Abstracts of

Royan International Twin Congress
13th Congress on Reproductive Biomedicine
5-7 September 2012

7th Royan Nursing and Midwifery Seminar
5-7 September 2012

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Contents

Scientific Board

Collaborators in Award Jury Board and 13th Congress on Reproductive Biomedicine and 7th Royan Nursing and Midwifery Seminar

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- Medical Ethics and History of Medicine Research Center

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Congress Chairperson

Maryam Niknejadi

Dear Friends and Colleagues,

We are delighted to welcome you to Royan International Twin Congresses, the 13th Congress on Reproductive Biomedicine and 8th Congress on Stem Cell Biology and Technology, in Tehran, September 5-7, 2012.

The Royan scientific meeting is always an outstanding annual event divided into two main sections: 1. "Reproductive Biomedicine" and 2. "Stem Cell Biology and Technology". We will have plenary sessions, symposia, poster sessions, and workshops addressing the latest researches on reproductive biomedicine. In our opinion, the best chance for accomplishing a satisfactory outcome is to integrate research findings into practical and clinical applications. This will have promising results for the future treatment of infertility as well as incurable or hard-to-cure diseases.

I would like to take the opportunity to introduce the Department of Reproductive Imaging, which has recently been established. This department offers clinical services and educational training courses, while applying modern modalities such as different types of sonography, three-dimensional sonohysterography (3D-SIS) and virtual hysterosalpingography for female infertility management in addition to Doppler ultrasound and interventional radiology for male infertility management. We hope that by sharing and exchanging scientific knowledge and clinical experiences among the Iranian scientists and their international colleagues, this congress can help ameliorate treatment in the most difficult situations of infertility.

Our scientific program relies on your contributions and new researches. We look forward to receiving your abstracts in all aspects from the reproductive biomedicine and reproductive imaging.

The local organizing committee will do their best to make you feel welcome and ensure that you remember the twin congress as a special event, from a scientific as well as a social point of view. This Congress could also be an extraordinary opportunity to enjoy the unique history, rich culture and beautiful natural scenery of Iran.

Best Regards,
Maryam Niknejadi M.D.
Reproductive and Biomedicine Congress Chairperson

Abstracts of

13th Royan International Congress on Reproductive Biomedicine

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Invited Speakers

Andrology

I-1: Screening of Subfertile Men for Testicular Carcinoma In Situ by An Automated Image Analysis-Based Cytological Test of The Ejaculate

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Background: Testicular cancer (TC) is usually diagnosed after manifestation of an overt tumour. Tumour formation is preceded by a pre-invasive and asymptomatic stage, carcinoma in situ (CIS) testis, except for very rare subtypes. The CIS cells are located within seminiferous tubules but can be exfoliated and detected in ejaculates with specific CIS markers.
Materials and Methods: We have built a high throughput framework involving automated immunocytochemical staining, scanning microscopy and in silico image analysis allowing automated detection and grading of CIS-like stained objects in semen samples. In this study, 1175 ejacu- lates from 765 subfertile men were tested using this framework.
Results: In 5/765 (0.65%) cases, CIS-like cells were identified in the ejaculate. Four of these had bilateral testicular biopsies performed and CIS was histologically confirmed in three. In total, 63 bilateral testicular biopsy were performed in conjunction with analysis of the ejaculates because of infertility work-up. Histological analysis of the biopsies for the presence of CIS yielded a test sensitivity of 0.67 and a specificity of 0.98. In addition, ejaculates from 45 patients with clinical signs of an overt TC were investigated and yielded a slightly lower sensitivity (0.51), possibly because of obstruction.
Conclusion: We conclude that this novel non-invasive test com- bining automated immunocytochemistry and advanced image analysis allows identification of TC at the CIS stage with a high specificity, but a negative test does not completely exclude CIS. On the basis of the results, we propose that the assay could be offered to subfertile men and other patients who are at increased risk of TC.
Keywords: Carcinoma In Situ, Testis, Semen Analysis, Diagnostic Test, Image Analysis

I-21: Ejaculatory Duct Obstruction

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Ejaculatory duct obstruction (EDO) underlies 1-5% of male infertility, although the diagnosis of EDO can be complex, treatment is well established and can be very effective.
Part of reason that this condition probably is underdiagnosed, is because of its rarity, subtle presentation and the concomitantly low index of suspicion held by physicians.
The causes of EDO are divided into congenital and acquired disorders. Clinically, EDO classically presents as hematospermia, painful ejaculation, or infertility.
In the past decade, transrectal ultrasound (TRUS) has replaced vasography as the main stay of diagnosis. Several adjunctive techniques now have been described for diagnosis of EDO, including seminal vesicle aspiration, seminal vesiculography, vesicle chromotubation.
The time- tested treatment for EDO is resection of the ejaculatory ducts (TUR-ED), which is performed in an outpatient setting, and the technique combines cystourethroscopy with resection of the verumontanum in the midline.
Complications from TUR-ED occur in 10-20% of the cases, and include watery ejaculate, hematuria, epididimitis, seminal vasculitis and low risk of incontinence or rectal perforation.

I-3: Human Y Chromosome Proteome Project: 2012 Update

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The Human Genome Project has generated a blueprint for the approximately 20,300 gene-encoded proteins potentially active in any of 230 cell types that make up the human body (human proteome). However, based on the UniProtKB/Swiss-Prot database content, about 6000 of these genes currently lack any experimental evidence

at the protein level; for many others, there is very little information related to protein function, abundance, subcellular localization, and interactions. The Chromosome- Centric Human Proteome Project (C-HPP) has been designed to map the entire human proteome in a systematic effort and to will enhance our understanding of human biology at the cellular level and lay a foundation for development of diagnostic, prognostic, therapeutic, and preventive medical applications. As of March 1, 2012, there are 20 international teams, including Royan Institute, focused on 18 different chromosomes (www.c-hpp.org). In Iran, the current efforts are focused on mapping the proteome of human chromosome Y. The Y chromosome is unique under many aspects and comprises 95% of the chromosome's length. It is always in the haploid state and full of repeated sequences but it is responsible for important biological roles such as sex determination and male fertility. Here, we present the most recent update of Y chromosome proteome on the platform of genomic Y sequence. Our strategy started with the definition of the proteins coded by the human Y chromosome, a list of missing/poorly characterized proteins, and proteomic profiling studies to identify those missing proteins. The project is set up to allow for a systematic exploration of the human Y chromosome proteome using antibody-based proteomics. Within this project, specific antibodies to human Y chromosome target proteins are being produced using a method involving the cloning and protein expression of protein epitope signature tags. The antibodies are subsequently validated using several approaches including siRNA. The antibodies are now being used to study expression profiles in target cell and in diseased and healthy testis samples. We also align the proteomics data set to the output of RNA-Seq and Real-Time polymerase chain reaction analysis data with defined expression thresholds. Owing to the integration of proteomic, genomic, transcriptomic, phenotypic and pathological data, novel findings are emerging from our studies. Several strategies to maximize the success of the human Y chromosome proteome project will be presented.

