group showed lower oocyte and 2-cell embryos versus control animals
Conclusion: In conclusion our data showed that, omega 3 (especially at dose level of 600 mg/kg) up-regulates the fertilization ratio. Moreover, administrating omega-3 enhances the diabetes-reduced ovulation.
Keywords: Ovary, Oocyte, Omega-3, 2-Cell Embryo

P-184: The Effect of Agnugol and Metformin Drugs on Oligomenorrhea in Patients with Polycystic Ovary Syndrome: A Randomized Clinical Trial

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Background: Polycystic ovarian syndrome (PCOS) is the most common endocrinopathy and cause of oligomenorrhea in women. The present study aimed to compare the effect of Agnugol and Metformin drugs on oligomenorrhea in patients with polycystic ovary syndrome.
Materials and Methods: This study is a clinical trial on 120 women with polycystic ovary syndrome and has oligomenorrhea, referring to a gynecology clinic Fatemieh Hamadan, was carried out in 1394. Women were availably selected and were randomly replaced in groups including 60 people by using permutation blocks (getting the medications Agnugol and metformin), and for 3 months were treated with the drug Agnugol and metformin. Data collection included demographic questionnaire and chek list was designed before and end of interventions, was completed by both groups. To analyze the data, descriptive statistics, chi-square tests, t test, ANOVA with repeated measures was used.
Results: The mean and SD of age was 39.45 ± 4.60 for women taking Agnugol, and 38.466 ± 0.84 for those taking metformin. Based on the between-subject results, the two Agnugol- and Metformin-taking groups were not significantly different in terms of menstruation length, cycle intervals, or the number of pads, P<0.005, meaning that the two drugs had similar effects on menstrual cycle regulation, menstruation length, and the number of pads. More side effects were reported in the group using metformin.
Conclusion: Agnugol and Metformin drugs in the treatment of oligomenorrhea patients with polycystic ovary syndrome have the same effect. Since Metformin is a chemical drug with side effects, Agnugol can be presented as its herbal alternative to treat oligomenorrhea.
Keywords: Drug Agnugol, Drug Metformin, Oligomenorrhea, Polycystic Ovary Syndrome

P-185: The Effect of Red Ginseng Water Extract on Oogenesis and Uterus Tissue of Adult NMRI Strain

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Background: Korean Panax ginseng ,belonging to the genus panax of the family Araliaceae is mainly used to maintain the homeostasis of the body, and the pharmacological efficacy of Korean ginseng identified by modern science includes improved brain function, pain-relieving effects, preventive effects against tumors as well as anti-tumor activity, enhanced immune system function, anti-diabetic effects, enhanced liver function, adjusted blood pressure, anti-fatigue and anti-stress effects, improved climacteric disorder and sexual functions, as well as anti-oxidative and anti-aging effects.Korean ginseng is found to have such main properties as ginsenoside, ploy acetylene,acid polysaccharide, anti-oxidative aromatic compound, and insulin-likeacid peptides. The aim of this study was to delibrate the effect of red ginseng water extract on reproductive system of female adult NMRI-mouse.
Materials and Methods: In this experimental study, we randomly divided 30 adult female mouse NMRI strain into 5 groups.ginseng water extract was prepered in distilled water at three doses and The groups were treated as follows for 30 days: i. Control (not gavaged), ii. Sham (gavaged water), iii.Treatment groups (gavaged100mg/kg,200mg/kg and 300mg/kg ginseng extract) . The mouses were killed 1 day after the last gavage and their ovaries and uterus tissue were separated and examined after the process of alcohol supply,molding,shredding as well asHematoxylin and eosin painting and the results were evaluated well via tukey-test ,ANOVA by SPSS program .
Results: The result of sections showed significant increase in oogenesis in three experimental groups and posetive impact on uterus tissue;

regular endometrium glands and appropriate thickness was observed.
Conclusion: Studies have found oral use of red ginseng water extract be effective on oogenesis and uterus tissue.
Keywords: Red ginseng, Oogenesis, Uterus

P-186: Detection of Toxoplasma Gondii in Paraffin-Embedded Fetoplacental Tissues of women with Recurrent Miscarriage

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Background: Congenital toxoplasmosis is an important cause of abortion worldwide. However, there is limited information on diagnosis of Toxoplasma gondii in women with recurrent miscarriage.
Materials and Methods: A total of 210 formalin-fixed, paraffin-embedded fetal tissues of women with recurrent miscarriage were collected from the archives of Avicenna Research Institute in Tehran, Iran. After DNA extraction, the presence of T. gondii was examined by nested polymerase chain reaction targeting the GRA6 gene.
Results: T. gondii DNA was detected in 3.8% (8/210) of the samples. Two of the positive samples were sequenced and deposited in GenBank (accession nos. KT735111, KT735112). Our sequences revealed 100% of similarity with T. gondii sequences that deposited in GenBank from Iran. Six patients had a history of more than three previous abortions; one patient had a healthy girl and another patient had two previous abortions. Abortions were occurred in the first trimester of pregnancy in seven patients and in the second trimester of pregnancy in one patient.
Conclusion: These findings suggest that toxoplasmosis may play a role in the etiology of recurrent miscarriage. However, further studies are needed to elucidate a clear relationship between T. gondii infection and recurrent miscarriage.
Keywords: Toxoplasma gondii, Paraffin Embedded Tissue, Recurrent Miscarriage, PCR, Iran

P-187: The Effect of Endometrial Scratching Injury on Pregnancy Outcomes in Women with Intrauterine Insemination Failures

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Background: Recently endometrial local injury has been proposed as a method for enhancing the implantation rate in ART cycles. Present study was conducted to determine the effect of endometrial scratch injury (ESI) on pregnancy rate in women with IUI failure.
Materials and Methods: 150 infertile patients with IUI failure attending to hospital during a 12-month period 2013 and 2014 were enrolled. They were randomly assigned in two groups. In the experimental group, all patients have undergone ESI at days 8-9 of stimulation phase in present IUI cycle (n=75). Patients in the control group have received no intervention (n=75). The IUI outcomes were compared between two groups.
Results: The chemical pregnancy rate was 10.7% and 2.7% in case and control groups, respectively without significant difference (P = 0.09). Also, no significant differences were found in terms of the clinical pregnancy and abortion rates between groups (P > 0.05).
Conclusion: According to our results, it may be concluded that ESI is not effective for increasing the pregnancy rate in women with IUI failure; however, further studies with larger sample sizes are needed to confirm or refute our result (IRCT201507271141N19).
Keywords: Infertility, IUI Failure, Endometrial Scratching injury

P-188: Evaluation of Outcome of Double Stimulation and Egg Collection in The Same IVF/ ICSI Cycle (Shanghai Protocol) in Poor Ovarian Responders

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Background: This study aimed to evaluate the efficacy of double stimulations during the follicular and luteal phases in women with poor ovarian response in IVF / ICSI cycles. The outcomes of present protocol were compared with the results of previous routine treatment.
Materials and Methods: This prospective study was performed during 2014 to 2015 at Royan Institute. All the patients who diagnosed as poor ovarian responders (POR) based on the Bologna criteria are eligible for participation in this study. Informed consent was taken from all eligible patients. In present protocol dual ovarian stimulation was performed according to Kuang et al. study. Double stimulations were performed during the follicular and luteal phases in women with by using Letrozole, Clomid, hMG and GnRh -agonist. The results of the present cycle were compared with previous cycles of patient by using appropriate statistical.
Results: 107 patients were eligible for the study. 12 patients did not respond to ovarian stimulation was canceled; however, the previous cycle in these patients was canceled due to no response. 28 patients did not return for the second stimulation or unwilling to continue this protocol due to no response for first stimulation. The cancellation rate was 37.3%. Totally in 67 patients the double stimulation was performed completely. The results show that the mean number of stimulation day, the number of follicles with sizes < 14 mm or > 17 mm on the trigger day, the number of retrieved oocytes, the number of MII oocytes and fertilization rates were not different between the first and second stimulation. However, the number of MI oocyts and the number of frozen embryos in the first stimulation was significantly higher than those of in second stimulation (P <0.001). Results related to ovarian stimulation cycle shanghai with previous cycles show that the patients regardless of protocol. In general, shanghai protocol than previous cycles, the number of oocytes obtained, the number of oocytes in MII, the number of MI oocytes and fertilization rate was significantly higher.
Conclusion: Based on the present results, it seems that the double stimulation in same cycle (Shanghai protocol) is appropriate for ovarian stimulation in patients with diminished ovarian reserve. However,

the outcomes of first stimulation was better than second stimulation, in generally the outcomes of this protocol were better than the previous routine treatment cycle of patients. According to limited studies in this area, it seems that clinical trials with larger sample sizes are needed to draw definite conclusion.

Keywords: Double Stimulations, Luteal-Phase Ovarian Stimulation, Mild stimulation, Poor Ovarian Response

P-189: The Effects of Acne, Hirsutism and Androgen Hormones Levels on Depression and Anxiety Levels in Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common disorder in reproductive ages that the most important symptoms of this syndrome include hirsutism, acne and increasing in androgen hormones. The aim of this study was to evaluate the relationship between anxiety and depression with acne, hirsutism and androgen levels.
Materials and Methods: This cross-sectional study was performed on 53 patients with polycystic ovary syndrome, diagnosed with Rotterdam criteria, and 50 healthy women as control group. The tools of data collection were a questionnaire for demographic information, Beck Depression and Anxiety questionnaire, clinical features and laboratory findings of hyperandrogenism.
Results: Average scores of acne (P=0.01) and hirsutism (P<0.001), total testosterone (P=0.007), free testosterone (P=0.008) in the patient group were meaningfully higher than the control group. The average SHBG level in patient group was (46.75 ± 30.58) and in control group was (51.86 ± 26.29) (P=0.3), which was not statistically significant. The average scores of depression (P=0.004) and anxiety (P=0.05) in the patient group were significantly higher than the control group. It wasn't found significant relationship between scores of hirsutism, total testosterone, free testosterone and sex hormone binding globulin with levels of depression and anxiety. Bu, it was found significant relationship between acne and levels of depression (r=0.31, P=0.03) and anxiety (r=0.31, P=0.03).
Conclusion: The prevalence of depression in patients with PCOS is more than normal women. There is high association of anxiety and depression with acne, but no association was found between depression and anxiety levels with androgens and hirsutism levels in our PCOS study group.

Keywords: Polycystic Ovary Syndrome, Depression and Anxiety, Acne, Hirsutism, Androgen Hormones

P-190: The Effect of Depression and Anxiety on Cognitive Performance in Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS), the most common and important endocrine abnormalities in women of childbearing age, has a prevalence of 2.2 to 26 percent. The prevalence of depression and anxiety has been reported differently in these patients. The objective of the present study was to investigate levels of anxiety and depression in this group of women and its association with cognitive function compared to healthy women.
Materials and Methods: In these cross-sectional study, 45 patients with polycystic ovary syndrome, diagnosed with Rotterdam criteria, and 45 healthy women as control group were selected. Anxious and depressed mood subjects were evaluated by Beck 2 Depression and Anxiety questionnaire. Also, cognitive function were assessed using the Montreal Cognitive assessment (MoCA).
Results: The average Beck 2 score in patients was 20.22 ± 9.73 and in control group was14.62 ± 8.16, P=0.004, which was statistically significant. Also, between the average Beck Anxiety scores in patients (17.17 ± 10.76) and control group (12.97 ± 9.90) was found statistically significant difference (P=0.05). Frequency of severe depressed and anxious mood, based on Beck criteria, in the patient group were 28.8% and 26.6%, and in the control group were 4 and 13%, respectively, which was found statistically significant difference (P=0.007, 0.02). Also, it wasn't found significant relationship between cognitive function and levels of depression (r=-0.03, P=0.81) and anxiety (r=-0.14, P=0.35).
Conclusion: The prevalence of depression in patients with PCOS is more than normal women, but despite of many universal reports, any relationship was not observed between depression and anxiety levels with cognitive function Cognition in our PCOS study group.

Keywords: Polycystic Ovary Syndrome, Cognitive Function, Depression And Anxiety

P-191: Sexual Function in Women with Polycystic Ovary Syndrom: Clinical and Hormonal Parameters

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Background: To determine the association of polycystic ovary syndrome (PCOS) and its clinical and hormonal parameters with sexual function.
Materials and Methods: This prospective, cross-sectional study conducted from 1st January 2012 to 31 December 2015 in outpatient clinic of the Research Institute of Endocrinology, Iran, among married women with PCOS, aged 18–45 years. The Female Sexual Function Index (FSFI) was used to evaluate sexual function, and serum levels of total and free testosterone, androstenedione, and sex hormone binding globulin (SHBG) were determined and employed to calculate FAI values. Univariate and multiple logistic regression analyses were performed in order to examine the association between sexual dysfunction and independent variables.
Results: In all, 783 participated, 492 women have full completed to FSFI questionnaire. Of these, 226 women (45.75%) met the criteria for sexual dysfunction. The mean age of women was 24.91 ± 4.90 years. No significant association between having a low score for any FSFI domain and having a low serum total or free testosterone or androstenedione and SHBG level was demonstrated. There was association between FAI > 4.5 and increase sexual function score but it isn't significant. Logistic regression analysis showed that there were significant associations between sexual function score and the literacy, alopecia and infertility.
Conclusion: No serum androgen level is predictive of female sexual

function but the low literacy, alopecia and infertility contributed to sexual dysfunction in women with PCOS. The burden of PCOS and sexual dysfunction suggests the need for further attention to this patient population.

Keywords: Polycystic Ovary Syndrome, Sexual Function, Androgen Levels

P-192: Prevalence of Spontaneous Pregnancy in Infertile Couples

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Background: Social problems of the infertile women are high. Studies show that 50% of infertile couples, whom do not conceive after 12 cycles, will conceive spontaneously in the next 36 months. The possibility that some infertile couples could conceive spontaneously is an important finding. This study was conducted to find the prevalence and causes of spontaneous pregnancy.
Materials and Methods: This is a descriptive cross sectional study. The setting for this study was all of cases registered between March 2012 to March 2013 at IVF unit in Ahvaz Imam Khomeini Hospital. The required information was collected from each file by a designed questionnaire. Descriptive statistics, T-test and chi-square test were used by SPSS software version 22. The conventional p-value of $\leq$ 0.05 was considered as overall significant level.
Results: Among the 658 couples were enrolled, the mean age of women men was 34.91 ± 7.58 and in male 30.52 ± 6.32 years. 91.4% couples lived in urban areas. 56.4% of couples were infertile for less than 3 years. 32 cases (4.79%) had a spontaneous pregnancy. Significant correlation between spontaneous pregnancy and years of duration of infertility was found (P=0.010), so 78.1% of cases with spontaneous pregnancy were less than 3 years of duration of infertility, mean age of couples with spontaneous pregnancy was significantly different from those without spontaneous pregnancy (P=0.048), so 67.7% of cases with spontaneous pregnancy were under 30 years old, female factors, male factors, combined male & female factors and unknown factors were 35.5, 27.4, 25.8 and 11.3%, respectively. 50% of cases had primary cause of infertility.
Conclusion: It can be concluded that in infertile couples with duration less than 3 years by fundamental care, follow-up and monitoring can be expected to will conceive spontaneous pregnancy. So it would avoid many psychological traumas caused by infertility and the potential risks that could threat couples' lives. But in infertile couples with duration more than 3 years and especially in unexplained infertility adequate treatment should be planned.

Keywords: Infertility, Spontaneous Pregnancy, Ahvaz

Genetics

P-193: A Family-based Study of Polymorphic Teratozoospermia by Exome Sequencing

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Background: Male infertility remains idiopathic in 30 to 50 % of cases due to its heterogeneous nature. One of the semen abnormalities leading to male infertility is polymorphic teratozoospermia which is defined as the presence of various types of morphologically abnormal spermatozoa in a sample. So far, certain phenotypes of this condition, such as "tapered head", "pyri form" and "amorphous", have not yet been associated with a genetic mutation. We have identified a large family with a host of consanguineous marriages in which seven people have been diagnosed with polymorphic teratozoospermia and all of whom share the amorphous phenotype. Our objective is to determine at least one novel pathogenic variant by performing a family-based exome sequencing study.
Materials and Methods: Semen analysis was performed on all the affected. Moreover, peripheral blood sample has been collected from the affected and a number of non-affected closely related. DNA extraction was performed by salting out according to standard protocol. Library was created using Agilent SureSelect Target Enrichment kit. Firstly, panel-based exome sequencing will be performed on one of the affected; in case we fail to detect a novel pathogenic variant, a whole-exome sequencing (WES) will be carried out on three of the affected. Data analysis will include investigating homozygosity regions of the genome, since the analysis of the pedigree shows an autosomal recessive pattern of inheritance. Identified pathogenic variants will be confirmed by Sanger sequencing in the non-affected.
Results: Semen Analysis indicated polymorphic teratozoospermia in all of the patients. We are currently awaiting the initial results of the sequencing to begin data analysis.
Conclusion: To our knowledge, this is the first family-based exome sequencing study on polymorphic teratozoospermia. Thus, we are hopeful that our results will help prepare an expert panel for this condition.

Keywords: Polymorphic Teratozoospermia, Clinical Exome Sequencing, Panel-Based Exome Sequencing, Whole-Exome Sequencing, Family-Based Studies

P-194: Evaluation of Exon 9 of DPY19L2 Gene in Globozoospermic Patients Referred to Royan Institute

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Background: Globozoospermia a rare phenotype of primary male infertility characterized by the production of majority of round-headed spermatozoa lacking an acrosome. DPY19L2 (12q14.2) is the major gene responsible for globozoospermia, because of its predominant testis expression, sperm head elongation and acrosome formation.

The most common genetic defect is a 200 kb homozygous deletion of the DPY19L2 gene in total globozoospermia. Our aimed is to study exon 9 in total and partial globozoospermia.
Materials and Methods: A total of 50 men with globozoospermia(20-100% round head sperm in spermogram) were considered as case group and 50 men with normal spermogram as control group. DNA was extracted from blood samples of selected individuals. In a first step, we screened for the deletion of DPY19L2 in all patients, in a second step, we sequenced exon 9 and intron boundaries in the non-deleted patients using specific primers and PCR technique. Ultimately sequencing was used to determine genetic changes of the mentioned area.
Results: Analysis of our results on total and partial globozoospermia show that the frequency of homozygous deletion carriers was 20 of 27 in total globozoospermic patients and none of the 23 partial globozoospermic patients had the whole DPYI9L2 deletion. Exon 9 was studies in cases with no deletion(60% of our case group) and no mutation could be found in them.
Conclusion: According to our data, 40% of globozoospermic patients had whole DPYI9L2 deletion. Several previous studies showed a very different prevalence of this deletion among globozoospermic patients. Although several variations of exon 9 of DPYI9L2 has been known and reported does far no change was seen in our population study. As a result exon 9 has no effect on globozoospermia in Iranian infertile men. DPYI9L2 is composed of 22 exons so evaluating of other exons and regulatory areas of this gene is recommended.
Keywords: DPYI9L2, Globozoospermia, Acrosome

P-195: Identification of Genes Involved in Male Infertility by Family-Based Exome Sequencing

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Background: Infertility is a socially important health problem. Male factor infertility is found in about 30-35% of the infertile couples. Out of several factors associated with male infertility, genetic factors account for 15% of all cases. Although male infertility is often solved with assisted reproductive technology (ART), an understanding of the genetic etiology of an infertile couple is critical for proper consulting and decision–making. Use of novel techniques, including next generation sequencing technology, in studies of infertility could help to detect the genes that are involved in infertility. While whole genome sequencing is still quite costly for most of the application, exome sequencing is a technique which is cheaper and only focuses on the protein coding portion of the genome. In family based study, identification of genetic variations will be more accurate and easier. In this approach the number of private variant will decrease, and the pathogenic variants are accurately detected.
Materials and Methods: To identify variants involved in male infertility, we have used a targeted exome capture and high-throughput DNA sequencing approach, and analyzed the entire exome of infertile affected individuals from multigenerational families. We have prioritized rare variants with a frequency of less than 1% in the population that are shared with affected within our family, as well as being potentially highly penetrate or functionally relevant.
Results: The exome sequencing output listed in total 200 variants that are private variants. By focusing on pattern of inheritance, we have candidate pathogenic variants in male infertility. Candidate variants and the segregation in the family were confirmed through Sanger sequencing.
Conclusion: The role of new candidate pathogenic variants will be confirmed through different functional assay. As a consequence, Identification of pathogenic variants underlying male infertility can uncover novel therapeutic targets as well as opportunity to opt for using advanced ART.
Keywords: Male Infertility, Exome Sequencing, Pathogenic Variants

P-196: Altered Gene Expression of Bone Morphogenetic Protein Receptor 1B (BMPR1B) in Granulosa Cells of Women with Polycystic Ovarian Syndrome

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Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorders that affects approximately 6-8% of women in reproductive age. It is the main cause of infertility due to anovulation. Transforming growth factor-β (TGF-β) secreted by oocyte, granulosa and cumulus cells, plays a critical role in normal folliculogenesis, oocyte maturation and female fertility by coupling to their receptors. Bone morphogenetic protein 15(BMP15) is one member of TGFβ family. It showed that altered expression of BMP15 gene was involved in PCOS disorder, as reported in oocyte, granolusa and cumulus cells of such patients. Therefore, we evaluated expression of BMP15 receptors, although we just reported BMPR1B expression in granulosa of PCOS patients in this abstract.
Materials and Methods: Granullosa cells (GCs) were isolated from four PCOS patients and six women with normal folliculogenesis as controls (male factor) who were undergone ovarian stimulation for IVF/ICSI program. GCs were isolated and purified from follicular fluid by using density gradient separation technique. RNA was extracted from GCs by TRIzol and followed to cDNA synthesis. The quantitative expression level of BMPR1B was evaluated by Real- time PCR.
Results: Our results shown the expression of BMPR1B was significantly decreased (5 fold) in PCOS patients compare to control group.
Conclusion: Of essential genes for folliculogenesis and ovarian function is BMP15 and its association with the pathogenesis of PCOS has been reported. BMP15 plays a pivotal role in pre-antral to antral follicle development. Decreased gene expression of BMPR1B may contribute to alteration in downstream genes which involved to oocyte maturation and competence. The results of this study are important for understanding the mechanism of follicular arrest and anovulation in PCOS patients.It will be considered to explore the gene expression of other TGFβ family members and their receptors in future study.
Keywords: Polycystic Ovarian Syndrome, Bone Morphogenetic Protein 15, Bone Morphogenetic Protein Receptor 1B, Gene Expression

P-197: Study of Genetic Alterations in Untranslated Regulatory Regions of NANOS1 in Azoospermic Men Referred to Royan Institute

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Background: Infertility is a problem affecting many couples with a child wish. In near half of these couples a male factor is responsible for the fertility concern. NANOS1 (nanos homolog1) gene is located on chromosome 10q26.11. Knockout mice of NANOS family genes were infertile. Nanos1 was first recognized in Drosophila and has a conserved function in germ cell development. NANOS1 protein is essential in the early stages of embryogenesis to protect the primordial germ cells from apoptosis. This study aimed to determine the association between any alterations in 5′ and 3′ untranslated regulatory region of NANOS1 gene and azoospermia.

Materials and Methods: A group of 50 patients manifesting non-obstructive azoospermia were tested for mutations in regulatory regions of NANOS1 gene, using standard PCR – sequencing method.

Results: Sequence analysis did not detect any mutations or single-nucleotide variations in 5′ UTR of NANOS1 in non-obstructive azoospermic patients.The results of PCR product sequencing in 3′ UTR, illustrated a single nucleotide variation with rs79664216 in just one case. Additionally another nucleotide variation with rs79664212 was also detected in the other case. These changes were not observed in control group.

Conclusion: Present data identified no mutations or SNPs in 5′UTR of NANOS1gene in azoospermic men. According to our result, variations in 3′ UTR with rs79664216 and rs79664212 might be a contributing factor for male infertility.These are preliminary data and should be confirmed by more investigations.

Keywords: Azoospermia, NANOS1, Primordial Germ Cells

P-198: The Use of Human Serum Albumin Signal Peptide and Codon Usage Strategy for High-Level Production of Recombinant Follicle-Stimulating Hormone in CHO Cells

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Background: Human follicle-stimulating hormone (hFSH) is a heterodimeric glycoprotein that stimulates the growth of ovarian follicles. This hormone is one of the most common and effective gonadotropin hormones in infertility treatment. The Chinese hamster ovary (CHO) expression system is a commonly used mammalian host for production of recombinant proteins. In this study, the effect of the Human Serum Albumin (hSA) signal peptide and codon usage strategy on the expression of this protein, in the CHO cell line was investigated.

Materials and Methods: At first, open reading frame (ORF) of FSH β subunit was modified based on codon us¬age in CHO cells. The FSH gene's innate signal peptide was replaced by the hSA signal peptide using the PCR technique. Subsequently, recombinant expression vector (pOptiVEC) will be linearized and transfected into the CHO-DG44 cells. Finally, recombinant FSH protein ex¬pression was evaluated using the Bradford's technique, SDS page, Western blotting and ELISA.

Results: The synthesized construct was confirmed using enzymatic digestion and sequencing techniques.

Conclusion: We expect that combined codon optimization and hSA signal peptide inclusion will lead to high-level FSH expression in CHO-DG44 cell line.

Keywords: Follicle-Stimulating Hormone, Codon Usage, Human Serum Albumin, CHO-DG44, Signal Peptide

P-199: Decreased Expression Levels of Histone Variants (H1T, H1T2) in Testicular Biopsies of Infertile Men with Spermatogenesis Failure

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Background: Epigenetic modifications are of important regulatory factors occur for development of gamete are considered to be critical during spermatogenesis. Histone H1t is a linker histone which binds to DNA. H1T and H1T2 are contributed in chromatin condensation as well as regulation of specific genes through spermatogenesis. Replacement of this histone H1 subtypes and hyperacetylation of histone H4 tail, facilitate the replacement of histones with sperm chromatin condensing proteins of TNPs and PRMs. The purpose of this study was to evaluate the presence of H1T and H1T2 proteins in the regulatory region of TNPs and PRMs genes.

Materials and Methods: Ethical approval and informed patient consent was gained from 12 infertile men referred to Royan Institute. Testicular biopsies were collected from patients through assisted reproductive techniques (ART) procedure. Based on pathological results samples were classified into the following three subgroups: obstructive azoospermia (as positive control), complete maturation arrest, and Sertoli cell only syndrome (negative control). Expression of H1T and H1T2 genes were analyzed by qRT-PCR and chromatin extracts of tissues evaluated for presence/absence of histone H1T and H1T2 proteins in regulatory regions of TNPs and PRMs genes using ChIP-Real Time PCR.

Results: Results showed lower expression of H1T and H1T2 genes as well as lower incorporation of H1T and H1T2 proteins on regulatory regions of TNPs and PRMs genes in two spermatogenic failure groups versus positive control.

Conclusion: In this study, it can be concluded that the decreased levels of H1T and H1T2 histone variants in testis tissues and failure in chromatin condensation have significant association with male infertility.

Keywords: H1T, H1T2, Epigenetics, Spermatogenesis Failure

P-200: Mir-22 as A Mediator of Sperm Chromatin Condensation

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Background: Sperm DNA integrity plays a significant role in sperm function, maintenance of proper epigenetic patterns as well as fertilizing capacity. It has been proved that during spermiogenesis, DNA binding histones are gradually replaced by protamines, resulting in condensation of the nuclear chromatin and protect the genetic material prior to fertilization. Protamines are specific nuclear proteins in mammalian sperm which require for sperm DNA packaging and structural stability. Sperm DNA damage and infertility is thought to be associated with incomplete protamination. Moreover, the role of microRNAs in the regulation of reproductive-related gene expression during spermatogenesis has been demonstrated. Therefore, the present study was aimed to investigate the relationship between mir-22 and protamine deficiency in subfertile men.
Materials and Methods: Semen samples were obtained from 28 couples referred to Fertility and Infertility Centre. Routine semen analysis was carried out according to WHO criteria. Protamine deficiency was determined by chromomycin A3 staining. The extraction of total RNA from sperms and complementary DNA (cDNA) synthesis was carried out. In order to evaluate the expression level of mir-22, protamine 1 and 2 real time-PCR analysis was performed.
Results: The percentage of CMA3-stained sample varied between a minimum of 13 and a maximum of 44 percent. The mean level of CMA3 staining (>30%) was significantly higher in sperm that expressed elevated level of mir-22 (P<0.05). The protamine 1 expression level had been increased dramatically in CMA3 positive (>30%) samples compared to CMA3 negative (<30%). However, no significant difference was found in the expression level of protamine 2 between two groups.
Conclusion: According to these results it is believed that higher expression level of mir-22 would increase protamin1/protamin2 ratio which may result in impairment of sperm chromatin.
Keywords: Sperm, Protamine, miRNA

P-201: Taxol-Treated "*In Vitro* Fertilized" Mouse Embryos Mimic: A Natural Cleavage Rate

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Background: Paclitaxel (taxol) as a microtubule-stabilizer is one of the most common anticancer drugs. Previous studies report that paclitaxel significantly improves survival and fertilization rates of post-thaw cumulus-free mouse oocytes. We studied the effect of taxol on mouse embryonic development through developing from 2-cell toward morula in IVF process.
Materials and Methods: We cultured 172 late 2-cell mouse embryos collected from 5 IVF cycles in different concentrations of taxol (10,5,0.5,0.2,0.1,0.05,0.01 μM) for 24 hours . The highest concentration without decrease in developmental potential to morula stage was selected for developmental assessment from late 2cell to the morula using 136 embryos as cases and controls.
Results: Among different concentrations,0.01 μM was selected for further evaluations. Significant increase in development to morula was observed after taxol treatment (31.34 %) compared with controls (8.6 %) (P <0.001), whilst the rate of 8-cell stage at the same time was significantly higher in control group (27.5%) than in Taxol treated group (5.9 %) (P <0.001).
Conclusion: Mouse embryos development to morula stage takes about 72 h *in vivo*, but IVF reduces cleavage rate and total cell number .Taxol treatment of late 2-cell IVF embryos helps to a more natural developmental rate toward morula.
Keywords: Taxol (Paclitaxel), Mouse Embryos, Morula, IVF

P-202: Gene Expression Analysis Of MMP26 in Women with Preeclampsia Using Cell Free Fetal RNA in Maternal Serum.