I-4: External Quality Assessment - A Necessity in The Andrology Laboratory

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Andrology laboratories need to produce reliable results for appropriate diagnostic and health care decisions. Since semen analysis is highly complex and procedurally difficult to standardize, quality control (QC) is essential to detect and correct systematic errors and high variability of results. The large discrepancies between assessments of sperm concentration and morphology in different laboratories underline the need for improved QC and standardization, with the goal of improving the quality and standardization of semen analysis and enhancing the comparability of results from different laboratories. Until there are universally accepted standard methods and definitions of motility and morphology, it will not be possible to compare results from different laboratories.
Whatever its size, each laboratory should implement a quality assurance (QA) programme, based on standardized methods and procedures, to ensure that results are both accurate and precise. In some countries, QA programmes are required by law, in others, by accreditation bodies or health insurance systems.
QC activities performed within one laboratory are referred to as internal quality control (IQC). External quality assessment (EQA) is the evaluation of results for the same samples in several laboratories. Quality assurance is a wider concept including optimization of the services provided.
External quality control (EQC) is an integral part of the complete QC process that monitors assay results, while external quality assurance (EQA) monitors all laboratory procedures relating to collecting and reporting data to ensure that laboratory processes are under control. EQC allows a laboratory to compare its results with those of others. It permits different methods to be evaluated and compared on a scale not possible in a single laboratory. EQC encompasses peer comparison and proficiency testing programmes, in which specimens presumed to be identical are sent to all participating laboratories for analysis. Laboratories submit their results to a central facility, where the data are examined for outliers, and means and standard deviations are calculated to characterize the performance of the participating laboratories.

Animal Biotechnology

I-5: Fifteen Years after Dolly: The Perspectives on Cellular Reprogramming

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It is a truly amazing time to developmental biology. During recent decades, three important breakthroughs have been developed: (i) isolation of stem cells from embryo, (ii) animal cloning by nuclear transfer (NT), and (iii) and induced pluripotent stem cells (iPS). Considering these three approaches of "Cellular Reprogramming", it seems that the required elements for cell therapy now exist. In one initial assumption and considering the exceptional practical advantages of iPS over the two other methods, the Yamanaka approach of direct cellular reprogramming is generally considered as the eventual method of choice for production of patient-specific therapeutic cells. However, comparative studies have established that the oocyte and egg are unique and can so efficiently reprogram somatic cell nuclei. Therefore, a better idea is that by focusing on the molecular mechanisms of oocyte/egg -mediated reprogramming, these molecules and pathways can be incorporated into the iPS route to improve the efficiency of direct cellular reprogramming. Therefore, perhaps, the future of stem cell therapy will conclude closer connections between these apparently distinct approaches.

Keywords: Cellular Reprogramming, iPS, Embryonic Stem Cell, Nuclear Transfer

Embryology

I-6: Follicle Development in Culture of Frozen / Thawed Human Ovarian Tissue

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The primary therapeutic goal for the oncology patient is survival. Recent advances in diagnoses and treatment of neoplasia have resulted in an ever-increasing number of patients being cured and resuming a normal life. However, recognized side effects of treatments used to eradicate malignancies are temporary or permanent sterility. The cryopreservation of ovarian tissue harvested before cancer therapy may preserve the reproductive function and/or restore fertility in women and young girls.
Good preservation of reproductive function, pregnancies, and births of healthy babies have been reported after ortho/heterotopic transplantation of cryopreserved ovarian tissue in patients with neoplastic disease.
In addition, long term culture and *in vitro* development of antral follicles to produce metaphase II oocytes for *in vitro* fertilization (IVF) may represent another alternative to overcome iatrogenic sterility.
Several developmental milestones already accomplished include follicle activation, preantral follicle growth, follicle differentiation, and oocyte maturation using cryopreserved human cortex.
The length of the follicular growth phase from the primordial to Graafian stage and the changes in the trophic requirements of the cells, cellular interactions, morphogenesis, and the sheer increase in bulk as the antrum forms are major challenges for contemporary cell culture technology.
At any age the human ovarian follicle reserve is composed of primordial follicles, the abundance and lack of differentiation of primordial follicles makes this population an ideal choice for *in vitro* growth to obtain fertilizable oocytes for potential use in asissted reproductive technologies (ART) and fertility preservation programs. The ability to develop these immature follicles fully *in vitro*, also, would facilitate greater understanding of the mechanisms regulating oocyte development.
It is assumed that complete follicle development from primordial to the pre-ovulatory stage in humans takes up to eight months and it has been calculated that the time needed for a follicle to grow from the primary to the pre-ovulatory stage to be 84 days. However, there is no solid evidence to show that this is a continuous period of growth; indeed, it is likely that follicles grow *in vivo* in a 'start-stop' manner in response to local influences. It appears that oocyte development can be supported within a rapidly developing follicle. Indeed, this ''accelerated'' growth has been demonstrated in other (non-human) culture systems and fully grown oocytes have been obtained.
Complete oocyte development *in vitro* from the primordial stage has been achieved in mice, but the larger size and longer growth period of human follicles has made the interspecies translation of these techniques difficult. The challenge now is to define the in-vitro conditions that facilitate a rate of growth that supports normal human oocyte development.
Culture conditions: The development of refined complex media is ongoing, and new evidence continues to contribute to literature. When supplemented with human serum acting as a protein support, a-MEM appears to be the most suitable medium for *in vitro* follicular development, when compared with EBSS or Waymouth's medium. Among the factors that regulate the initiation and progression of primordial follicle development, KL and bFGF seem to be essential for the progression from primordial stage to primary stage; VEGF and GDF-9 are fundamental for the achievement of the secondary stage follicle; insulin, IGF-I, and IGF-II act as trophic factors for follicles and simultaneously stimulate follicular growth; N-acetyl cysteine (NAC), a free-radical scavenger, working in combination with FSH, plays an important role in follicle growth; and gonadotropins, FSH, and luteinizing hormone (LH) are essential for progression from the preantral stage to the antral follicle stage.
The approaches involve incubating small pieces of cortex, isolation and culture of growing follicles, and isolation and incubation of follicles isolated from cultured cortical strips.
It is recognized that, whereas cortical strip culture supports human follicle activation and growth to the secondary stage, follicle integrity and oocyte survival are only maintained for a relatively short period of time. Therefore to develop further, follicles must be released from the stromal environment, but many observations appear to confirm that local ovarian factors indeed inhibit development of follicles. The question remains as to whether rapid in-vitro growth results in the production of competent oocytes. It has been noted that the presence of stromal cells is vital during initial growth.
For culture of isolated follicles, preantral follicle isolation from cortical tissue is usually achieved by either mechanical dissection, enzymatic isolation, or a combination of both. Enzymatic isolation commonly uses collagenase and DNase to liberate follicles from stromal tissue, yielding many more follicles than mechanical dissection, and it has also been associated with enhanced steroidogenic potential in culture. Collagenase has been associated with follicle damage and poor survival in large mammals. Mechanical isolation, using fine needles, has the advantage of preserving follicular integrity by maintaining the basal lamina and thecal layers of the follicle but the procedure is protracted and laborious. The development progression of human follicles following isolation from the cortex is notable however isolation of follicles is much more difficult in humans than in other mammals because human cortical tissue is more compact and fibrous when compared with commonly used animal ovaries.
Cortical strip culture removes follicles from the *in vivo* endocrine and paracrine processes regulating growth rate; however, follicles will still be subject to the effect of follicle interactions and the influence of stromal cell

factors. It is clear that tissue shape and stromal density are important factors which regulate follicle growth initiation in-vitro, as solid cubes of cortical tissue show a lower rate of growth initiation. In contrast, when stromal cells are removed and the tissue is cultured as flattened 'sheets', the initiation rate is greater and follicles grow faster. The physical environment of the follicles within the cortical tissue affects their response to stimulatory and inhibitory factors and therefore influences their ability to grow.

Once follicle growth is initiated within the strip, they can develop to multilaminar stages. At this point, the cortical strip environment becomes inhibitory to further growth. Therefore, strip culture does not support optimal development of all stages and a multi-step culture system is required to support complete development. Pre-antral follicles can be mechanically isolated from the cortex culture system after six days and placed within an individual culture system for further development to antral stages. In addition, with tissue culture systems, the use of high oxygen concentration appears to be important for facilitating oxygen diffusion to the follicles within tissue segments, as it is believed that oxygen deficiency may cause centralized necrosis. Central necrosis is thought to be the main cause of damage to tissue and cells and may reduce the ability of oocytes to continue to develop.

Measures to support the three-dimensional follicular architecture and thereby maintain intra-follicular cell association in-vitro have been employed in the culture of mammalian follicles for almost 20 years and it has been demonstrated, largely using rodent systems, that the use of suspension cultures, mineral oil sheaths, hydrophobic membranes and follicle encapsulation result in the promotion of follicle growth and the attainment of developmental milestones. The ability of the system to promote human follicle growth *in vitro* from the earliest stages at rates that are accelerated in comparison with the *in vivo* environment is indeed promising but we need to know if oocytes produced by these systems are deleterious to oocyte epigenetic health and normality.

Significant progress has been made but further optimization is required to routinely complete the *in vitro* development of the stages detailed here. Translation of any *in vitro* human follicle growth system into a clinical setting will require rigorous testing to determine the normality and health of *in vitro* grown oocytes before the application of IVF procedures.

I-7: Human Ovarian Tissue Cryopreservation: State of The ART

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In recent years, advances in the diagnosis and treatment of childhood, adolescent and adult cancer have significantly improved the survival rate and life expectancy of cancer patients. However, chemotherapy and radiotherapy treatments are gonadotoxic and may induce the loss of ovarian function and fertility with consequent premature ovarian failure (POF). A number of factors determine the level of ovarian damage caused by chemotherapy: the type of drug, administration protocol (duration and dosage) and the patient age. Cyclophosphamide and other alkylating agents are the most toxic factors for the ovary, producing a decline in primordial follicle density which is exponential as the dose increases. Moreover the effect of chemotherapy on female gonadal function is related to the patient's ovarian reserve: younger patients are less likely to experience severe POF. Ovarian damage from radiotherapy depends on the dosage and irradiation field. In particular more than 30 Gy hypothalamic/pituitary radiation produces impaired secretion of gonadotropins, 2-5 Gy ovarian/uterine radiation results in half of follicles being lost with consequent impaired gonadal function and more than 5 Gy abdominal radiation causes irreversible ovarian failure.

Different options are available to preserve fertility in cancer patients and give them the opportunity to restore fertility and also to become mothers when they have recovered from disease. The choice of the most suitable strategy for preserving fertility depends on the type and timing of therapy, the type of cancer, patient age and the partner status.

Ovarian tissue cryopreservation is an important strategy for conserving both steroidogenic and gametogenic functions and it is the only option available for pre-pubertal girls, women with hormone-dependent tumours and women who cannot delay the start of chemotherapy. Cryopreservation of ovarian tissue could be applied in different malignant diseases (systemic, extra-pelvic and pelvic) and in benign haematological, autoimmune or genetic diseases. Other benign diseases, such as ovarian endometriosis or recurrent ovarian cysts are also indications for ovarian tissue cryopreservation.

Ovarian tissue can theoretically be frozen using two different approaches: as fragments of ovarian cortex or as an entire ovary with its vascular pedicle. To date, human ovarian tissue cryopreservation has so far been almost exclusively limited to avascular cortical fragments. The cryopreservation of ovarian tissue is a complex procedure because the heterogeneous cytological composition (oocytes, granulosa and stromal cells) of the ovarian tissue makes the adaptation of protocols for ovarian tissue cryopreservation difficult. Many factors influence the effect of ovarian cryopreservation, such as cryoprotectant, frozen carrier, cortical sample size and freezing procedure. The standard method for ovarian tissue cryopreservation is slow freezing/rapid thawing. This results in good preservation of all types of follicles and is the method of choice for the cryopreservation of ovarian fragments. Another method, albeit experimental, is the vitrification of cortical fragments. Vitrification is technologically promising. It is simpler and one cryocycle is less time-consuming and cheaper than the conventional freezing method. However, results have shown that vitrification can guarantee the storage of viable follicles after warming, but conventional freezing is more effective. Moreover during the vitrification procedure the tissue is cooled at an extremely rapid rate, because it is brought into direct contact with liquid nitrogen, particularly when specific "open carrier" for ultrarapid cooling are used. There is also a hypothetical risk of disease transmission

through contact with accidentally contaminated liquid nitrogen. Therefore, for cryopreservation of human ovarian tissue, conventional freezing is more promising than vitrification at present.

The main aim of ovarian tissue cryopreservation is to re-implant cortical ovarian tissue after thawing. Tissue is re-implanted into the pelvic cavity (orthotopic site) or a heterotopic site such as the forearm or the abdominal wall once oncological treatment is completed and the patient is disease-free, in order to restore ovarian function and normalize levels of gonadotrophins. The advantage of orthotopic transplantation is that follicles are in their natural micro-environment and can develop with fewer problems. Moreover, it allows for a natural pregnancy to occur. To date worldwide, 19 children have been born as a result of orthotopic transplantation of frozen/thawed ovarian tissue. Until now all pregnancies obtained after transplantation of cryopreserved ovarian tissue were observed in adult patients at the time of harvesting. However there is no reason to doubt the capacity of pre-pubertal ovarian cortex to develop and function correctly after re-implantation. Animal studies have demonstrated that puberty and cyclic hormonal activity can be restored by re-implantation of fresh and frozen-thawed pre-pubertal ovarian tissue in both pre-pubertal and adult mice. In humans only two experiences have been reported. For heterotopic transplantation there are many unresolved issues. One of them is the optimal site for transplantation. In theory, the optimal site should be vascularised, because the rapid revascularization of the graft is crucial for the survival of ovarian follicles. In addition the site of re-implantion should be easily accessible without any invasive procedure, because repeated ovarian transplantation and/or egg retrieval may be necessary if early graft exhaustion is expected. Another concern related to transplanting ovarian tissue to the heterotopic site is the environmental factors that can affect the follicular development such as temperature, pressure, space for follicular growth, peritoneal fluid, cytokines, angiogenic factors, and hormonal milieu, so the oocyte maturation process appears to occur differently than in the orthotopic environment.