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Background: Nucleic acid fragments released from the placenta into the mother's circulation provide a rich source of novel biomarkers for the prediction of pregnancy complications. Preeclampsia that affects 5 to 10% of pregnant women worldwide, is a pregnancy complication which is a major contributor to the maternal and neonatal mortality and morbidity. It has been suggested altered placental expression of matrix metalloproteinases (MMPs) may cause shallow cytotrophoblastic invasion and incomplete remodeling of the spiral arteries and ultimately lead to preeclampsia. Our aim was evaluation of MMP26 gene in preeclamptic women compared with normal pregnant women.
Materials and Methods: 10 ml Peripheral blood samples were obtained from pregnancies with PE (28-32 week, and Gestational age-matched controls. Plasma was isolated and cell free fetal RNA extraction from blood was performed using the QIAamp Circulating Nucleic Acid Kit. Reverse transcription of Cff-RNA was performed using VILO SuperScript kit. The expression of MMP26 gene were evaluated with Real-time PCR method.
Results: Our results have shown that expression level of MMP26 gene was higher in comparing control group. But the expression of MMP26 was not statistically significant, and probably the reason is low number of samples.
Conclusion: MMP26 RNA level in plasma of preeclamptic women was higher but not significant compared to healthy women.
Keywords: Preeclampsia, Cell Free Fetal RNA, MMP26

P-203: Generation of High Expressing CHO Cell Line for The Production of Recombinant Luteinizing Hormone Using Human Serum Albumin Signal Peptide and Codon Usage Strategy

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Background: Luteinising hormone (LH), is a gonadotrophic hormone that plays a crucial role in regulating the function of the testis and ovary organs. Up to day, this hormone have produced in different ways and also in various cell lines. Chinese hamster ovary (CHO) cells, are the most commonly used mammalian hosts for recombinant proteins production. In this study, in order to expression optimization of recombinant LH (rLH) protein, the human serum albumin (HSA) signal peptide and codon usage strategy were used.
Materials and Methods: Firstly, the beta subunit of LH hormone was changed based on codon us¬age in CHO host. The synthesized gene in the vector PGEM-B1 was purchased and cloned into the expression vector pOptiVEC by PCR. The alpha subunit was already present in this vector. Also, the gene innate signal peptide is replaced by the HSA signal peptide. In continuous, recombinant expression vector will be linearized and transfected into the CHO-DG44 cells. Finally, rLH protein ex-pression was analysed by Bradford's technique, SDS page, Western blotting and ELISA.
Results: The construct was confirmed by enzymatic digestion and sequencing.
Conclusion: It is expected that codon optimization and HSA can be more effective for high production of recombinant proteins.
Keywords: Luteinising Hormone, Codon Usage, Human Serum Albumin, Expression Optimization, CHO-DG44

P-204: Evaluation Associated Polymorphism CXCR1 Gene with Postpartum Uterine Diseases in Dairy Cows

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Background: Uterine diseasescaused by bacteria in cows mainlyoccurduring parturition, estrus or by means of artificial insemination. These diseases cause to economical lossesin the herd.Control of environmental health, maternity and …. helps tocontrol of postpartum disease. Breed choosingin some diseases such as mastitis because of low heritability and lack of phenotypical data has not been successfulto reduce rate of disease . So the ability to detect and remove sensitive animals is extremely useful and treatment costs and other costs can be reduced.When an infection in utrineoccures, genetic markers are released and response to inflammation, soaccording to thesese markers we can identify resistant or susceptible cattles.CXCR1 is one of the strongest markers and this marker is a chemokine that is necessary for the migration of neutrophils to the site of the infection. The aim of this study was to determine SNPs from a segment of CXCR1 gene in dairy cows and evaluate relationship between CXCR1 gene and postpartum uterine diseases. In this study blood samples were collected from 10 cows with metritis, 10 cows with endometritis, 10 cows withretained placenta and 10 cows without uterine infection.
Materials and Methods: DNA was extracted and the gene encoding the receptor of interleukin-8 (CXCR1) on a 311 bp segment was identified by RFLP-PCR and polymorphisms of CXCR1 gene in the blood and the relation of this gene with uterine disease was evaluated. The results of enzyme digestion in this studywere that if two bands of 150 bp and 161 bpwereseen it was G allele and if three bands of 150 bp, 111 bp and 50 bpwere seen it was the C allele.
Results: According to the findings of this study and the existence of three bandswith molecular weights of 150bp, 111bp, and 50bp in all samples with uterine disease except samples with retained placenta, an insertionoccured at nucleotide956. All normal samples (controls) onlyhadbands of 161bp and 150bpand there was no evidence of band with the weight of 50 bp. This finding indicates the existence of an incision site occurred by Hinf 1 enzyme and genotype GG.
Conclusion: In this study, samples with postpartum uterine diseases had genotypeGC that indicated changes at the level of CXCR1 gene that caused changes in the expressionof thereceptor of interlukin 8 (CXCR1) anddisrupted the chemotactic ability of IL-8 for inducing neutrophils migration to the infected site, thereforethey were sensitive to the uterine infections. In this study,According to the phenotypicalobservations and molecular tests it seems that a relation exists between postpartum uterine diseases and CXCR1 gene.
Keywords: Metritis, Endometritis, Retained Placenta, CXCR1, Dairy Cattle

P-205: Comparison of Sperm HIF-Alpha between Infertile Men with Varicocele and Fertile

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Background: Varicocele is a testicular vascular disease that causes ischemia, dysfunction of arterial blood flow and hypoxia. In damaged tissues, hypoxia leads to activation of hypoxia-inducible factor-1 alpha(HIF-1 alpha) which is one of the hypoxic pathway markers. There, we aimed to compere HIF-1 alpha between fertile and infertile men with varicocle.
Materials and Methods: Semen samples were collected from 25 fertile and 25 infertile men with varicocele and assessed accordingly WHO-2010 after RNA extraction and cDNA synthesis, HIF1 alpha was assayed using Real -Time PCR.
Results: We observed significant different between fertile and infertile men with varicocele for semen parameters. Expression of HIF-1 alpha was higher in fertile men with varicocele compared to fertile.
Conclusion: The result of this study show that level of HIF-1 is higher in infertile men with varicocel due to pathogenic condition in this individual. However further studies need for confirming this result in the large population.
Keywords: Varicocele, Hypoxia, Hypoxia-Inducible Factor-1 Alpha, Semen Parameter

P-206: Evaluation of Intron-Exon Boundary Sites of 2 Exons in DNAH1 Gene in Infertile Men with Immotile Short Tail sperm Defect

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Background: Immotile short tail sperm (ISTS) defect is a syndrome which causes male infertility. Patients with ISTS defect have immotile short-tailed sperm. It has shown that mutations in DNAH1 gene, which encodes an inner arm heavy chain dynein, leads to male infertility from the abnormalities of sperm tail. Purpose of this study was to evaluate the genetic variations of intron-exon boundary sites of 2 exons (31 and 74) of DNAH1 in infertile men with ISTS. These regions are consensus splice sites and it has been shown that homozygous mutations in these regions lead to sperm abnormalities.
Materials and Methods: In this study, 30 Iranian infertile men with ISTS defect and 30 Iranian normozospermic men as controls were recruited. It took 3 years to collect patient's samples. Initially, DNA was extracted from peripheral blood, and after PCR reaction and sequencing the results of sequenced segments were analyzed by Finch TV.
Results: Sequence analysis results did not identify any mutations or single-nucleotide polymorphisms (SNPs) in intron-exon boundary sites of these 2 exons in case and control groups.
Conclusion: Although our data revealed no variations, but due to the high expression of DNAH1 gene in human sperm tail and considering the fact that DNAH1 is a big gene and recent studies revealed that mutations in this gene lead to male infertility, evaluation of other exons and regulatory areas of this gene is recommended. On the other hand it is suggested that genetic variations were detected in intron-exon boundary sites of DNAH1 gene in patients with family history of male infertility.
Keywords: DNAH1 Gene, ISTS Defect, Male Infertility

P-207: Altered Incorporation of Chd5 Protein to Chromatin of Testis Tissues of Infertile Men

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Background: Spermatogenesis is a considerable step in male fertility. During the spermatogenesis process, a series of epigenetic events occur. Chromatin remodeling is one of the most important epigenetic events through many developmental and cellular mechanisms. Members of Chromodomain-helicase-DNA binding family (Chd) are involved in chromatin rearrangements, among them, Chd5 is a histone acetyltransferase which hyperacetylates histone H4 and causes to histone removal, expression and replacement of transitional proteins (TNPs) and protamines (PRMs) to condense chromatin. The aim of this study was to investigate the presence of Chd5 protein in the regulatory region of TNPs and PRMs genes.
Materials and Methods: Testicular biopsies were collected from 12 infertile men through assisted reproductive techniques (ART) procedure referred to Royan Institute and consent was obtained from patients according local ethical approval. Based on pathological features these samples distributed into 3 groups: obstructive azoospermia (positive control), complete maturation arrest and Sertoli cell only syndrome (negative control). The qRT-PCR was used to determine the expression of Chd5 gene and quantitation incorporation of Chd5 to the promoter regions of TNPs and PRMs genes were evaluated by ChIP-Real Time PCR.
Results: Our results showed significant decreased in incorporation of Chd5 protein on the promoter regions of TNPs and PRMs genes in complete maturation arrest and Sertoli cell only syndrome groups, compared with the obstructive azoospermia patients.
Conclusion: This study indicates that decreased expression of Chd5 can be associated as a marker for spermatogenesis failure.
Keywords: Chromatin Remodeling, Chd5, Epigenetic, Spermatogenesis.

P-208: Embryo Toxicity Assessment Using Treatment of Human Embryonic Stem Cells with Three Anti-Cancer Drugs (Bortezomib, Lapatinib, Paclitaxel)

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Background: hESC-based systems are used to model certain aspects of development in the human pre-implantation embryo and have the potential to be used for embryo toxicity screening. We aim to estimate safe dosage of 3 anticancer drugs (Bortezomib, Lapatinib and Paclitaxel) for embryonic development. Bortezomib is a novel dipeptide boronic acid and a potent and selective inhibitor of proteasome with antitumor activity. Lapatinib is a dual receptor tyrosine kinase inhibitor for ErbB1 and ErbB2. Paclitaxel is a well-known drug for cancer treatment which acts as a microtubule stabilizer, so it probably interferes with chromosomal segregation during cell division.
Materials and Methods: A chromosomally normal hESC line, RH6, was cultured in feeder free condition and treated with 0.1, 0.05, 0.01, 0.005 μM of Bortezomib and 0.5, 0.2, 0.1, 0.05 μM of Lapatinib for 24 hours. Paclitaxel treatments were performed for 24, 26, 28 and 30 hours with 0.01 μM concentration. Cell viability using Fluorescence-Activated Cell Sorting was evaluated by Propidium Iodide assay.
Results: When cells treated with 0.01 μM of Bortezomib and 0.2 μM of Lapatinib for 24 hours there was no significant difference between the treatment and the control groups while the viability of the cells decreased with increasing in concentration of these drugs. Paclitaxel treatment for 28 hours showed the most viability with no significant difference from control. Shorter and longer exposure periods were cytotoxic.
Conclusion: Regarding the time dependent effect of Paclitaxel and its possible hazardous effects on the cells during different phases of cell cycle, it must not be administrated during pregnancy.According to our preliminary findings 0.01 μM of Bortezomib and 0.2 μM of Lapatinib seem to be safe for embryo development. More assessments including pluripotency potential of treated hESC is under investigation.
Keywords: Human Embryonic Stem Cells, Bortezomib, Lapatinib, Paclitaxel, Cytotoxicity

O-209: Improving The Preimplantation Devel-

opment of *In Vitro* Fertilized Mouse Embryos following Lapatinib Treatment

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Background: One of the major reasons for implantation failure and spontaneous abortion of embryos obtained from IVF is high incidence of chromosomal abnormalities in preimplantation development that mostly are diploid-aneuploid mosaic. Lapatinib (GW572016), a potent ATP-competitive inhibitor that simultaneously inhibits both EGFR and HER2, leads to marked inhibition of cell division with subsequent apoptosis. We hypothesize more sensitivity of aneuploid cells to lapatinib according to reports for more sensitivity of aneuploid cells to some anticancer drugs. To explore the effect of lapatinib on preimplantation embryo development, we evaluate the effect of lapatinib in mouse embryos obtained from IVF.
Materials and Methods: To determine the nontoxic dose of drug, 132 late two-cell mouse embryos were included in control group and treatment with 0.02, 0.05, 0.1, 0.2, 0.5 μM of lapatinib for 12 and 24 hours. To investigate the effect of drug on preimplantation embryo development, lapatinib treatment with selected nontoxic condition was performed in two separate experiments. In the first one 88 embryos were included, 45 treated with lapatinib and 43 as controls; 24h after drug removal developmental rate were studied. Regarding exclusion of these embryos for molecular evaluation, in the second experiment, 44 embryos were equally included in treatment and control groups; 48h after drug removal developmental rate were studied.
Results: In terms of embryo survival, 0.2 μM for 24h was determined as appropriate treatment condition for further evaluations. In the first experiment, development to 8-cell stage in treatment group was significantly higher than controls (40% versus 18.6%, $P<0.05$). In the second experiment, 50% of treated embryos reached to morula compared with 18.8% for controls ($P<0.05$).
Conclusion: The treatment of late two-cell mouse embryos with 0.2 μM of lapatinib for 24 h improved the growth and development rate of IVF embryos in comparison to control. Cytogenetic and mechanistic investigations are ongoing.
Keywords: IVF, Lapatinib, Preimplantation, Morula

P-210: Efficacy of N-acetyl Cysteine on Implantation by Effect on HOXA9 Gene Expression: A Double Blinded Randomized Placebo Controlled Trial

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Background: N-acetyl cysteine (NAC) as a nutritional supplement is a precursor of glutathione-biosynthesis, therefore it has been greatly applied as an *in vivo* and *in vitro* antioxidant. Recently, studies showed that co-administration of folic acid and NAC can improve pregnancy and ovulation rates in infertile patients. On the other hands, high expression of HOXA9 can be result in successful implantation rates. Therefore, we evaluated the expression of this gene in NAC administration during *in vitro* fertilization (IVF) cycle.
Materials and Methods: A single center, double blinded, placebo controlled, randomized trial study was performed with 30 women (age: 22-40) with at least two repeated implantation failure (RIF) history. Subjects received either NAC or placebo with both effervescent tablets having similar color, size and appearance. Expression of HOXA9 was evaluated on the day of Window of Implantation (WOI) by biopsies from endometrium. Thirty RIF patients were randomized in two groups of A and B to receive NAC 1200 mg/day or placebo, respectively for at-least 9 weeks before starting ovarian stimulation. They donated their tissue (Pipelled based biopsy from endometrium) on specific day (19-21) of their cycle. Then patients were undergone ovarian stimulation (using NAC) ended to IVF. Total RNA-extraction and cDNA synthesis were performed from tissue samples, then the gene expression was evaluated by using Real Time PCR.
Results: Our results have shown that expression level of HOXA9 gene was different in comparing both groups on the day of WOI. The expression of HOXA9 was statistically significant (P value= 0.025).
Conclusion: There is a significant difference in expression of HOXA9 in comprising two NAC supplementation and placebo groups during IVF protocols for RIF patients.
Keywords: Implantation, Recurrent Implantation Failure, N-acetyl Cysteine, HOXA9

P-211: Study of Genetic Alterations in Transcription Factor Binding Site of SPATA33 in Azoospermic Men Referred to Royan Institute

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Background: Male infertility affects >20 million men worldwide and represents a major health concern. Male factors account for about 40% of infertility in the world. Genetic mechanisms have been considered as a significant cause of some cases of male infertility. A novel gene SPATA33(Spermatogenesis associated 33), is located on chromosome16q24.3. SPATA33 was mainly expressed in the postpartum and adult mouse testes. Its increased expression during the first wave of the spermatogenesis, indicated that SPATA33 may be associated with the meiotic process. The Present study aimed to determine the association between the alterations in transcription factor binding site of SPATA33 gene and azoospermia.
Materials and Methods: In this study, a total of 50 Iranian non obstructive azoospermic(NOA) men were considered as case group. Control group comprised 50 fertile men who had at least one child. Nucleotide variations were analyzed in both case and control groups by standard PCR sequencing technique.
Results: PCR product sequencing results illustrated a single nucleo-

tide variation with rs112536073 in both cases and control groups. Additionally a nucleotide variation with rs459920 detected in 33 case and was also observed in 20 control samples. The frequency of this variation is significantly (P<0.05) higher in NOA patients compared to control group.
Conclusion: This is the first report on transcription factor binding site of SPATA33 gene investigation in male infertility. Although the variation (rs112536073) seems to be a more frequent variant in our population, our result suggest that the single nucleotide polymorphism (rs459920) can be assumed as a contributing factor for male infertility
Keywords: Azoospermia, SPATA33, Spermatogenesis