The main drawback of the cryopreservation of ovarian cortical strips is the ischemia that occurs at the time of ortho/heterotopic transplantation. Because these small cortical pieces are grafted without any vascular anastomoses, they are completely dependent for their survival on the time necessary for establishment of neovascularization after grafting. Therefore, the reduction of the ischemic interval between transplantation and revascularization is essential for maintaining the viability and functional lifetime of the graft. To this end, cryopreservation of the whole ovary with an intact pedicle and vascular supply could potentially overcome this problem because reperfusion will occur immediately upon re-transplantation and anastomosis. This strategy is still experimental and no human studies of whole ovary transplant after cryopreservation have been performed to date.

Ovarian tissue cryopreservation offers real hope for fertility preservation to young cancer patients and it should be offered before gonadotoxic chemotherapy in all cases where there is a high risk of POF.

I-8: Sperm Chemotaxis towards Progesterone, A Guiding Mechanism That May Be Used to Select The Best Spermatozoa for Assisted Reproduction

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Background: Spermatozoa are able to sense an attractant molecule gradient and as a consequence, orient their movement towards the source of the attractant. This mechanism is known as sperm chemotaxis. In recent years, our laboratory contributed to the knowledge of several features of mammalian sperm chemotaxis. These include the size and physiological state of the chemotactic sperm population, the biological sources of attractants, the identity of a physiological attractant candidate, the species specificity of the phenomenon, some signal transduction pathways by which chemotaxis is induced, the chemotactic pattern of movement, and the verification of sperm chemotaxis under vivo conditions. The aim of the project was to characterize the sperm chemotactic response and its potential applications for assisted reproduction.

Materials and Methods: In order to study mammalian sperm chemotaxis we first developed a method to objectively assess sperm directionality and additional kinetic parameters. This method consists on a device (a chemotaxis chamber) and a videomicroscopy and image analysis system. The chamber has two wells (W1 and W2) that are connected by a bridge over which a capillary space filled with culture medium is formed by adding a coverslip. The cells are loaded in W1, while the attractant in W2, which immediately diffused from W1 to W2 forming a unidirectional long-lasting gradient across the bridge. Cells freely swimming over the bridge are digitally recorded and the tracks evaluated by computer image analysis which includes a software developed in our lab.

Results: We first characterized the sperm chemotactic response under *in vitro* conditions. We described for the first time the chemotactic response in animal sperm from mouse, rabbit and bovine. In addition, spermatozoa respond to several biological sources of chemoattractants like follicular fluid, oviductal fluid and conditioned medium of the egg-cumulus complex. To elicit a chemotactic response spermatozoa must have accomplished capacitation, whereas only a small subpopulation of spermatozoa (~10% of the cells) are chemotactic at any given time. Follicular fluid that has been obtained from one species may attract spermatozoa of other species, suggesting that chemotaxis may not be a species-specific phenomenon. Several sperm attractants have been reported, however, we observed that progesterone seems to be of physiological importance. Thus, a small quantity of progesterone, which is secreted by the cells surrounding the egg, is able to attract spermatozoa. Moreover, the chemotactic response was suppressed when either egg conditioned medium was depleted of progesterone or the sperm progesterone receptor was blocked. We next investigated the molecular mechanisms that lead to the sperm chemotactic response towards progesterone.

Thus, the transmembrane adenylyl cyclase-cAMP-protein kinase A pathway and soluble guanylate cyclase-cGMP-protein kinase G pathway, calcium mobilization and protein tyrosine phosphorylation appear to be involved. Chemotactic spermatozoa swimming towards an attractant source are indistinguishable from non chemotactic sperm, but we observed that they showed a unique pattern of movement when they returned towards the source of a chemotactic concentration of progesterone. Next we observed under in vivo conditions that the chemotactic mechanism helps to transport sperm to the fertilization site, thus validating the *in vitro* observations. As whole, the results obtained along years lead us to design a new sperm selection assay based on sperm chemotaxis towards progesterone.

Conclusion: Human spermatozoa are able of chemotactic response to very low levels of progesterone. This steroid has been considered a physiological attractant since by the time of ovulation it is secreted by the cells surrounding the egg. Only capacitated spermatozoa (those ready to fertilize the egg) may show chemotactic behavior. Therefore, a sperm population enriched with capacitated spermatozoa by means of chemotaxis may be considered a physiological selection procedure that may be useful for assisted reproduction technologies.

Keywords: Sperm Chemotaxis, Progesterone

I-9: Activation and Selection of Sperm for IVF and ICSI

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Background: During spermatogenesis the sperm nucleus exchange histones against cystein rich protamines, which stabilize the sperm chromatin and protects the DNA against damages. At ejaculation the age of the patient, handling routines, processing of the semen sample and the storage of the final sperm suspension might damage the DNA.

Sperm chromatin stability and DNA damages are evaluated with different methods are time-consuming, requires expensive equipment's and does not take into account the individual intra-variability in semen quality. Conventional ICSI has therefore been shown to transfer paternally derived defects to the children. Furthermore, the selection of a healthy sperm for ICSI, via IMSI (Intracytoplasmic Morphologically-selected Sperm Injection), is very time-consuming and requires additional equipment.

However, the sperm's ability to bind to the zona pellucida can easily be evaluated via the hyaluronan- binding assay (HBA), and if the HBA score is above 70% normal IVF can be used in border-line male factor prepared sperm suspensions. The denudation of oocytes with non-toxic ICSI-Cumulase does not interfere with the sperms binding and fertilization ability, thus denuded oocytes could potentially be fertilized high HBA scored sperm. Thus we could reduce the use of ICSI and subjective selection of sperm for ICSI.

Since human sperm also have receptors for a growth factor involved in the metabolism and motility of sperm it might be suitable for activation of sperm motility of immotile sperm from the testis?

I will give an overview of the effects of GMCSF on the male reproductive tract, how it affects the performance of sperm and how it can be used for physiological selection of motile testicular sperm for ICSI.

Denudation of oocytes with a highly purified, non-toxic, pathogen-free and recombinant human hyaluronidase solution (ICSICumulase) does not interfere with the sperm binding receptors on the zona pellucida. Thus if the sperm express zona pellucida binding sites on their plasma membranes they bind to and fertilize the oocyte. Spermatozoa that express such receptors are more mature, better morphology, higher chromatin stability, lower frequency of aneuploidy to receptors on the zona pellucida.

A high zona binding competency correlates to the pregnancy rate, thus border-line sperm suspensions with a high HBA score can be used for insemination of ICSICumulase denuded oocytes. The sperm have direct access to the zona pellucida facilitating the fertilization step and there seems to be no differences between the fertilization rate between cumulus-intact or denuded oocytes.

Sperm express receptors for GMCSF on their heads and mid-pieces and when exposed to this growth factor they become highly hyperactive. Even immotile testicular sperm become highly motile facilitating the selection of progressive sperm for micro-injection. These embryos have a higher implantation rate leading to high ongoing pregnancy rates.

Conclusion:

1. Denudation of sensitive oocytes with non-toxic, recombinant human hyaluronidase is a more physiological, resulting in better embryo quality and higher pregnancy rates.
2. The denudation does not interfere with the sperm's ability to bind and fertilize oocytes. Borderline sperm suspension, with proven oocyte binding ability, can therefore be used for IVF, thus the natural selection of sperm reduces the subjective selection of sperm occurring at ICSI.
3. GMCSF is a natural growth factors found abundant in both the female and male reproductive tract. The sperm has receptors for GMCSF in the head and the midpiece. GMCSF initiates hyperactivation of sperm from both normozoospermic and male factor patients.

In immotile testicular sperm GMCSF initiates motility, which facilitates and shortens the time for selection of sperm for ICSI.

If such sperm are used for ICSI it increases both the fertilization and clinical pregnancy rates.

I-10: QA in The ART Laboratory

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The establishment of a high quality ART program requires a multi-disciplinary effort, involvement and commitment of all staff members - team work. It is of vital importance that all aspects of the treatments are taken into consideration and that you already from the start use international guidelines, standards and indicators to initiate the construction of a well-organised ART program that meets the highest international standards and

requirements.
In order to obtain and maintain an optimal level of patient care and success rates, a total formal quality management (TQM) system must be implemented. Professional international and domestic guidelines, accreditation bodies and quality management models can be used. Implementation of a TQM system is associated with a huge workload and requires investments. However, due to increased standardization and efficiency of all processes and procedures, improved transparency and traceability of all actions performed, the quality of service improves substantially, which is of benefit for both the customer and the staff. The TQM system covers all aspects of the clinic and the procedures, from facility parameters, initial evaluation of a patient, stimulation protocols, culture and embryo transfer techniques, gestational period, to parameters in connection with the delivery of the baby. TQM is not only designed to detect and eliminate problems, but also to constantly improve the clinic's performance by incorporate latest know-how and techniques, as well as to make all procedures and processes more effective and reproducible.
It is important to produce an organizational chart, which clearly shows the hierarchy, the line of communication, authority and responsibility. This avoids many unnecessary misunderstandings, conflicts and leads to a more effective communication and interaction between different staff categories (Job descriptions).
Standardization is obtained by the introduction of standard operational procedures (SOPs) that can be of technical (equipment's) or clinical type. The SOPs are upgraded yearly and the latest international technologies and bench mark processes and procedures are incorporated in a never-ending project.
International defined key performance indicators (KPI) are used to evaluate the program, which reduces misinterpretations and facilitates communication and auditing. Unfortunately, many clinics only focus on the ultimate outcome - pregnancy rates, which do not assess the overall quality of the ART program.
A couple that undertakes a treatment in a "controlled" environment will be given an optimal and safe treatment in a safe environment by highly educated, skilled and professional staff members. One should also remember that the procedures are performed within facilities especially designed for ART, equipped with calibrated certified ART-equipment's, using embryo-tested disposables and media products, proven to give high reproducible and consistent pregnancy rates. The couples are therefore given high value for money, but it also simultaneously reduces the time spent for auditing and increases the inflow of patients to the clinic.

I-11: Dedifferentiation of Mouse Fibroblast Cells by Chemical Induction

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Induced pluripotent stem cells (iPSCs) generated by ectopic expression of four transcription factors have great promises for regenerative medicine in humans. Since the initial report of iPSCs by viral transfection, ample efforts have been made in the generation of iPSCs through non-viral approaches. Small molecules offer the advantages of low cost without genomic modification and have been used to induce cell reprogramming for lineage trans-differentiation and for maintaining pluripotency of stem cells. Stem cells and cancer cells share many common features, implying that there are similar underlying mechanisms in their development. Chemicals that can activate common pathways in both cancer and stem cells may lead to interesting de-differentiation or trans-differentiation processes. We therefore investigated the possibility of reprogramming somatic cells with carcinogens at non-genotoxic levels. By in silico high-throughput screening of the Sigma-Aldrich's inventory for cancer research, we identified 16 candidate chemicals and treated B6/129 mouse embryonic fibroblasts (MEFs) at passage 3. The protocol was consisted of a 16-day treatment period followed by 5 days of recovery. From recovery day 2, colonies appeared at an efficiency of 0.02%. These colonies were positive for both alkaline phosphatase and surface specific embryonic antigen-1 (SSEA-1) at a comparable level to those of mouse embryonic stem cells (ESCs). Global gene expression analysis with a 38K gene MEEBO microarray revealed that the induced colonies expressed 122 genes that are ESC-enriched, including down-regulated somatic markers and up-regulated stem cell markers. In conclusion, combined chemical treatments herein trans-differentiated or de-differentiated MEF to an intermediate state within the mesodermal lineages.

I-12: Nuclear Reprogramming in Bovin Somatic Cell Nuclear Transfer

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Somatic cell nuclear transfer (SCNT or cloning) returns a differentiated cell to a totipotent status; a process termed nuclear reprogramming. Reproductive cloning has potential applications in both agriculture and biomedicine, but is limited by low efficiency. To understand the deficiencies of nuclear reprogramming, our research has focused on both candidate genes and global gene expression patterns in cloned bovine embryos/offspring as compared to those generated by conventional reproduction. We also studied the telomere restoration, growth patterns, behavior, reproduction, and milk and meat compositions of cloned animals. Cloned animals that survived beyond the first month of life are generally normal and healthy. We found aberrant expression patterns in both imprinted as well as X-linked genes in term cloned calves. The expression profiles of cloned blastocysts, however, closely resembled those of the naturally fertilized embryos but were considerably different from those of their nuclear donor cells. Our findings suggest that cloned embryos have undergone significant nuclear reprogramming by the blastocyst stage. However, it is possible that during re-differentiation in later development gene expression aberrancies occur. Additionally, small initial nuclear reprogramming errors may be manifested during subsequent development.

I-13: Blastocyst Culture and Freezing

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It should now be beyond doubt that blastocyst culture results in better pregnancy rate (PR). This calls for fewer embryos to be transferred, and indeed blastocyst culture is of most value when the intention is to put back one embryo at a time.
The PR with one top-grade blastocyst is 45-65% in different studies. If two top-grade blastocysts are transferred, the PR usually does not differ, but the rate of multiples increases manyfold. Given a good freezing programme, the cumulative PR after one fresh and one frozen single blastocyst transfer exceeds that of a fresh two-blastocyst transfer.
Culture can be done open, or in drops under oil. The use of a low-oxygen (”three-gas”) incubator is beneficial.
ET shall be performed on day 5. PR decreases if fresh ET is done on day 6, but embryos frozen on day 5 or day 6 perform equally when transferred in a freeze-thaw cycle. Preferrably, this should be a spontaneous or a FSH stimulated cycle. Oestrogen / progesteron cycles may result in a higher rate of miscarriages.
Only top-grade or next to top-grade blastocysts should be frozen to ensure good results. The fact that there are fewer day 5 embryos to be frozen than after 2-3 days culture is an advantage rather than the opposite, as the PR per embryo is higher.
Puncturing of the expanded blastocyst is crucial and can be done just as well with a needle as by laser. Most studies have shown that vitrification is superior to slow-freezing, but these studies often have surprisingly poor results for slow-freezing with low surviving rates and low PR. At our unit, 76% of thawed blastocysts survive and are transferred with a PR after transfer of a single blastocyst of 56%, which is the same as the comparable fresh transfer.
Where three or more embryos are transferred, there is no advantage of blastocyst culture. Indeed, it only results in even more multiple pregnancies.