P-212: High Expression of Human Chorionic Gonadotropin Genein CHO DG44 Cellsby HAS Signal Peptide and Fc Fusion Tag Methods

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Background: Human chorionic gonadotropin (hCG) is a glycoprotein produced by the embryo after implantation to increases the maintenance of the corpus luteum. It is made of 2 subunits as α (alpha), and β (beta) subunits. As well as the urine of pregnant women as its main source, also it can produce by cultures of cells like CHO (Chinese Hamster Ovary) dhfr minus DG44 using genetic engineering. Applying of fusion tags such as Fc tag that is fused to the carboxyl-terminus of protein of interest, dramatically effect on whole expression, stability and straight means for single-step purification of proteins. In this study the effect of HAS (human serum albumin) signal peptide, optimization of hCG beta subunit sequence according to CHO expression system, and Fc fusion tag was surveyed on overall expression of hCG.
Materials and Methods: In this study, the hCG signal sequence was replaced by the hSA (Human Serum Albumin) signal peptide sequence, the sequence of hCG beta subunit codon optimized and the Fc sequence added at 3' end of alpha and beta subunit and finally the recombinant gene construct was cloned in poptivec modified vector as a host and expression vector.
Results: The exact sequence of final construct was confirmed by digestion and sequencing methods.
Conclusion: Here, we have brought up the strategy that make it possible to extreme secreted protein quantity in a facile manner, including Fc fusion tag. Additionally, with precise design of the expression vector and optimization of parameters including signal peptide, codon optimization, cell type, and media, sturdy overexpression can be readily obtained for most secreted proteins.
Keywords: CHO DG44, Fc Tag, Signal Peptide, Codon Optimization

P-213: Expression Profile of SEPTIN and TUBULIN Gene Families in Testes of Men with Non-Obstructive Azoospermia

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Objective: Alteration in genes involved in spermatogenesis, like Septins, lead to infertility in men. Septins belong to a conserved family of cytoskeletal proteins with GTPase activity that take part in cell polarity, morphogenesis, compartmentalization, cell cycle progression, and vesicle trafficking by interacting with several cytoskeletal proteins. In humans, sperms with abnormal expression patterns of SEPT4,6,7 and 12 are more prevalent in infertile men. Although it has been shown that SEPT12 forms a filamentous structure with SEPT7,6,2 and 4 at the sperm annulus. During spermatogenesis, SEPT12 interacts with α- and ß-tubulins forming SEPT12-microtubule complexes which is critical for sperm structure and function, and its reduced expression disrupts the organization of α-/ß-tubulins. Also, SEPT12 is colocalized with SPAG4 near the nuclear periphery in round spermatids and in the centrosome region in elongating spermatids.
Materials and Methods: We analyzed 2 microarray libraries of infertile men obtained from Gene Expression Omnibus (GEO). GEO2R software were used for the analysis of microarray. After applying t-test, we extracted the differential gene expressions of each infertile cases against normal group (p-Value<0.05). Analysis applied on azoospermia patient include Meiotic Arrest(MEI), post meiotic arrest, pre meiotic arrest(PRE) and Sertoli Cell Only Syndrome (SCOS). A heatmap of microarray results using Multiple Array Viewer for SEPT and Tubulin families and LaminB1, SPAG4 transcripts, was drawn.
Results: The expression of the SEPT7 in MEI, PRE and SCOS patients were significantly higher compared to control group; while the expression of the SEPT12, Tubulin α8, Tubulin β 4B in MEI, PRE, SCOS patients, and Tubulin α3 in SCOS patients and the expression of SEPT4, SPAG4 and Tubulin β3 in all patient groups were considerably downregulated.
Key words: Male infertility, SEPT, Septins, Tubulin, Azoospermia

P-214: Evaluation of Genetic Variations in Exon 7 of SEPTIN12 Gene in Infertile Men with Immotile Short Tail Sperm Defect

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Background: The immotile short tail sperm defect (ISTS) as a kind of teratozoospermia, is an autosomal recessive disease that severely affects axonemal complex and sperm flagella accessory structures. SEPTIN12 is a potential sterile gene in human which belongs to a family of polymerizing GTP-binding proteins and is critical for sperm normal development. SEPT12-microtubule complexes are required for sperm head and tail formation. Mutations and genetic variants of SEPTIN12 have been observed in teratozoospermic men with distinctive sperm pathology including defective sperm tail elongation. In the current study we investigated exon7 of SEPTIN12 gene variants in infertile men with ISTS defect.
Materials and Methods: In this study 30 infertile men with ISTS (above 60%) and 30 normozoospermic men as control group were recruited. To investigate genetic variations, DNA was extracted from peripheral blood samples using salting out method and mutational

analysis was performed by direct sequencing of exon 7.
Results: No mutations or single-nucleotide polymorphisms (SNPs) were found in exon 7 of SEPTIN12 gene in case and control groups, however an intronic variant (rs: 7201715, T>C), was observed within intron 7-8 in homozygote form in 2 patients with ISTS. This variation was also identified in 3 normozoospermic men heterozygously.
Conclusion: Although obtained data indicated no association between exon 7 of SEPTIN12 gene variations and ISTS abnormality, because of SEPTIN12 essential role in sperm tail development, evaluation of other exons of this gene is recommended. Moreover, the T>C intronic variation is located within the binding site of hnRNP K splicing factor in pre-mRNA. Thus bioinformatics analysis would reveal the possible impact of this variation on splicing process.
Keywords: ISTS, SEPTIN12, Genetic Variation

P-215: Early Detection of Cancer Using Circulating microRNAs in Peripheral Blood by Bioinformatics Approaches

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Background: MicroRNAs (miRNAs) are a class of non-coding RNAs that regulate many cellular processes including tumorigenesis. Circulating miRNAs are known as novel and less invasive markers in many malignancies such as cancer. Recent studies have shown that some specific miRNAs are deregulated in blood of early stage cancer patients compared to healthy controls. In this study, we aim to design subsets of circulating miRNAs can detect each type of cancer from unaffected controls and other types of cancers with high accuracy.
Materials and Methods: We used miRNA expression profiles from the cancer genome atlas (TCGA) and analyzed 6104 next-generation sequencing (NGS) data related to 14 different types of cancer tissues encompassing 5493 cancer samples and 611 healthy controls. We were using feature selection algorithm and support vector machine with 10 fold cross validation as machine learning method for improving detection accuracy. After that we intersected results with available breast cancer blood miRNome data.
Results: By focusing on five miRNAs, we could separate all cancer samples from all normal samples with 97% accuracy. We obtained subsets with maximum 5 members and also acceptable accuracy for each cancer type. The highest accuracy received for thyroid carcinoma (98%) and kidney renal clear cell carcinoma (97%) with subset of three and two miRNAs, respectively. We also could classify samples in 3 classes (breast invasive carcinoma, normal breast tissue and all other normal and cancer tissues) just with 3 miRNAs.
Conclusion: Using these bioinformatics approach we identified various subsets of miRNAs that could distinguish every type of cancer from unaffected controls. These subsets have potential to be evaluated in blood samples of each cancer type. But large prospective studies are needed to validate their true value in a particular clinical scenario.
Keywords: Cancer Detection, microRNA, Bioinformatics Approach

P-216: The Effect of CHD5 Gene Expression during Spermatogenesis in Infertile Men

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Background: The genetic causes of spermatogenetic failure still remain largely unknown. It has been estimated that more than 2300 genes play a role in spermatogenesis. Mutations in each of these genes could theoretically cause male infertility. Infertility caused by spermatogenesis defects can be based on failure of spermiation, partial failure in chromatin condensation, abnormal sperm head morphology, immotility of epididymal sperm. CHD5 acts as a master regulator of the histone-to-protamine chromatin remodeling process during spermiogenesis. In this study we aimed to evaluate the expression of CHD5 in different group of infertile men with spermatogenesis defects
Materials and Methods: To examine the expression of CHD5 in testis biopsies, of azoospermic or oligospermic infertile men [hypospermatogenesis as control group (n=10), maturation arrest (n=10), sertoli cell only syndrome (n=10) and oligoasthenoteratoazoospermia (n=4)] who referred to Royan institute. we carried out real-time, quantitative reverse transcriptase Real Time-PCR (RT-PCR) with synthesized cDNAs made from extracted RNA of testis biopsies different groups.
Results: Infertile men with spermatogenic defects shown lower CHD5 expression compare to controls. We also observed the clinical grade of spermatogenesis failure was correlated inversely with CHD5 expression.
Conclusion: In this study, we shown CHD5 mediates a cascade of molecular events underlying histone removal during spermiogenesis. CHD5 deficiency leads to disruption of these biological processes and subsequently we assume to increase histone retention in both spermatids and sperms.
Keywords: Spermiogenesis, CHD5, Chromatin Remodeling, Infertile Men

P-217: Decreased Incorporation of Human Testis/Sperm-Specific H2B (hTSH2B) on Chromatin of Testis Tissues of Men with Spermatogenesis Failure

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Background: Chromatin remodeling is one of the most interesting events through germ cell production and early embryo development. During mammalian spermatogenesis somatic histones are partly replaced by testis-specific histones, transition proteins (TNPs) and finally by protamines (PRMs). The human histone H2B variant TSH2B is highly expressed in testis and plays important function in the chromatin transition during spermatogenesis. This histone variant is 85% homologous to somatic H2B that has been detected in only ~30% of mature spermatozoa. The aim of this study was to investigate the incorporation of TSH2B variant on regulatory regions of TNPs and PRMs genes.
Materials and Methods: After evaluation of the expression of TSH2B variant in previous study we decided to investigate epigenetic role of this histone variant in regulation of chromatin condensing genes of TNPs and PRMs. for this respect consent was obtained from infertile men referred to Royan Institute, according local ethical approval and then testis tissue samples were collected from three groups including complete maturation arrest, sertoli cell only syndrome, and hypospermatogenesis (as positive control), based on pathological features. Chromatin immunopercipitation (ChIP) coupled with real time-PCR was performed to evaluate the incorporation of TSH2B into regulatory regions of TNPs and PRMs genes.
Results: Our findings revealed decreased incorporation of TSH2B into regulatory regions of mentioned genes in the groups with spermatogenesis failure vs. positive control.
Conclusion: This study implies that disruption of TSH2B incorporation in chromatin is associated with spermatogenesis failure.
Keywords: Male Infertility, Epigenetic, Spermatogenesis, Chromatin Remodeling, TSH2B

P-218: Designing A Panel of Circulating MicroRNAs for Early Detection of Breast Cancer

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Background: MicroRNAs are a new class of small non-coding RNA that have a regulatory role in the post-transcriptional level of gene expression. Aberrant expression of miRNA is related to many diseases such as cancers so it can be used as a biomarker for diagnosis and prognosis of cancer. Circulating microRNAs are stable in body fluids such as blood, serum, plasma and etc. We investigated to find the best group of candidate microRNAs and designing a panel for early detection of breast cancer.
Materials and Methods: We analyzed miRNome data from the cancer genome atlas (TCGA) with a computational approach. In the first step we analyzed 6104 samples of 14 different type of cancers and normal tissues, and then differential expression between 778 Breast cancer samples, 87 control samples and all other cancer and normal samples were done. We also performed differential expression between candidate list with 105 Microarray circulating miRNA data set from invasive ductal carcinoma to validate candidate list.
Results: We found that miRNAs can separate normal samples from their tumors so miRNA can be a good biomarker. The candidate list with a threshold of adjusted P values less than 0.001 and absolute log fold changes more than one were selected. A list of 54 differentially expressed miRNAs can separate Breast Cancer from all normal tissues with a good P value. As regards 16 out of 54 are significantly deregulated in all cancers, we focused on just other 38 miRNAs by consideration of different features and then compared this list with circulating miRNAs data set to achieve the final candidate list of miRNAs consist of 17 miRNAs for early detection of breast cancer.
Conclusion: Although miRNA profiling in whole blood is a potential biomarker for early detection of breast cancer, clinical validation of these new candidate biomarkers in wet lab is necessary.
Keywords: MicroRNA, Biomarker, Breast Cancer, Early Detection, Bioinformatics

P-219: Expression Pattern of Apelin Gene in Reproductive Tissues of Rat during The Pregnancy

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Background: Apelin is an adipokine that plays a role in the regulation of many biological functions in mammals including the neuroendocrine, cardiovascular, immune systems, glucose homeostasis and obesity. To date there is no report about the expression pattern of apelin gene in reproductive tissue and its association with sex hormone fluctuation during the pregnancy.
Materials and Methods: Serum and tissue samples were taken from pregnant rats on days 1, 7, 14 and 21 of gestation and also 7 days after parturition. Apelin gene expression was examined in rat reproductive tissues (ovary, uterus, oviduct) and, brain and adipose tissues using real-time PCR and ΔΔCt analysis method. Serum progesterone, steradiol and Apelin were measured using ELISA method.
Results: Apelin gene expression was increased at days 14 and 21 of gestation in ovary, brain and adipose tissues, while it decreased after parturition. Its expression in oviduct and uterus was increased during the pregnancy in a time dependent manner and then decreased after parturition. apelin gene expression pattern was similar to its changes in serum during the pregnancy. apelin gene expression in different tissues had significant positive correlation with estradiol and negative association with progesterone levels.
Conclusion: Based on our results which demonstrated that apelin gene expression changes in reproductive tissues of pregnant rats during the pregnancy in concomitant with change in its serum level we concluded that this hormone may has role in molecular changes of reproductive tissues during the pregnancy.
Keywords: Apelin, Reproductive Tissues, Rat, Pregnancy

P-220: Altered Gene Expression of Bone Morphogenetic Protein Receptor 1B (BMPR1B) in Granulosa Cells of Women with Polycystic Ovarian Syndrome

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Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorders that affects approximately 6-8% of women in reproductive age. It is the main cause of infertility due to anovulation. Transforming growth factor-β (TGF-β) secreted by oocyte, granulosa and cumulus cells, plays a critical role in normal folliculogenesis, oocyte maturation and female fertility by coupling to their receptors. Bone morphogenetic protein 15(BMP15) is one member of TGFβ family. It showed that altered expression of BMP15 gene was involved in PCOS disorder, as reported in oocyte, granolusa and cumulus cells of such patients. Therefore, we evaluated expression of BMP15 receptors, although we just reported BMPR1B expression in granulosa of PCOS patients in this abstract.
Materials and Methods: Granullosa cells (GCs) were isolated from four PCOS patients and six women with normal folliculogenesis as controls (male factor) who were undergone ovarian stimulation for IVF/ICSI program. GCs were isolated and purified from follicular fluid by using density gradient separation technique. RNA was extracted from GCs by TRIzol and followed to cDNA synthesis. The quantitative expression level of BMPR1B was evaluated by Real- time PCR.
Results: Our results shown the expression of BMPR1B was significantly decreased (5 fold) in PCOS patients compare to control group.
Conclusion: Of essential genes for folliculogenesis and ovarian function is BMP15 and its association with the pathogenesis of PCOS has been reported. BMP15 plays a pivotal role in pre-antral to antral follicle development. Decreased gene expression of BMPR1B may contribute to alteration in downstream genes which involved to oocyte maturation and competence. The results of this study are important for understanding the mechanism of follicular arrest and anovulation in PCOS patients.It will be considered to explore the gene expression of other TGFβ family members and their receptors in future study.
Keywords: Polycystic Ovarian Syndrome, Bone Morphogenetic Protein 15, Bone Morphogenetic Protein Receptor 1B, Gene Expression.