Ethics and Reproductive Health

I-14: The Impact of Disclosure Decisions on Donor Gamete Participants: Donors, Intended Parents and Offspring

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To discuss the psychological impact of disclosure decisions on donor gamete participants including gamete donors, intended parents, and the children conceived through these third party reproductive techniques.
In the past decade, there has been a dramatic increase worldwide in the number of children born as a result of gamete donation. The growing demand for these programs has resulted in a trend toward greater openness and less anonymity in gamete donation and increasing calls for disclosure to offspring about their donor conception. Recent research on how parents of donor offspring make decisions about disclosure to offspring finds that even when couples disagree initially about whether or not to tell, they ultimately come to the same conclusion (whether they decide to tell or not to tell). Studies are also beginning to consider the psychological needs of gamete donors and how changing policies such as legislation giving offspring access to donor’s identity may influence intended parents’ disclosure decisions. While the psychological well being of donor-conceived children does not seem to be impacted by parents decisions to disclose or not, a recent prospective study concluded that families may benefit from openness about the child’s genetic origins.
In conclution, the increasing numbers of participants in programs of third party reproduction around the world appear to have accelerated the trend toward greater openness. Gamete donors, intended parents and offspring do not appear to have been negatively affected by this change.

I-15: The Emotional Impact of Infertility and Assisted Reproduction: Are Psychological Support and Counseling Helpful?

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To describe the emotional impact of infertility and its treatment and to identify the typical accompanying psychosocial stressors. Gender differences in the coping styles of men and women confronted with these stressors and effective treatment interventions will be discussed.
In recent years, the number of couples seeking treatment for infertility has grown substantially around the world. Several factors account for this demand including the postponement of childbearing in women, a growing public awareness of treatment options, and the availability of innovative techniques in assisted reproduction. While no longer considered psychogenic, the impact of infertility and its treatment is described as a state of ongoing distress. Infertile couples, often overwhelmed by the physical, financial and emotional demands of infertility treatment, may experience anxiety, depression, marital discord and social isolation. As a result, psychological support and counseling is an integral part of many fertility treatment centers. Women describe infertility as the most upsetting experience of their lives while men report less psychosocial distress. Prolonged treatment increases couple conflict and stress, but ultimately infertility results in a closer marital relationship. In addition to providing support for couples in crisis, fertility counselors provide guidance for those negotiating the sometimes ethically and psychologically complex decisions regarding assisted reproduction.
In conclution, psychological support is an integral part of fertility treatment.

I-16: Assisted Reproduction and Multiple Gestation: What Are The Psychological Consequences?

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To describe the social and historical impact of ART on multiple gestations and to discuss psychological issues unique to these families.
At the time of the birth of the Dionne quintuplets in 1934, only 33 cases of quintuplets had been reported in the literature and none of the quintuplets survived more than 50 days. Spontaneous higher order multiple gestation is still rare but the number of iatrogenic multiple pregnancies has dramatically increased as a result of assisted reproduction. Despite pretreatment counseling about the medical and emotional risks of having multiples, many infertile couples continue to want that experience and their religious affiliation, education and knowledge of multiple gestation outcomes were not predictors of that desire. The transition to parenthood of multiples can be difficult and couples often find themselves overwhelmed by the social, psychological and financial pressure of raising multiples. Mothers of multiples may experience social stigma, isolation, stress and depression. On the positive side, couples report increased marital solidification as a result of dealing with the stress of raising multiples.
In conclution, though raising multiples may provide unique rewards, couples are often unprepared for the stresses and challenges involved in raising them.

I-17: The Ethics of Using IVF and PGD to Conceive A Savior Child

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Until the advent of preimplantation genetic diagnosis (PGD) in the 90's of the last century, the only efficient means to find out whether an offspring has inherited a genetic disease from a couple who are carriers of the defective gene is through prenatal diagnosis (PND). However, if the fetus is found to have the genetic disease, presently the only available treatment is to abort the fetus, and this is not always acceptable to many people for a variety of religious and cultural reasons. PGD was originally developed as an alternative to PND in order to select genetically disease-free embryos *in vitro* before their transfer to the woman's womb, and in the past decade, it has widely used for couples at risk of monogenic diseases such as cystic fibrosis, thalassemias, X-linked diseases such as Duchenne muscular dystrophy and chromosomal structural abnormalities such as translocations and aneuploidies. Recently, PGD has been used in combination with human leukocyte antigens typing (HLA-typing) to select embryos that are not only disease-free but also tissue-matched to be compatible with an existing child. Since the child is capable of donating its cord blood for stem cell transplantation to save an afflicted sibling's life, it is dubbed a "Saviour Child". The first PGD/HLA-matched "Saviour Child" was created in 2001 to save its sibling with Fanconi Anemia, and the technology has since been extended to treat other conditions including thalassemias, immunodeficiencies, ataxia telangiectasia, Omenn syndrome etc. Many parents who have a child afflicted by a genetic disease that they have passed on are able to reproduce another child who is disease-free and HLA-matched as a cord blood donor to save its sick sibling. While many afflicted families have welcome PGD/HLA-typing as a medical "gospel", the technology has also raised many ethical and social quandaries, and the public in different parts of the world has far from reaching a consensus regarding its uses. This paper addresses some of these ethical issues.

I-18: The Ethics of Managing Extremely Premature Neonates(EPRN) between 22-24 Weeks

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When faced with a preterm delivery of an extremely premature neonate between 22-24 weeks (EPRN), doctors are confronted by a three-fold uncertainty of age, viability and prognosis that influence their decision whether or not to resuscitate the neonate. Firstly, in the delivery room the preterm neonate's age is seldom precisely known, and successful resuscitation depends on the neonate's accurate age; secondly, human fetal viability is uncertain and is determined by fetal lung development that takes place between week 22 to 25 of gestation as well as varying geographic contexts and institutional technical capabilities. Thirdly, EPRN presents with the uncertainty of long term morbidity. Outcome studies suggest that neonates on the high end of the "viability spectrum" have a survival rate about 40-50%, and for those who survive, about the same percentage will have moderate to severe long term morbidities including cerebral palsy, blindness, deafness, other neuro-sensory deficits, chronic lung diseases and mental and motor retardation. These uncertainties are translated into a three-fold dilemma of whether or not to treat the neonate, who should make the decisions and on what moral grounds are the decisions made. This paper attempts to address some of these questions.