P-221: DNA Methylation of H19 Imprinted Gene and Male Infertility

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Background: Infertility affects 10.9% of all couples of reproductive age, with a male factor being detected in 30–50% of the cases. Proper sperm production is associated with the establishment of an appropriate epigenetic state in developing germ cells. The association between abnormal spermatogenesis and epigenetic disturbances in germ line-specific genes remains to be demonstrated. Imprinted genes are expressed in a parent-of-origin-specific manner and they rise fully methylated from sperm or oocyte depending on the sex. Recent studies have shown the role of DNA methylation in correct spermatogenesis. Studies concerning the associations of aberrant DNA methylation in spermatozoa with idiopathic infertility are not scarce. Having this background, we studied the methylation patterns of H19 imprinted gene in sperm of control and Oligozoospermic patients.
Materials and Methods: We studied the methylation of H19 Imprinted gene and LINE-1 repetetive elements as control, by Combined Bisulfite Restriction Analysis (COBRA). Briefly the digested and undigested bands represent the methylated and un-methylated alleles.
Results: According to our results, the lowest methylation percentage of H19 imprinted gene belongs to 3 out of 23 cases with sperm features under normal range (2 cases Oligoasthenoteratospermia and 1 case Oligoteratospermia). However our results showed that the median of methylation percentage is not statistically significant between case and control group.
Conclusion: Above results suggest that the methylation of imprinted and even non-imprinted genes as a marker for fertility status should be investigated with more participants in next studies.
Keywords: DNA Methylation, Male Infertility, Genomic Imprinting

P-222: Altered Expression and Function of BRDT as Transcription Factor in Testis Tissues of under ART Infertile Men Referred to ROYAN Institute

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Background: Spermiogenesis stage of spermatogenesis is including to many epigenetics events leading to sperm chromatin condensation. Approximately, 85% of DNA in human male gamete is packaged into nucleoprotamine and 15% of it is remained as nucleohistone. At post-meiotic stages, a global chromatin hyperacetylation gives the signal for BRDT's first bromodomain to direct the genome-wide replacement of histones by transition proteins. BRDT binds via its two bromodomains to acetylated histones at the promoters of specific meiotic and post-meiotic genes and facilitates their activation at the appropriate time. The aim of this study was to evaluate the expression of BRDT gene and incorporation of BRDT protein in promoter regions of TNP1, TNP2, PRM1 and PRM2 in sticular biopsies of infertile men with spermatogenesis failure.
Materials and Methods: The study was included total of 45 infertile men who referred to ROYAN Institute. This samples categorized into 3 groups: obstractive azoospermia (positive control), complete maturation arrest at second spermatocyte/spermatid level and Sertoli cell only syndrome (negative control). Samples of testicular tissues extracted by TESE/micro TESE procedures performed in an attempt to obtain sperm, also for this respect, consent was obtained from participants. Quantitative mRNA expression of BRDT was performed by real-time PCR. Furthermore, incorporation of BRDT on the regulatory regions of TNP1, TNP2, PRM1 and PRM2 was evaluated by ChIP-real time PCR.
Results: Statistical analysis showed that BRDT gene expression was significantly higher in obstractive azoospermia group compared to complete maturation arrest and Sertoli cell only syndrome groups ($p \leq 0.05$). The incorporation of BRDT in regulatory regions of TNP1, TNP2, PRM1 and PRM2 in complete maturation arrest was signifi-

cantly highen than the other groups.
Conclusion: The finding in this study declared an association between altered expression/ incorporation of BRDT and spermatogenesis failure and male infertility.
Keywords: Spermatogenesis, Male Infertility, Epigenetic, BRDT Gene

P-223: Comprehensive Gene Expression Profiling and Gene Ontology Analysis of Male Infertility in Human and Mouse

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Background: Ten to fifteen percent of couples face infertility after 1 year of unprotected sexual intercourse which, in almost %50 of cases, is related to male factors. Genetic factor is the cause of 15-30% of male infertility and 15-25% of infertile men consider of idiopathic and also semen parameters of 40% of infertile men are normal. Gene expression profile study can lead to identify the gene expression patterns, genetics interactions and new markers. Gene ontology (GO) and genetic network analysis can find new functions and relations in genes.
Materials and Methods: We analyzed 8 microarray libraries of human male infertility and 6 libraries of mouse with a gene knockout involved in infertility were downloaded from Gene Expression Omnibus (GEO). Affymetrix and GEO2R software were used for the analysis of microarray. After applying t-test, we extracted the differential expression genes of each infertile cases against normal groups ($P<0.01$). The comparison of common genes, GO, KEGG pathway and Gene Set Enrichment Analysis applied on three classes include of: (i) azoospermia, oligozoospermia and teratospermia, (ii) meiotic arrest(MA), post meiotic arrest(PMA) and sertoli cell only syndrome(SCOS) and (iii) human and mouse.
Results: The maximum similarity was between azoospermia and oligozoospermia and the most common biological process was immune response. In common downregulated genes between MA, PMA and SCOS we revealed 14 genes (ODF1, OAZ3, FSCN3, GGN, ADAM29, ACTL7A, CCDC153, GSG1, CC1N, TPPP2 and ABHD1) were involved in spermatogenesis. We achieved 38 differential expression genes with meiosis function in azoospermia cases.
Conclusion: This is a bioinformatic study on transcriptome level, and these results need to confirm by experimental assay like real-time PCR, to find a biomarker for male infertility and also several alteration can occur in the post transcription level, which should identify by some techniques such as western blot and ELISA.
Keywords: Male Infertility, Bioinformatics, Microarray, Gene Expression Profiling, Gene Ontology

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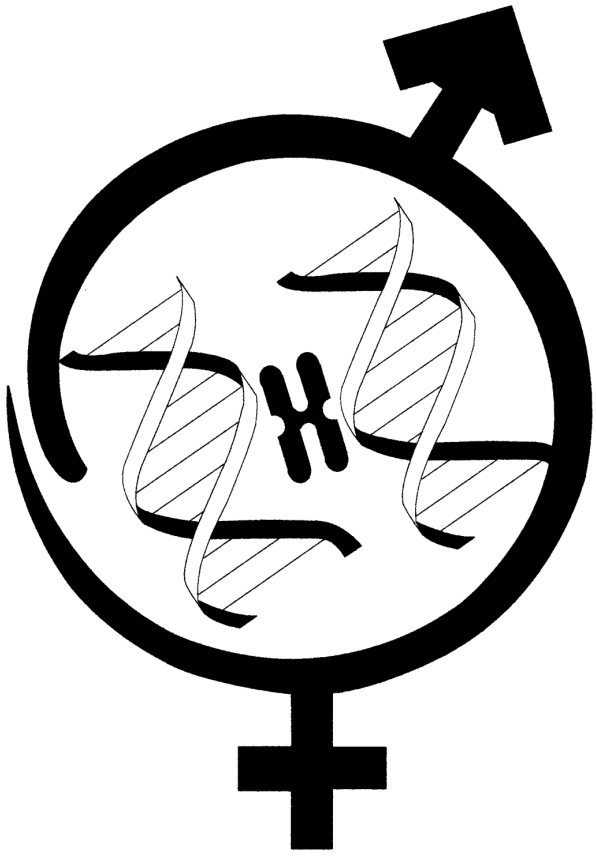

Royan Institute

Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

Invited Speakers

I_{nm}-1: Iran IVF Midwives Questionnaire

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Background: The objective of this market research study was to evaluate "the patient-learning and nurses/midwives -teaching experience when using Gonal-f Pen for infertility treatment".
Material and Methods: This prospective, observational study was conducted across more than 30 Iran infertility clinics. Following instruction, patients and nurses/midwives used the redesigned Gonal-f pen and completed questionnaires to evaluate their experiences about the device after the simulated injections. Data from nurses/midwives questionnaires are presented using descriptive statistics (SPSS Dimensions). Scales rating key performance indicators used a 5-point scale where 5 was the best and 1 was the worst possible outcome.
Result: A total of 1591 questionnaires were included in the study. Of these, 810 reported that they had previous experience of IVF. Significantly more patients reported that less training was required to use the prefilled Gonal-f pen than a syringe and lyophilized powder with very high satisfaction level (49%). Significantly more patients rated the prefilled Gonal-f pen as easier in terms of use (49-51%) compared with their current method. Most (51%) patients "strongly/somewhat agreed" that they were comfortable with the number of steps involved in preparing and giving the injection, and 50% believed that using the new pen device would ensure that they set the correct dose and administered it in full when they self-injected at home. 52% of Midwives considered the redesigned pen easy to learn and believed it would be easy to teach patients how to use.
Conclusion: In this market research study with infertile patients and infertility Midwives, the redesigned Gonal-f pen was perceived as easy to learn, easy to teach how to use, and well accepted.
Keywords: Infertility, Gonadotropin, Gonal-F Pen, Prefilled Pen Device

I_{nm}-2: Human Ovarian Tissue Banking and Fertility Preservation

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There is a concerning increase in cancer diagnosis according to the Iranian Cancer Society. Today, due to medical advances, many cancers are treatable with timely diagnosis and follow up. Therefore, many cancers are no longer considered as incurable. Although, in many cases, chemotherapy and radiotherapy aim to save lives, premature ovarian failure and reduction of follicular reserve is undeniable. By taking into consideration the probable infertility of cancer patients, preservation of their reproductive ability prior to onset of cancer treatment is crucial. Different methods of assisted reproductive techniques that include oocyte, embryo and ovarian tissue cryopreservation have helped these patients. The most appropriate technique is selected according to the patients' circumstances. Ovarian tissue cryopreservation technique has a long history of use, in this technique numerous follicles at different stages of maturity are preserved without delays to cancer treatment. In addition, for single or young girls this is the best choice to preserve their reproductive ability. From December 2000 until 2010, the researchers at Royan Institute conducted a wide range of investigations on ovarian tissue cryopreservation with the intent to provide fertility pres¬ervation to cancer patients that were considered to be candidates for these services. In 2010, Royan Institute established the Royan Human Ovarian Tissue Bank as a subgroup of the Embryology Department. Since its inception, approximately 180 patients between the ages of 7- 47 years have undergone consultations. Ovarian samples were cryopreserved from 50 patients (age: 7-35 years) diagnosed with cervical adenocarcinoma, breast carcinoma, Ewing's sarcoma, opposite side ovarian tumor, endometrial adenocarcinoma, malignant colon tumors, as well as Hodgkin's lymphoma, major thalassemia and acute lymphoblastic leukemia.

I_{nm}-3: Fertility Preservation for Non-medical Reasons

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As a real, female fecundity decreases in the middle of the third decade. Ovarian ageing causes a progressive decline in pool of primordial follicles, which can lead to menopause as a result of quantitative decline, and apart from this, decline in the quality of oocytes. Advances in reproductive medical and biology, oocytes can be collected by gonadotropin stimulation with minimal risk (<1%) of OHSS and then unfertilized oocytes can be cryopreserved. In recent years ART techniques have been used for individuals undergoing chemotherapy or radiotherapy, now it is a new way to use this procedure for non-medical aspects. This kind of intervention- often named to as "social freezing" is complete different dimension than medically indicated cryopreservation or "medical freezing", we should aware of complicated pregnancy after age 40 (such as: gestational diabetes & pre-eclampsia and IVF may be associated with a higher risk of epigenetic abnormalities, therefore this precedune should be critically discussed, and finally decisions should be made on a case-by case basis.
Keywords: Fertility Preservation/ethics, Non-Medical Reasons

I_{nm}-4: Ethical Issues in Fertility Preservation

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Recent developments in cryobiology including vitrification have given us the opportunity to preserve the oocytes, ovarian and testicular tissue. These techniques together with sperm and embryo cryopreservation brought a new era entitled: "fertility preservation" (FP). It is defined as preserving the reproduction ability to use in the future when is the right time for the client. This procedure can be devided into two types: 1) for medical resons mostly in patients with cancer and 2) for non medical reasons which can be called: "social fertility preservation".
FP for medical reasons: Cancer treatments like chemotherapy or radiotherapy destroy reproductive tissue so may reduce fertility or even make the patient infertile. So, it is advisable for young people to do FP if they want to have a child in the future. The most important ethical point here is the duty of the physician to give the apropriate information to the patients. Oncologist is ethically and even in some

countries legally obligated to provide information about fertility preservation for the patients and refer them to a FP center. If they don't maybe the patient becomes infertile and the oncologist is responsible for this complication. Other ethical points are: 1. Possibility of transmission of cancer cells to the offspring, 2. Low life expectancy after cancer treatment and possibly FP becomes useless in some cancers, 3. Resource allocation and support of the FP costs by health care system, 4. Informed consent specially in minors considering better outcome of some cancers in children but difficult FP procedures in them, 4. Gamete and embryo disposition and consent for the fate of them and 5. Posthumous reproduction and posthumous donation.

There are ethical issues for every FP method. For embryo cryopreservation: It is used for matures who already are couples and their cancer is not too invasive so, the patient has the time for doing one or two in-vitro fertilization IVF cycle and also the cancer is not sensitive to hormones because most of the IVF medications are hormonal. Under these condition it is the most promising method. For sperm cryopreservation: It is used for mature men who are currently single and want to save the sperm for future use. This method is also promissing. Oocyte cryopreservation is used for mature women who are currently single and their cancer is not too invasive so, the patient has the time for doing one or two IVF cycle because the IVF procedure for oocyte retrieval procedure is same for single women. The cancer must not be sensitive to hormons. For immature males instead of sperm, testicular tissue cryopreservation is used which is in the research phase and not too promissing. For these patients, informed consent should be taken from their guardians with informing the patients as far as they understand. Ovarian tissue cryopreservation is used for immature females or patient whose cancer is too invasive cancers not permitting IVF cycle or are sensitive to hormones. This procedure is also in research phase and not too promissing for the patient.

FP for social reasons: used for women who want to postpone their pregnancy for social reasons like not finding the apropriate partner or socio echonomic reasons. The age of pregnancy is increasing and this means people are willing to get pregnant in advanced ages that fertility declines in those ages. Social FP brings increased gender equality; increased control over reproductive destiny; more time to find a suitable partner, to complete education and to achieve financial and psychological stability before embarking on parenthood. The problem is to inform people or not. It seems that people have a right to know about possibility to freeze oocytes and do FP but some argue that it may change the trend of the society and the age of marriage increases to older ages. But if there is no information about FP, most of the FP clients will be old women with no ovarian reserve that the best stimulation protocols can not retrieve any oocyte with acceptable quality. Misguides must be prevented. Even the fertility preservation name can be a misguide because we only do the cryopreservation and maybe no pregnancy happens with the frozen embryos or gametes. Detail counselling should be provided and specific tests to be offered to the candidates befor starting the protocol. Age limit of 35 years seems to be a good limitation and women under 35 should give the chance to themselves to be pregnant naturally.

Keywords: Fertility Preservation, Cryopreservation, Ethics, Law, Social

I_{nm}-5: Lifestyle and Female Factor Infertility

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Most studies report a BMI greater than 27kg/m² and a BMI less than 17kg/m² is associated with increased ovulatory dysfunction and resultant infertility. For women with an elevated BMI, subfertility appears to be related to insulin resistance leading to insulin excess. Hyperinsulinemia may lead to androgen excess by reducing sex hormone-binding globulin synthesis, thereby increasing free testosterone, and by stimulating ovarian androgen production rates. Excess androgen, in turn, is a major factor leading to altered hypothalamic-pituitary and ovarian physiology and anovulation. Obesity-associated hyperleptinemia may be an additional factor involved in anovulation, not only through the induction of insulin resistance, but also through direct impairment of ovarian function. Inadequate levels of exercise associated with obesity may be a more common cause of anovulation and subsequent infertility than exercise-associated anovulation. Vigorous/intense physical activity was associated with ovulatory infertility. Other causes of decreased female fecondability are tobacco use, heavy alcohol intake (≥14 drink per week), stress and anxiety, but no clinical trial has demonstrated definitively that reducing stress prior to infertility treatment improves pregnancy rates.