I-19: Informed Consent of Infertile Couples in Iran: Legal Controversies

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Informed consent is considered the most important step in clinical interventions. According to the Beauchamp's definition the consent is: "...an autonomous act by a patient or research subject to expressly permit a professional person to perform a medical action on the patient or to include a person in a research project...". This definition is widely accepted, but recently, the position of the consent is changed to a legal document which is necessary to protect the physician or researcher. For obtaining a good consent, it must be ensured that the patient understands the nature of their condition and the risks and benefits of the treatment procedure and its alternatives, and agrees to it voluntarily. In Iran due to current penal code, the consent is in controversy. There are 3 expressions related to the informed consent in penal code of Iran: 1. Permission "Persian: EZN" article 318 of penal code, 2. Consent "Persian: REZAYAT" article 59 of penal code and 3. Disculpation "Persian: BARAAT" article 60 of penal code.
It seems that all the 3 articles can be summarized in "informed consent" with the following conditions:
1. Full information about the disease and treatment procedure including duration, possible pain, complication and … which should be presented both verbally and written in the consent.
2. Informed consent is not a contract but it is the process of making decision between patient and physician which means "the patient knows what is going on".
3. Informed consent cannot disculpate any body; the malpractice can be evaluated if the patient claims. If the physician makes a mistake, he will be punished regardless of presence of an informed consent signed by the patient.
4. There is no need for husband consent in the case of wife's treatment, but as having a baby cannot be considered as "a treatment", the consent for assisted reproduction must be given by both.
5. Informed consent can be taken back in any time, but it seems that the consent in assisted reproduction can be taken back just by wife or husband.
6. According to the law, the consent for therapeutic abortion can be given only by the pregnant woman and there is no need for the husband's consent. This includes the consent for pregnancy termination.
7. One of the most important issues in informed consent is the patient's capacity. Before getting the consent the physician must be confident that the patient understands the situation and can communicate with the doctor. As using assisted reproductive technique is an intentional act for a couple, so, the capacity is not an issue in this regard. Therefore, no patient without capacity can participate in assisted reproduction programs.

Female Infertility

I-20: Sclerotherapy in Recurrent Endometrioma

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Endometriosis as a common hormone-dependent gynecologic disease is seen in 10-15% of women of reproductive age. On the other hand, 20-50% of women with infertility have endometriosis and 30-50% women with endometrioma are infertile. One of severe form of endometriosis is endometrioma. Surgery is considered as essential treatment for endometrioma with the recurrence rate of 10%. Many researches showed that the second surgery which performed on these patients my lead to premature ovarian failure (POF) or decreased ovarian reserve. Another option for treatment of endometrioma is sclerotherapy using several materials such as ethanol 50-800, methotrexate, tetracycline and anastrazole. Most of studies in this field used ethanol in two methods: 1. Aspiration of endometrioma, irrigation, injection of ethanol and repeated aspiration after 10 minutes. 2. Retention of ethanol after irrigation. It seems that retention is more effective. In a clinical trial study, we investigated ethanol for the treatment of recurrent endometrioma before IVF. The result showed that pregnancy rate was insignificantly increased in patients treated with sclerotherapy compared to control group (33.3% vs. 15%, p>0.05). The results pointed to a better trend toward the ethanol. We think that in the future sclerotherapy may be used instead of repeated surgery more than before.

I-21: The Expression of TLRs in Follicular Cells of Poor Ovarian Responder

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Background: One of the most annoying problems in IVF is poor ovarian response. It is anticipated that 5-18% of all IVF cycle are affected by poor response to ovarian hyperstimulation. Poor response to goandotropin may lead to decline in the pool of embryos for transfer or cryoperservation, and decrease pregnancy rates. Different mechanisms explain poor ovarian response for example decreased number of FSH receptors in granulosa cells, anti-FSH IgA and IgG potentially exerting a local FSH antagonizing effect in maturing follicles, the presence of a specific FSH receptor-binding inhibitor in the follicular fluid, the higher FSH threshold to stimulation follicle development. As mentioned above, likely immune system is involved in the mechanism of poor responder and affects the steroidogenesis. First line of immune system is pattern recognition receptors (PRRs) as a compartment of innate immunity. The most important group of PRRs which also identified in female reproductive tract is Toll like receptors (TLRs) family. So far, TLR1-10 are characterized in human. TLR1, 2, 4, 5, 6 and TLR10 are expressed on the cell membrane and they recognize lipid and protein ligands. TLR3, 7, 8 and TLR9 can detect nu-

cleic acid from pathogens are found in endosomal compartment. In addition these receptors recognize a broad range of endogenous ligands including heat shock proteins, hyaloronan, host RNA, and reactive oxygen species (ROS). So TLRs play abundant immunologic and physiologic roles in reproductive system. Therefore, the aim of this study is to investigate TLR1.2, 3, 5, 7, 8 genes expression in follicular cells obtained from ovarian poor response women in compare to normal women.

Materials and Methods: All procedures were approved by the Royan Ethics committee and informed consent was obtained prior to the collection of samples. Forty patients (20 infertile ovarian poor responder patients and 20 normal women with male factor infertility) underwent controlled ovarian stimulation with monitoring E2 levels and pelvic ultrasounds. Gonadotropin doses were then adjusted accordingly and monitoring was continued until patients received 10,000 IU hCG intramuscular. Oocyte retrieval was performed approximately 36 h after hCG. The follicular fluid was obtained from the largest follicle (>18 mm) visualized on ultrasound before using any flushing medium. This follicle was aspirated with a 17-gauge Cook needle attached to 100 mm Hg pump-operated aspirator. It was the first puncture of the oocyte retrieval. The follicular fluid was transferred to a sterile Petri dish, and the oocytes were then removed. The follicular fluid was placed into a 15-mL conical tube and centrifuged at 300g for 5 min. The supernatant was removed for further proteomic study. Total RNA was extracted separately from cellular pallet in each group using TRI reagent and treated with DNaseI. First strand cDNA synthesis was performed using oligo dT primers and Superscript II reverse transcriptase system. RT-PCR and Quantitative PCR was performed using the prepared cDNA and primer for TLR1-10. Relative TLR expression quantities were compared between two groups. The threshold cycle values were normalized against the threshold value of human β-actin. Differences in normalized expression values between samples were tested for significance using ANOVA statistical test. The results were expressed as mean ± SEM. The level of statistical significance was set at $p < 0.05$.

Results: TLR1-10 genes were expressed in follicular cell of both, case and control groups. The mean relative expression of TLR1, 2, 4, 6 genes were significantly higher in poor ovarian responder, TLR5 and 8 expressions were higher in poor ovarian responder but not significant. TLR 3 , 7, 9 and TLR10 was lower in patients with poor ovarian responder in compare to normal women.

Conclusion: Our findings suggested that TLRs are involved in pathophysiology of ovarian poor response. It's been proposed that inflammatory markers such as IL6, IL8 and TNF-α are vary in poor responders. Since these markers are produced by TLRs signaling, therefore it's possible that these changes are a result of TLRs activation in poor responders.

Keywords: Follicular Cells, Innate Immunity, Ovarian Poor Response, TLR

I-22: Quality of Life in Patients with Endometriosis

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Endometriosis is defined as presence of endometrial tissue outside of the uterus. It is a common disorder of women in reproductive age. It is estimated to occur in ten percent iof women in this age and even more in patients with infertility and pelvic pain. Endometriosis varies in appearance from a few minimal lesions to massive ovarian endometriotic cysts that distort the tubo-ovarian anatomy and extensive adhesions and involvement of bowel, ureter, and bladder. Extra pelvic lesions are seen but with much less occurrence. This disease can decrease ovarian reserves of ovums and chance of premature menopause is increased especially with bilateral ovarian involvement. This complication is seen after surgical treatment of endometrioma and should be discussed with patients' before operation and a full consent should be taken.