I_{nm}-6: Cord Blood Banking: from The Beginning to The Present

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The use of umbilical cord blood stem cells has increased significantly in the past 20 years. Today, doctors all over the world are realizing the power of newborn stem cells and recommending family banking to their patients.

When faced with the illness of a child or loved one, families want hope and doctors want options. Banking newborn stem cells provides both.

Doctors recommend banking for a variety of reasons. Umbilical cord blood is used today to treat many life-threatening diseases including leukemia, certain other cancers and blood, immune and metabolic disorders. And using your own family's cord blood can have significant advantages in treatments, including accessibility, declining GVHD, fewer complications and improved medical outcomes. Stem cells are ushering in an age of regenerative medicine. They are all over the news, because they will transform the next 50 years of medicine in ways that are now inconceivable. In coming decades, stem cells will be reversing diseases ranging from Alzheimer's to autism, re-growing organs and tissues.

Emerging the Cord Blood Stem Cells Banks has significantly provided and increased enjoying these sells in curing diseases seemed lethal previously. This has turned the use of these cells in a vast variety of options as the best alternative, regardless the limited number of the cells found in a unit of blood which was previous believed to be merely applicable for the children. But nowadays, applying 2 or even 3 blood units of cord blood can be applied in adults' stem cells transplantation, in case they have been found of proper match in their genetic structure. That is why today we witness an increase in the number of stem cells transplantations among adults compared to those of children's.

At present, there have been more than one millions of cord blood samples banked in various cord blood banks all around the world and more than 30,000 transplantations have been performed taking advantage of these stem cells.

Oral Presentations

O_{nm}-1: Effect of Garlic on Dysmenorrhea in Women with Endometriosis

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Background: Endometriosis characterized with the proliferation of endometrial glands and stroma outside of pelvic. Endometriosis associated with inflammatory reactions and the formation of fibrotic tissue by symptoms associated with this disease, chronic pelvic pain, dysmenorrhea, dyspareunia, pain during bowel movements and infertility. Surgery and hormone therapy are current treatments for this disease, that they have high recurrence rate and side effects. Due to the side effects of hormone therapy and surgery, and people's willingness to use complementary medicines, the study aimed to investigate the effects of garlic tablet is on dysmenorrhea in women with endometriosis.
Materials and Methods: This study is a triple blind clinical trial .120 women20-45 years that had been referred to the Reproductive Health Center in Emam Khomeini and they have dysmenorrhea associated with endometriosis included to this study. The samples were randomly assigned to two groups. 60women (intervention group) and 60 women (placebo group). Before entering the study, pain intensity was assessed with a Visual Analogue Scale pain (VAS). The score 4 and more were included. Garlic tablets or placebo daily for 12 weeks, the samples were given. A researcher the end of 4 weeks, at the end of 8 weeks, and then at the end of 12 weeks by phone and asked the pain scores on based of VAS. Statistical software SPSS (version 22) was used for data analysis.
Results: The mean scores of two groups in baseline were similar (P> 0.05). The results showed that dysmenorrhea pain scores in the intervention group after the intervention in the 4weeks, 8weeks and the 12weeks decreased significantly (P<0.05). Repeated measurement test showed that changes significantly in the intervention group than in the placebo group (P<0.05). None of the subjects did not mention side effects of the drugs.
Conclusion: Garlic can be used as an effective herbal remedy for problems including inflammation and an imbalance between oxidant and antioxidant factors like endometriosis.
Keywords: Garlic, Endometriosis, Dysmenorrhea

O_{nm}-2: Fetal Choroid Plexus Cyst among Pregnancies following ART: from Diagnosis to Counseling

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Background: The purpose of this study was to specify the outcome of isolated fetal choroid plexus cyst and to clarify its clinical importance in order to provide counseling for prenatal care.
Materials and Methods: This cross sectional study was carried out using the archive of Royan Institute in Tehran, Iran, between April 2009 and December 2012.In this period of time all prenatal sonographies following ART cycles were evaluated by using a computerized database and isolated CPC who was detected in the fetuses were recruited.. Mother serum screening tests, fetal echocardiography and amniocentesis were assessed in patients with isolated CPC until birth. The neonatal outcomes were followed-up by phone call to all individuals.
Results: A total of 6240 pregnancies were assessed and isolated CPC was present in 33 fetuses resulted from ART cycles. Results of double test (n=17), triple test (n=1) and fetal echocardiography (n=8) were normal. Quadruple test result showed 3 abnormal out of 14 cases that all had normal karyotypes. Two samples were dropped out due to preterm birth (PB). We found that outcomes of all remaining fetuses (n=31) were normal and no anomaly was detected until birth.
Conclusion: This study showed that isolated CPC is a benign regressive condition with no clinical significance, especially when mother serum screening is in normal range. Regarding releasing information to parents , they could be informed of definite regression of the cyst by 25-28 th week of gestation .
Keywords: Ultrasonography, ART, Fetus, Anomaly

O_{nm}-3: The Impact of FSH Concentration on The Outcome of ART

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Background: The number and quality of oocyte and embryo play an important role in the art outcomes. There are numerous factorsaffecting oocyte and embryo quality whereas sperm parameters and hormone levels can be considered as the most important of them. Serum folotropin stimulating hormone levels provide a powerful concept for predicting ovarian response. In this regards, there is a direct correlation between the number and quality of oocyte and the higher quality of emberyos. Therefore, "Egg Quality" is associated with the probability of emberyo implantation. This prospective study was conducted to evaluate FSH level as a predictive factor in the success of ART.
Materials and Methods: A total of 206 ICSI patients who were referred to Qom infertility institute were taken into consideration between 2014and2016. They were divided into two groups and their serum FSH level was measured (at day 3 of menstrual cycle).In these two groups, FSH, LH, estradiol (E2) levels and pregnancy rate were also evaluated. All patients had a rather acceptable spermogram
Results: Pregnancyrate was correlated with FSH levels (P < 0.05). FSH concentration in groups 2 (8>ng/ml) showed lower quality oocytes [dark central granulation, aggregation of smooth endoplasmic reticulum (sER)] and had a lower quality embryo compared with the group 1 (>8ng/ml)
Conclusion: The serum FSH is a reliable predictor of egg quality and can be called as one of the most important factors in Art and its success. Basal FSH levels can be used as the most accurate marker for the ovarian response in women with normal ovarian reserves undergoing IVF-ET
Keywords: ART, Pregnancy Outcome, Serum FSH

O_{nm}-4: Evaluation of Pain Experienced and Side Effects during Sonohysterography

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Background: Saline infusion sonohysterography and other intrauterine investigations methods may cause uterine cramping, pain and

vasovagal reactions. The purpose of this is to evaluate the pain perception and side effects in women undergoing sonohysterography for the assessment of uterine and tubal patency
Materials and Methods: In this prospective clinical trial, a total of 152 women undergoing sonohysterography were evaluated. The examination was scheduled in the early follicular phase of menstrual cycle, before day 17. The indications for sonohysterography included infertility, thick endometrium in transvaginal sonography and abnormal uterine bleeding. Pain perceptions of patients were measured at inflation and deflation of balloon catheter by mean of a 17-point visual analog scale (VAS). Side effects and complications related to procedure were also assessed. The outcome measures were pain perception after inflation and deflation of balloon catheter, vasovagal reactions and late side effects.
Results: Eighty-nine percent (136 of 152) procedures were performed correctly. The mean age was 329 6012, and mean infertility duration was 70489 5083 years. The mean pain scores at inflation and deflation of balloon were 105/ ± 1053 and .439 ./3 respectively. Pelvic pain did not occurred in 3505 % of patients after inflation of balloon and in 175 after deflation of balloon. None of patients had severe pelvic pain (8 to 17 VAS) at inflation and deflation of balloon catheter. None of the patients experienced severe vasovagal reactions (manifesting as vomiting and syncope).
Conclusion: Our data support that sonohysterography is a safe, well tolerated examination with a very low rate of side effects and rare complications.
Keywords: Sonohysterography, Pain, Vasovagal Reaction

O_{nm}-5: Moderating Role of Extraversion and Quality of Martial Relationship on The Relationship between Attachment Styles and Adjustment to Infertility

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Background: Infertility is a disease of the reproductive system. Infertility is a severe and chronic stressful experience which affects couples, especially women. Although epidemiological findings estimate high prevalence of infertility, the knowledge regarding psychological factors influencing adjustment to it is limited. Adjustment to infertility is influenced by several mediating as well as moderating factors. The present study was aimed to investigate moderating role of extraversion and quality of martial relationship on the relationship between attachment styles and adjustment to infertility.
Materials and Methods: This end, A total of 192 infertile women referred to the Infertility treatment clinic at Royan Institute participated in this study. Participants were asked to complete demographic information questionnaire, Adjustment to Illness Scale (AIS), Adult Attachment Inventory (AAI), Eysenck Personality Questionnaire (EPQ-RS), and Golombok-Rust Inventory of Marital State Questionnaire (GRIMS). Structural equation modeling analysis was used to test the hypothesize Moderation model Using SPSS (version 18) software.
Results: The results indicated significant positive correlations between secure attachment styles with adjustment to infertility and the significant negative correlations between insecure attachment styles with adjustment to infertility. Extraversion and quality of martial relationship also showed significant positive correlations with adjustment to infertility. However, extraversion and quality of martial relationship did not showed a moderating role on the relationship between attachment styles and adjustment to infertility.
Conclusion: Based on the results of the present study, it can be concluded that attachment styles revealed a unique strong correlation with adjustment to infertility. The results can be practical in making preventive programs and protocoling psychological interventions for infertile women.
Keywords: Infertility, Attachment, Extraversion, Martial Relationship, Adjustment

O_{nm}-6: Pelvic Inflammatory Disease: Threatening Women's Reproductive Health

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Background: Pelvic inflammatory disease (PID) is a clinical syndrome caused by ascending microorganisms from the vaginal and endocervical. More than 85% of PID cases are due to sexually transmitted disease or bacterial vaginosis. The Center for disease control and prevention estimates that 800,000 women in the United States affected by pelvic inflammatory disease (PID) annually. This disease as a major threat for women's reproductive health worldwide and has potential long-term side effects, especially tubal infertility, ectopic pregnancy, and chronic pelvic pain.
Materials and Methods: This study was a review of Persian and English full articles published over the years (2000-2014). In order to achieve the published articles, the SID, Magiran, Medlib, Pubmed, Sciencedirect, Google scholar, Scopus, were searched. Search was performed using the pelvic inflammatory disease, reproductive health of women, infertility, ectopic pregnancy, chlamydia and gonorrhea and prevention of pelvic inflammatory disease key words.
Results: Salpingitis due to pelvic inflammatory disease is the cause of 10-20% of infertility cases, 5-10%, of women are experienced ectopic pregnancy and 20-30% complicated with chronic pelvic pain. In developed and developing countries, about 10-15% of women of reproductive age have had at least one episode of PID.
Conclusion: The cost and complexity of screening programs is still considered as a barrier. The World Health Organization has concluded that vaccination against chlamydia and gonorrhea for prevention of pelvic inflammatory disease and its long term side effects is a top priority in the world.
Keywords: Pelvic Inflammatory Disease, Reproductive Health of Women, Infertility, Ectopic Pregnancy

O_{nm}-7: Aloe Vera and Sperm Count, Motility and Morphology

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Background: Aloe vera is a medicinal plant that is claimed to have hypoglycemic effect with fewer side effects and without toxicity.Aloe vera-derived ingredients were not found to be toxic in oral studies using rats. The aim of this study was to determine the validity and/or invalidity of Aloe vera gel on enhancing the reproductive activity in male rats.This study investigates the validity and/or invalidity of Aloe Vera gel on enhancing the reproductive activity in male rats.
Materials and Methods: In this experimental trial, 33 adult male rats were randomly assigned into three groups. The experimental groups received only Aloe vera orally for 60 days of treatment in two

different sublethal doses; 100 mg/kg as high dose and 50 mg/kg as low dose, whereas the control group received distilled water. Sperm samples were collected from the distal region of the epididymis and used for evaluation of sperms were count, motility and morphology (23). An average of 200 sperm was counted on each slide under a fluorescent microscope.

Results: Treatment of male rats with Aloe vera resulted in a significant decrease in cauda epididymal sperm count of the low and high dose-receiving groups as compared to the control group (31.27 ± 3.03) (P<0.001). The average percentage of sperms' progressive motility showed a marked decrease in both the low and high dose-receiving groups in comparison to the control group . In addition, the percentage of sperms with normal morphology decreased significantly in both of the dose-receiving groups as compared to the control group (94.09 ± 2%) (P<0.001).

Conclusion: Based on the results of this study, some of the fertility parameters such as sperm count, motility, testosterone level, were decreased in the rats treated with Aloe vera.

Keywords: Aloe Vera, Sperm Count, Motility, Morphology

O_{nm}-8: Study of Auriculotherapy Effect on The Outcome of ART in Infertile Women who Refer to Om-e-Leila Fertility and Infertility Center, Bandar-Abbas, 2014-2015

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Background: Infertility is a disease of the reproductive system defined by the failure to achieve pregnancy after 1year of regular unprotected sexual intercourse. Infertile couples experience stress and depression. Some studies indicated that Auriculotherapy can reduce stress. So the aim of this study was to determine the effect of Auriculotherapy on the stress levels and ART outcome in infertile women.

Materials and Methods: This study was a clinical trial. The participants were 56 infertile women, aged 20-45 years, contributed into two groups (Control & Intervention).in control group, the patients received routine treatment, but in Intervention group, the patients received Auriculotherapy for 8 to 12 session during their cycle, in addition to routine treatments. Both groups completed Newton questionnaire in 3 stages. After the data collection analysis was conducted by SPSS software.

Results: The results has shown that the mean score of stress in intervention group decreased significantly, both prior to embryo transfer and prior to pregnancy test stages (P=0.032, P=0.035).As well as the results showed that the pregnancy rate and clinical pregnancy rate (gestational sac and fetal heart rate in US view) in intervention group were more than control group. But the pregnancy rate difference between two groups was not significant (P=0.21), although the clinical pregnancy rate difference between two groups was significant (P=0.036).

Conclusion: The results of this study indicated that Auriculotherapy can be an effective method to reduce the stress and improve the outcome of ART.

Keywords: Stress, Auriculotherapy, Infertility, Iran

Poster Presentations

P_{nm}-1: Application of Instructional Technology in Infertility Education and Assisted Reproductive Technologies (ART)

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Background: Instructional technology is widely used in all aspects of medical Education (including nursing, midwifery, and reproduction health). Applying and using common instructional technologies and adopting instructional design patterns in medical education can make a great contribution in medical simulations and education and improvement of the clinical skills of reproduction experts, midwifery students and also patients. The present research has been conducted with the purpose of application of instructional technology in 4 dimensions (instructional design and instructional approaches dimension, technological dimensions, psychological and instructional aspects dimension, and communication patterns dimension) in infertility education and assisted reproductive technology.
Materials and Methods: The research method is based upon electrically searching for keywords in scientific and specialized databases over a 20-year period between 1996 to 2016.
Results: In this paper, patterns of instructional design (Gagne and Keller), instructional approaches (patient-centered treatment and problem-based approach), information technology (telemedicine, electronic health and mobile health) and communicative patterns and communicative frameworks (patient centered communication and clinical methods) in infertility education and assisted reproductive technology are studied.
Conclusion: Based on the results of this study, we may conclude that instructional technology can help reproduction health specialist who want to teach communicative skills, clinical skills and autobiographical to infertile couples who want to use ART in terms of education, instructional design, and instructional approaches. In terms of technology, instructional technology can be really useful for exchange of authentic information in order to diagnose and treat infertility and also use of m4RH which helps change the behavior of patients and improve the relationship between wife and husband. By considering context factors such as family, culture, spirituality, confirmation and response to emotions and values through respect and fulfilling demands and priorities of the infertile couples, instructional technology provides a means for communication and interaction between reproductive health experts and suffering families and experience of infertile patients. Instructional technology can also be effective from psychological and instructional dimensions by utilizing infertility psychology and treating it from behavioral, emotional, communicative, social and cognitive approaches through the use of cognitive-behavioral and rational-emotional theories. It can also use instructional theories to educate, train and adjust children born through ART.
Keywords: Instructional Technology, Instructional Design, Infertility Education, Psychology, Assisted Reproductive Technologies (ART)

P_{nm}-2: The Psychological Effects and Consequences of IVF Treatment

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Background: IVF treatment can cause a stress generating experience for infertile couples. There are various clinical reports on the emotional effects of IVF treatment. Common emotional reactions to infertility and her treatment include depression, anger, sin, frustration and sorrow. According to studies, women show high levels of anxiety and men show high levels of losing confidence during IVF treatment. The present research has been conducted with the goal of investigating the psychological effects and consequences of IVF treatment.
Materials and Methods: The research method is based on electronically reviewing and searching for keywords in scientific and specialized databases over a 12-year period between 2004 to 2016.
Results: In the results of this paper, first the psychological models of infertility, -mental consequences of infertility, mental disorder factors and the conceptual framework of infertility psychology were investigated in 4 levels of personality traits and life partner attachments, concerns, intervention and psychological disorders. Finally, the emotional-psychological consequences of infertility were investigated.
Conclusion: Based on the result, the stress level reaches its peak during the IVF treatment cycle and if IVF treatment fails, life satisfaction will reach its lowest level. Losing confidence and self-esteem, getting a negative picture of your body, a sense of defect and loss of merit and sinking in treatment are some mental consequences of IVF treatment, while losing contact with social networks, losing contact with spouse, losing social status, a sense of social shame and getting alienated from the world are some social consequences of IVF treatment. Thus, psycho-social infertility consulting helps patients discover, realize and fight the problems associated with infertility and her treatment.
Keywords: Infertility, Infertility Treatment, Psychology, *In Vitro* Fertilization, IVF

P_{nm}-3: An Overview of The Consultation Continuity of Infertility

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Background: Infertility is a medical crisis - psychological and social counseling and infertility is an integral part of its treatment. While infertility centers offer a range of treatments to couples, research has shown that even in couples with infertility treatment incentive to leave rates high. Available evidence indicates that the level of care in the treatment of infertility can have a big impact on dropout rates. Consultations at the time of discontinuation of therapy, Impact on the couple's decision. This study aimed to review the interventions of advisers in maintaining the fertility treatment was performed
Materials and Methods: Narrative review present study, using Mesh and Text word , Related Keyword identified and bases , Springer, SID, Iranmedex, Magiran, Pubmed, Google scholar, Cochrane, science Direct search strategy using any database, Search journals Was. 107 articles were obtained in the initial search screen after title and abstract, duplicate articles unrelated to the research question have been deleted. Ultimately, 14 were in the study which contains 2 clinical trial, 3 Cohort study, 4 and 5 case-control study was cross-sectional study.
Results: The authors were organized in six categories: 1. Select the type of advice and counseling infertility patients required based on gender; 2. Consulting and correcting misconceptions Associated with the cause of infertility; 3. Consulting treatment of sexual dysfunction and enrich the relationship between the spouses; 4. Consulting and the

global deal community; 5. advice on gamete donation and surrogacy; 6. evidence-based psychological interventions
Conclusion: The findings of this review suggest that the mental health system as involvement in a process of self-care intervention can be an effective way to bring down the dropout rates of infertility treatment. Availability holistically integrated team of medical and mental health during treatment in infertility clinics and regular attendance is essential interventions.
Keywords: Infertility Counseling, Continuity of Care, Leaving Care

P_{nm}-4: Menopause as A Disease: The Analysis of Medicalization about The Health-Related Issues

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Background: Menopause is one of the most important periods in the women's life; which has a major share in the formatting of women's ideas about themselves. It has significant effects on their physical, psychological and social health. According to historical records, in the past three decades, women's lives increasingly are medicalization and menopause women become more depending to medical knowledge.
Materials and Methods: We conducted a comprehensive search in the literature that were published in 2000 to 2015; and in the SID, PubMed, Scopus and Google Scholar databases by using the key words such as menopause, social constructionism and medicalization.
Results: This paper seeks to show how and in what way normal experience and events of life, such as menopause, enter to the realm of medical sovereign; the process that is called as "medicalization" in sociology. Mental nature and constructionism of menopause is analyzed, in this paper and is shown how and why menopause is considered as "estrogen deficiency disease" in the medical literature. Menopause with what procedures and mechanisms have been confiscated in favor medicine in Iran. Qualitative data mining is used to collect and analyze the data in this study.
Conclusion: This paper presents a literature review and shows that presume biomedical models for health-related issues, causes ignoring the true nature and characteristics of these issues. Thus, the menopause should be considered as a socio-cultural construction and confirmed on the effect of cultural, social, economic and political factors. Although menopause has its own needs, but it isn't just a biomedical phenomenon and should not be dealt with as a disease or disorder, it doesn't need to medical interventions.
Keywords: Disease, Social Constructionism, Medicalization, Health-Related Issues, Menopause

P_{nm}-5: Ethical Issues and Challenges of Egg Donation as Essential Knowledge Need in This Topic

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Background: Egg donation received from a volunteer in some cases, of infertility, for fertility is the only possible option. Despite donate organs and tissue to help resolve some of the problems and sufferings of the people, in turn, discussions a lot about the ethical and religious implications of the use of this technology will follow. Considering the topic importance, we conducted a literature review by using principlism approach and by following the principles ethical Quartet's, to address ethical issues and challenges in egg donation.
Materials and Methods: Literature for the period 2005 to 2014 was searched in the electronic databases of Google Scholars, Science Direct, and PubMed using the following keywords:¬"Egg Donation","Infertility" and "Medical Ethics".
Results: A total of 84 articles were identified. Of the articles related to the concept of ethical issues around egg donation, a total of 20 articles were selected for the final analysis. Donor selection, confidentiality in egg donation, and financial compensation for egg donation risks are the most important ethical challenges of egg donation.
Conclusion: Findings have implications for policy, healthcare organisations, and raising awareness about ethical issues and infertile family training needs in particular. Patients under egg donation should be informed about potential ethical issues and challenges, so that it can be discuss with their healthcare providers prior to this option treatment. Besides, planning and legal definition of financial relations between the two sides seems to be very useful in order to prevent any type of abuse. Recommendations for practice development and further research are made.
Keywords: Egg Donation, Infertility, Medical Ethics

P_{nm}-6: Using Beck Questionnaire to Detect Postpartum Depression among Mothers Conceived by Assisted Reproductive Techniques (ART) in Comparison with Naturally Conceived Mothers

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Background: The aim of this study was to compare the rate of PPD in mothers who naturally conceived with mothers who had infertility history using Beck questionnaire.
Materials and Methods: Four hundred six mothers with infant 3 to 9 months old [308 mothers with normal pregnancies referring to Tehran health centers as control group and 98 women who became pregnant by the use of assisted reproductive techniques (ART) at Royan institute as ART group] were investigated in this historical cohort study. General questionnaire including age, education, occupation, age of infants, the number of twin births in the past, the number of children, delivery method, history of infant hospital admission, lactation status, maternal depression history and cause of infertility (for ART group) was completed by each mother. The valid questionnaire of Beck was used to measure depressive symptoms. SPSS software version 20 and Levene, One-way ANOVA, t test and linear regression, Tukey and Dennett's statistical tests were used for data analysis. The significance level was considered less than 0<05.
Results: The age range of mothers were 51-17 years with the mean ± SD (28.87 ± 5.18) years. Also, the age range of infants were 3-9 months with the mean ± SD (5.18 ± 1.3) months. The percent of post-

partum depression were 19.2%, 23.1% and 7.1% in all mothers, control and ART groups, respectively. The rate of postpartum depression was statistically lower in women who had become pregnant after infertility treatment(P =0.0001). Linear regression analysis showed that history of infertility, education level, number of children and history of maternal depression are influencing factors on PPD.
Conclusion: It seems that PPD in mothers conceived by ART is lower than those naturally conceived using Beck questionnaire. Although this finding needs to be confirmed using special questionnaire to detecting PPD such as Edinburgh postpartum depression scales (EPDS).
Keywords: Postpartum Depression, Assisted Reproductive Technology, Natural Pregnancy, Beck Questionnaire

P_{nm}-7: Sexual Dysfunction in Women Undergoing Fertility Treatment: Prevalence and Associated Risk Factors

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Background: Sexual dysfunctions are one of the most difficulties for infertile women, which can be as the cause of infertility.This study investigated the prevalence of this disorder and associated factors in order to improve infertility treatment process and the quality of life of women referring to infertility center.
Materials and Methods: A cross sectional study was performed on 200women who referred to Kargar infertility center of Yazd.. Data collection tool was a questionnaire contained two parts; demographic characteristics and infertility information. Also, data for sexual dysfunction was obtained through diagnostic interview based on the international classification DSM-IV. For data analysis, logistic and linear regression analysis were used. The P<0.05 was considered significant
Results: Most of women (80.9%) suffered from primary infertility and the mean duration of infertility was 50.2 ± 8.4 months. The prevalence of sexual dysfunction was 44% (n=88); including dyspareunia in 20% (n=40), impaired sexual desire and lack of orgasm in 15.3% (n=30 patients), vaginismus in 11.2% (n=22) and lack of sexual stimulation in 10.6% (n=20). Binary logistic regression analysis showed that age, sexual satisfaction and history of mental illness had a significant effect on the probability of experiencing the sexual dysfunction.
Conclusion: There is a high prevalence of sexual dysfunction among infertile women. Considering the interaction between sexual dysfunction and infertility, professional health care centers should be sensitive to this effect. Also, more attention must be paid on marital relationships, economic and social situation and infertility characteristics in order to prevent sexual dysfunction development through early screening and psychological interference.
Keywords: Sexual Dysfunction, Infertility, Women

P_{nm}-8: Effect of Infertility on Sexual Function: A Cross-Sectional Study

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Background: Sexual dysfunction is an important psychological disorder that may increase in infertile couples. To evaluate the effect of infertility on sexual function in women attending in private and public institutions in YAZD during 2014.
Materials and Methods: In a cross - sectional study evaluated the sexual function among 300 women attending in health care centers of YAZD western of Iran during 2014. Participants were divided in two groups, fertilities and infertilities women. Data was collected by trained research midwives using demographic and FSFI questionnaires. SPSS software was used to analyse the data of this project. Differences were regarded statistically significant with an alpha error of 0.05.
Results: The mean age was 25.26 ± 4.7 years in fertile and 30.74 ± 9.07 in infertile women. Significant difference was reported in mean age between fertile and infertile women (p=0.014). The Mean± SD of all demissions of female sexual function was difference between fertile and infertile women. Sexual function was lower in infertile women.
Conclusion: All dimensions of sexual function were lower in infertile women in compared with fertile women. Further research should be done on this subject and ways to address such problems should be found.
Keywords: Infertile Women, Sexual Dysfunction, FSFI Questionnaires

P_{nm}-9: Psychological Consultation in Infertility

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Background: In all cultures, infertility is a stressful experience and critical threatening to the stability of individual, couples, family, and community. Failure in fertility affects deeply on various aspects of life infertile couples and that need to psychological consultation. In many studies, the negative impact of infertility on marital and sexual functioning fitness, anxiety and depression and reducing the quality of marital life has been shown. The aim of this review study is effect of psychological consultation on infertile couples.
Materials and Methods: Review study
Results: The various descriptive studies have suggested infertile couples, suffer various forms of emotional stress that eventually could disposed them to depression and it is possible to further reduce the chances of get pregnant. Many couples consider certainly infertility as a major crisis and stressful event in life, in finally, infertility becomes to the bio-psycho-social crisis that threatens the mental health of infertile couples. It should be emphasized that the most physical therapy infertility alone is not enough and attention to the psychological needs of Infertile, couples an essential part in infertility therapy.
Conclusion: The goal of psychological consultation is recognition and help to people for more adaption with existing conditions. The objective of infertility counseling as well as search, understand and solve the problems caused by infertility and its treatment and find ways to deal effectively with the problem, and also, that infertile couples be encouraged to express feelings and thoughts about their problems and share their problems.
Keywords: Counseling, Infertility, Mental Health, Psychological Problems, Couples

P_{nm}-10: The Effect of Malaria Infection, Treatment and Prevention on Infertility

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Background: Malaria is a life-threatening mosquito-borne disease. It caused by parasitic protozoans of the genus Plasmodium and affected on millions of people around the world.
Materials and Methods: A search in the PubMed database and Google scholar was performed on relevant studies published from 2000 to 2015. Articles that were written in English and relevant to the topic were enrolled in this study.
Results: In general, biologically effects of malaria on human and animals' fertility is negative: reduced coital frequency, increased spontaneous abortions, higher probability of still births, and a decrease in general maternal health. Malaria infection is associated with decreases semen quality and severe oligozoospermia, necrozoospermia or azoospermia in men. Plasmodium spp. decrease motility, concentration and viability of sperms in infected males. Also, there was a decrease in serum levels of testosterone and increased cortisol level in infected mice compared with control group. Moreover some studies have shown that fever resulted from malaria infection has an inhibitory effect on the reproductive system of males. One of the indirect effects of malaria infection on reproduction in humans is infertility of due to antimalarial drugs. There are many studies indicated that antimalaria drugs such as chloroquine, quinine, quinacrine and pyrimethamine have been associated with adverse effects on reproductive functions. Chloroquine was shown to reduce testosterone secretion, sperm motility and average number of fetuses of cohabited females. Pyrimethamine was shown to inhibit of spermatogenesis and causes male infertility in mice. Maximum tolerable dose of quinine to injected BALB/c mouse caused morphological changes in the testes and suppressed spermatogenesis. Moreover it is showed that environmental DDT exposure in young men living in high endemic malaria area is related with impaired semen quality.
Conclusion: Therefore, results of this systematic review demonstrated that complications of malaria may be disrupting on human reproductive system.
Keywords: Malaria, Plasmodium spp., DDT, Infertility

P_{nm}-11: Relationship eetween Spiritual Health and Mental Health in Couples Undergoing in Assisted Reproductive Therapy: Systematic Review

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Background: Assisted reproductive therapy are created stressful conditions for infertile couples, some couples in this situation may experience mental health problems. Religion and spirituality health is one of the factors that have a key role in the vulnerability of people to mental illness which in recent decades has received considerable attention. The aim of this study was to systematically review the relationship between spiritual and mental health in couples undergoing in assisted reproductive therapy.
Materials and Methods: In the current study, English and Persian databases including Magiran, Iranmedex, SID, Google Scholar, scincedirect and PubMed were searched, using keywords such as "spiritual health", "assisted reproductive therapy", "infertility", and "mental health". Related articles published during 1995-2016 were assessed. Relationship between spiritual health and mental health in couples undergoing in assisted reproductive therapy was evaluated.
Results: As the results indicated, spiritual health have a high correlation with mental health and have a positive effect on it by creating hop, peace, meaning and aim in the patient's life.
Conclusion: So far several studies have been conducted on the relationship between mental health and spiritual health. In many of these, spiritual health has a positive impact on mental health. It seems that we can improve quality of care and mental health by considering spiritual health in treatment program.
Keywords: Mental Health, Spiritual Health, Infertility, Assisted Reproductive Therapy

P_{nm}-12: The Study of Self-Concept and Self Discrepancy among Women Receiving and Donating Oocyte who Referred to Royan Infertility Center in 2014

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Background: A technology for fertility assistance is the use of donated oocyte. Although the data about medical aspects of this process is available, the knowledge of psychological affairs related to this therapy is limited. Self-concept and self discrepancy are able to investigate the psychological aspects of every person and therefore, we decided to study these measures on women who receive and donate oocyte.
Materials and Methods: In this cross-sectional investigation, carried out in 2014, 53 women were divided randomly into two groups including donors and receivers. The data were collected by a three part questionnaire including demographic questions, self-concept scale and self discrepancy scale questions (consisting of "ideals" and "have to be" self discrepancy data). The data were analyzed through SPSS19 and by use of statistical tests including Kruscal-wallis and ANOVA.
Results: The mean age of the cases in receiving and donating groups was 32.6 ± 5.3 and 29.33 ± 3.7, respectively. These two groups did not show any significant difference in their demographical characteristics. The self-concept and self discrepancy data were poor. There was no significant difference between the two groups regarding the mean scores of self-concept data (P=0.572), whilst, there was a significant differences between the two groups regarding the "have to be" part of self discrepancy data (p=0.019). But no significant difference was found between the two groups about the "ideal" part of the self discrepancy data (P=0.22).
Conclusion: According to the achieved results, the officials should provide supportive psychological programs for infertile women receiving oocyte. It is also necessary to take some measures for evaluating the emotional aspects of oocyte recipients.
Keywords: Self-Concept, Self Discrepancy, Oocyte Recipient Women, Oocyte Donor Women

P_{nm}-13: A Review of Traditional Medicine and Complementry Medicine on Infertility

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Background: The lack of fertility in terms of social and economic importance of the family quite a lot. the prevalence of infertility in Iran and around the world and different methods of treatment and economic times therefrom caused many problems for infertile couples have. In the various communities such as Iran is a very important phenomenon was childbearing for the durability of the family and divorce prevention it has very important role. infertility may cause violence against women become . Given the importance of different recommendations in traditional medicine iran to treat infertility is . in women is one of the most important foods that temper of strong is like honey, figs and nuts which for uterine cold is appropriate .food warm temper raises the quantity and quality of semen and In fertile usde to. Homeopathy is used to treat people who have hormone-related infertility and also in study of five infertile women that are treated in the hospital of Gynecology and Obstetrics . Acupuncture and traditional Chinese medicine and Western medicine semen has been increased and improved. Acupuncture has improve endocrine basic level and increased the number of oocytes. Acupuncture also helps to reduce the stress of infertility.
Materials and Methods: A literature search was conducted on pubmed.
Results: Integrates a variety of medical procedures to better assist the treatment of infertility.
Conclusion: According to the survey it can be concluded that the integration of traditional medicine, complementary medicine with western medicine to help infertile couples spend less time Kmtrvdr our costs better.
Keywords: Infertility, Traditional Medicine, Iran, Acupuncture, Homeopathy

P_{nm}-14: Ovulation Induction And Risk of Ovarian Cancer in Women: Review Article

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Background: Infertility is one of the medical problems in today's world , the use of fertility treatment has increased markedly in recent decades. However, various concerns associated with the use of fertility drugs, especially with the potential risk of cancer is increased. The aim of the present study was a comprehensive overview of studies in the field of infertility, ovulation induction and its impact on risk of ovarian cancer in women.
Materials and Methods: This study is a review of all articles of observational, published during the years (2000-2015) in both Persian and English with full text were examined. Studies through databases SID, magiran, Medlib, Persian and databases for articles pubmed, Scopus, Google Scholar, Science Direct in order to achieve a level of published articles in the journals were obtained. Search using valid key words infertility, infertility, ovulation induction, cancer, gynecological cancer, clomiphene citrate, human chorionic gonadotropin, infertility, ovarian cancer. Search strategy was used in titles. At the end of the study articles were enrolled.
Results: Ovulation induction therapy, especially long-term use increases risk of ovarian cancer . However, these findings are based on quantitative studies and, have not been established in a recent study cohort. Safety ovulation induction, especially in the light of the delay in fertility and increased use of infertility remains a concern.
Conclusion: Given that the cause of cancer in women widely known with hormone. And conflicting results of studies in this field, further evaluation is required in order to induce ovulation drugs on the risk of cancer.
Keywords: Infertility, Ovulation Induction, Fertility Drugs, Ovarian Cancer, Women

P_{nm}-15: Confidentiality in Embryo Donation (A Cross-Sectional Study in Tehran)

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Background: Confidentiality in embryo donation is one of the most controversial issues related to the use of this assisted reproduction technique. Although the existing evidence insist on the need to expose the truth to newborns. This issue is important from two perspectives: First, the responsibility of Embryo Donation service providers in which the evidences suggest that the measures taken by the authorities may not ensure the interests and rights of the natives of embryo donation and so the second respect or their social parents' responsibility arises in which the attitudes of applicants of embryo donation about the privacy and disclosure issues would be helpful.
Materials and Methods: The present study is a descriptive study through interviews with 100 couples applying for embryo donation regarding the confidentiality of the process. The research instrument was a questionnaire that was designed based on the Likert scale in which the applicants attitude towards the recitation of embryo donation issues to result children and why they agree or disagree with this issue and also evaluate their attitude towards embryo receiving from familiars. Validity was based on content validity and its reliability was calculated using Cronbach's alpha coefficient (equal to 0.7) and test-retest method (equivalent to 0.97). Data analysis was performed using SPSS version 18. To summarize the data, we used the mean, standard deviation, and frequency and to determine the factors affecting the applicants' decision to disclose the embryo donation issues for the resulting children, the logistic regression model was used.
Results: The present study showed that the majority of applicants (93%) have no intention of informing the child how was born and issues such as inclination for existence of honest relationships among family members, consequences of secrecy in the family, respect for the birthright of man to his identity and even the necessity of understanding the genetic origins based on medical necessity were not considered as a reason for the requirement to disclose the truth by majority (65%). Also the majority (62%) believed that to be informed of child will lead to his/her teen and would endanger the child' intention for recognition of his/her biological parents and family independence.
Conclusion: Since man has a major benefit in knowing their genetic origin and their identity and authenticity recognition could play an essential role in the future life of born from embryo donation, the organizations providing services related to the field of assisted reproductive should support this demanding right. In this regard, the couples who are going to use donated embryos should take ready before any remedial action to address the issue of children have a right to know their biological origins. Also the social support to encourage and support to notify the recipients of the identity of their biological children is so important.
Keywords: Embryo Donation, Infertility, Confidentiality, Anonymous

P_{nm}-16: The Beauty of what Price The Cost of Infertility for Women

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Background: What is clear Vmhsvs serious changes in different aspects of lifestyle in creating and expanding the scope of the problem is not only ineffective but also scientific research showing that changes in how Ypvshsh clothing, housing, nutrition, health,Agriculture and ... all play a significant role in the creation of infertility and other problems, physical and spiritual, of course, have today.
Materials and Methods: Review, the study valid written dozens of articles.
Results: Many Women to Get out of the House when not in use and use of Cosmetics, many of them have become a major Nuisance Denied being aware of the fact that in these Cosmetics Chemicals called barium there is an Element of the Alkali metals That many Substances and its Salts are highly toxic in HumansIt is Widely used in Industry and in the Manufacture of plastics, Poaps and Cosmetics, including lipstick also some nail polish, Nunscreen, Moisturizers, Shampoos and hair spray used Dygrmvrd Directly enter the body through the pores. The Negative effects of these Pompounds in preventing the implantation of the embryo in the womb, abortion and a variety of cardiovascular diseases has been proven to cause disturbances in hormones cause the body's natural reproduction.
Conclusion: No doubt provide a correct pattern and can be tailored to Islamic criteria Family life provide material and psychological health of family members have a significant role.
Keywords: Lifestyle, Infertility, Cosmetics

P_{nm}-17: Counseling Aspects of Gamete Donation Programs in The Infertility Treatment: A Narrative Review

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Background: Infertility is considered as a crisis in life and can cause extreme stress for infertile couples. The using of donated gametes or embryos is required in some cases infertility treatments. Egg and sperm donation for couples less fertile provides an additional chance. The aim of this narrative study was to determine exploring of counseling aspects of Egg Donation Program for the treatment of infertility.
Materials and Methods: Related Keywords revealed By Mesh terms and text word. It was searched in databases: Google Scholar Science Direct, SID, The Cochrane Library, Central Register of Controlled Trials (CENTRAL), MEDLINE, Up-to date, Magiran, Iranmedex, and Irandoc. Keywords was: gamete donation and counseling, infertility in women, egg donation. Finally, researcher found 293 papers and 2 books and guidelines, the data were extracted from 82 articles and two books.
Results: The findings were classified in two main categories and 13 subcategories: First category: basic problems of infertility including following seven subcategories: 1) The definition of infertility; 2) the causes of infertility; 3) the treatment of infertility; 4) donation indications; 5) a variety of ways to donate; 6) Complications of infertility; and 7) factors affecting gamete donation. Second category: counseling aspects of Egg donation includes six subcategories: 1) Medical advice; 2) Psychological counseling; 3) Genetic counseling; 4) Legal advice; 5) Ethical advice; and 6) The role of midwives in counseling.
Conclusion: These findings suggest that in addition a variety of medical issues, infertility is influenced by the whole range of personal characteristics, religious, psychological, social, legal, financial and moral issues. The patients undergo assisted reproductive therapy require to counseling and examination in all aspects of counseling. It seems that it does not conducted in most cases. so, it is suggested that in order to enhance infertility programs and to improve the reproductive health of couples undergoing ART, Informed midwives in infertility and assisted reproductive therapy as well as efficient in counseling, be established in clinics to provide related services.
Keywords: Infertility, Sperm Donation, Egg Donation, Integrated, Counseling

P_{nm}-18: The Factors of Affecting on The Gamete Donation Program Process in Infertility Treatment: A Narrative Review

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Background: Some infertile couple use to gametes or embryo donation for their infertility treatment. The aim of this study was to reviewing of the factors of affecting on the gamete donation program process in infertility treatment.
Materials and Methods: Related Keywords revealed By Mesh terms and text words. It was searched in databases: Google Scholar Science Direct, SID, The Cochrane Library, Central Register of Controlled Trials (CENTRAL), MEDLINE, Up-to date, Magiran, Iranmedex, Irandoc. Keywords was: gamete donation and counseling, infertility in women, egg donation. Finally, researcher found 293 papers and 2 books and guidelines, the data were extracted from 82 articles and two books.
Results: Effective factors in the process of gamete donation: 1) negative factors: donor restrictions, removal or withdrawal of donors, lack of information about the attitudes of the infertile couples and barriers, poor knowledge of infertile couples, the negative attitudes of gynecologists / midwives, lack of professional experience and a good knowledge of egg donation in nurses, necessity to spend considerable time, religious infertility and "pattern of confidence in the Emotional relationship", fear of disclosure for treatment, lack of acceptance of the child as the biological child, physical complications, costs of treatment 2) positive factors: duration of infertility, failure of previous treatment, age, and female infertility factor.
Conclusion: The study findings showed several factors are effective even on the acceptance or rejection of third party reproduction in couples undergoing assisted reproductive techniques such as: the amount of information and awareness of Odd and staff, beliefs and how to tackle religious of couples, socioeconomic, physical and emotional. Motivation and acceptance or rejection of gamete donation among couples, should be assessed by counseling before therapy.
Keywords: Gamete Donation, Egg Donation, Midwife Counseling, Third Party Reproduction, Infertility

P_{nm}-19: Treating Infertility with Herbal Medications

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Background: Herbal medicines may enhance fertility by supporting the natural functions of the ovulation and fertility process. Herbs are generally safe to use. However, because some herbs should not be taken during pregnancy. Therefore it is preferable to consult a health-care provider who is familiar with herbs and how they affect different aspects of your fertility.
Materials and Methods: This article is an overview of treating infertility with herbal medications.
Results: The effects of herbal medicines are generally cumulative, and the clinical effects of treating the infertile couple are usually seen after 60-120 days. Herbs are also cycle-dependent. They require the entire menstrual cycle to be effective, and work best with multiple cycles. This means that if, for instance, a woman decides to have an *in vitro* fertilization: IVF transfer within a week, she should avoid herbal treatment.In general, it is appropriate to treat any type of infertility condition with herbal medicines. This includes advanced maternal age, luteal-phase-defect, premature ovarian failure, male factor, or unexplained symptoms. Clinical observers have reported impressive results when mixing herbs with gonadotropins during intrauterine insemination (IUI) and IVF cycles
Conclusion: A number of herbal supplements are available that are helpful in supporting fertility for both women and men. For women, vitex (chasteberry), red clover and other herbs traditionally used to help restore hormonal balance are combined with the same vitamins and minerals found in a prenatal vitamin The combination offers a comprehensive fertility supplement for women. For men, clinically proven supplements are available to help improve sperm parameters such as count, motility, and morphology.
Keywords: Infertility, Herbal, Medications

P_{nm}-20: Development and Psychometric of Health-Related Quality of Life Questionnaire for Polycystic Ovary Syndrome (PCOSQ-50)

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Background: The determinants of the health-related quality of life of women with Polycystic Ovary Syndrome are not fully understood. The aim of this study was to develop a comprehensive instrument to assess the health-related quality of life of Iranian women with PCOS and to assess its psychometric properties.
Materials and Methods: We used a mixed-method, sequential, exploratory design including both qualitative (in depth interview aimed to define the components of health-related quality of life questionnaire (PCOSQ)) and quantitative approaches (aimed to assess the psychometric properties of PCOSQ).
Results: A preliminary questionnaire was developed including 147 items which emerged from the qualitative phase of the study. Considering the optimum cut-off points for content validity ratio (CVR), content validity index (CVI) and impact score (IS), items of preliminary questionnaire were reduced from 147 to 88 items. Finally by excluding highly correlated items using the exploratory factor analysis, a 50- item questionnaire was obtained. The Kaiser criteria (eigenvalues more than 1) and Scree plot tests demonstrated that six factors are optimum with an estimation of 47.3% of variance. Assessment of psychometric properties of the questionnaire demonstrated a mean CVI =0.92, CVR = 0.91 , the Cronbach's alpha for whole questionnaire=0.88(0.61 to 0.88 for subscales), the Spearman correlation coefficients of test-retest =0.75, the intra class correlation coefficient (ICC) for the PCOS questionnaire subscales ranging from 0.57 to 0.88. Eventually the final questionnaire included 50 items, rated on a 5-point Likert scale, in six factors, including 'psychosocial and emotional', 'fertility', 'sexual function', 'obesity and menstrual disorder', 'hirsutism' and 'coping'
Conclusion: The PCOSQ-50 is a valid and reliable instrument for the assessment of quality of life of women with PCOS. This new questionnaire will assess any obscure aspects that have hither to not been addressed by previous questionnaire.
Keywords: Polycystic Ovary Syndrome, Quality of Life, Development, Psychometric

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International Journal of Fertility and Sterility (Int J Fertil Steril)
Guide for Authors

Aims and Scope: The ***"International Journal of Fertility & Sterility"*** is a quarterly English publication of Royan Institute of Iran. The aim of the journal is to disseminate information through publishing the most recent scientific research studies on Fertility and Sterility and other related topics. ***Int J Fertil Steril*** has been certified by Ministry of Culture and Islamic Guidance since 2007. It has also been accredited as a scientific and research journal by HBI (Health and Biomedical Information) Journal Accreditation Commission since 2008. **This open access journal holds the membership of the Committee on Publication Ethics (COPE).**

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