There are many diagnostic modalities for endometriosis such as combination of some markers and imaging techniques such as TVS, TRS, and MRI. Imaging techniques have a high sensitivity and specificity for ovarian endometriosis but not for peritoneal or deep infiltrative endometriosis (DIE). The gold standard for diagnosis of endometriosis is laparoscopy and histopathologic evaluation of lesions. Many classification systems were perposed but most of them are subjective and correlates poorly with pain symptoms but may be of value in infertility prognosis and management.

Medical treatments are not indicated for patients with endometriosis and infertility but should be considered for those with pain and as a adjuvant after surgical treatment. In those patients with infertility laparoscopic treatment of endometriosis or controlled ovarian hyperstimulation with intrauterine insemination (COH-IUI) and assisted reproductive technology (ART) are the best modalities. ART is the method of choice for those with severe distortion of tubo-ovarian anatomy. Because hormonal suppressive treatment does not cure endometriosis recurrence or persistence of endometriosis can be expected in nearly all patients after the cessation of medical treatment, and this is positively correlated with the severity of endometriosis.

The main goal of laparoscopic treatment of patients with pain is to resect all endometriotic lesions as much as possible. It is the most difficult pelvic operation and should performed by an expert laparoscopist. When endometriosis causes mechanical distortion of the pelvis surgery should be performed to achieve reconstruction of normal pelvic anatomy. Surgical management of minimal and mild endometriosis appears to offer a small, but significant, benefit with regard to fertility outcome. Sometimes patients should be operated by a team of expert gynecologic laparoscopist and urologist or colorectal surgeon especially in thse with bowel and ureter involvement.

Even with advance surgery and medical treatment there is a real chance of recurrence of the disease and this subject should be discussed with the patient. She should be advised about this chronic disease that potentially affect her quality of life and should be informed about the potential complications of the disease and medical or surgical treatments. Coping with endometriosis as a

chronic disease is an important component of management. Psychiatric consult may be helpful in those patients with intractable pain and those with depression following to the disease.

I-23: The Role of Hysteroscopy in Patients with Repeated IVF Failure

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The success of reproduction, although gradually increasing over the years, many couples also had been left frustrated following repeated failed attempts. Implantation of the embryo, can only take place in a receptive uterus and inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures. Endometrial receptivity can be reduced by morphological and/or molecular variables. Endometrial morphological irregularity like uterine polyps, septa, leiomyomata and adhesions can prevent implantation. Hysteroscopy is one of the earliest approaches to direct study of the uterine cavity in case of repeat abortions, infertility and especially in repeated IVF failures. Also There are many evidences for the benefit yielded by hysteroscopy in improving pregnancy rates in patients with repeated IVF failure, even in the case of morphologically normal endometrium . It has been shown by recant data that hysteroscopy in the cycle preceding a subsequent IVF attempt nearly doubles the pregnancy rate in patients with at least two failed IVF attempts compared with starting IVF immediately. Injury-induced inflammatory response facilitates the transition of a non-receptive uterus into a receptive uterus which may increase pregnancy rate in these cases. In conclusion it seems legitimate to perform hysteroscopy in women who have had IVF-ET failures .

I-24: Recurrent Implantation Failure

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Recurrent implantation failure (RIF) has many definitions, but usually authors consider RIF, the failure to achieve a pregnancy fallowing 2-6 IVF cycles in which more than 10 high grade embryos were transferred to the uterus. There are a lot of causes for RIF but two main causes of that are related to embryo and endometrium. Chromosomal abnormalities, zona hardening and problems in culture media are causes of RIF due to embryo development.And the factors such as polyps, fibroids, endometriosis and thin endometrium can affect implantation related to endometrium. The relationship between ART failure and thrambophilia remain largely inconclusive.The exact mechanism by which thromobophilia can cause implantation failure is not fully understand, But many Theories are affected such as: impaired vascularization, impairment of trophoblast function and immunologic mechanism, other causes of RIF may related to presence of hydrosalpinx, endometriosis, adenomyosis. About the treatment of RIF, there are controversies. The aim of all infertility specialists is to provide an evidence bored treatment for these patients.Removing or occlusion of hydrosalpinx and doing hysteroscopy before any additional treatment, and injury of endometrium just before COH, assisted hatching, blasto cyst transfer, zift, PGD, treatment by heparin in thrombophilia and co - culture, … but all of them need more research for approval.

I-25: Management of Hydrosalpinx in ART

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Hydrosalpinx, is one of the severe manifestations of tubal disease, is associated with significantly lower implantation and pregnancy rates, increased spontaneous abortion and ectopic pregnancy.This condition is usually due to PID but may also result from peritonitis of any cause or tubal damage from previous surgery.The mechanism for poor outcome in patients undergoing IVF is not yet clear. Many theories have been reported, including the embryotoxic effect, endometrial hostility, mechanical washout of embryos. several methods for treatment of hydrosalpinx Such as neosalpingostomy, salpingostomy, proximal tubal ligation, ultrasound-guided transvaginal aspiration of hydrosalpinx, hysteroscopic placement of an Essure and ultrasound-guided transvaginal aspiration and sclerotherapy have been used in practice. The decision to repair or remove fallopian tubes usually made intraoperatively based on the severity and prognosis for an intrauterine pregnancy. IVF is preferred for older women and for those with male factor or other infertility factors. Patients with poor-prognosis hydrosalpinges are better served by salpingectomy followed by IVF. laparoscopic salpingectomy prior to IVF showed an increase pregnancy, and live birth rates. Laparoscopic tubal occlusion is an alternative to laparoscopic salpingectomy in improving IVF pregnancy rates. Proximal occlusion of a hydrosalpinx by hysteroscopic placement of an Essure device may offer an alternative to laparoscopic surgery in patients with severe pelvic adhesion. A clinician may also be faced with the situation of identifying a hydrosalpinx for the first time during stimulation in ART cycle. In these situations aspiration of hydrosalpinges during oocyte collection may be effective in improving pregnancy rates. Finally in a prospective clinical study, ultrasound-guided transvaginal aspiration and sclerotherapy with 98% ethanol was performed before the IVF cycle. The results showed that sclerotherapy could improve outcomes of IVF, with no recurring sign of the hydrosalpinx and no adverse effect on ovarian reserve or responsiveness during the IVF procedure.

I-26: The Role of Stimulating Agents and Protocols in Prevention of Severe OHSS

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The goal of ovarian Stimulation for assisted reproductive technologies (ART) is obtaining multifollicular growth ovarian hyper stimulation syndrome (OHSS) is one of the most Severe and also potentially Life-threatening complications, which happens in 1-10% of IVF cycles.
The most common risk factors which are related to OHSS are polycystic ovarian syndrome (PCOS), younger age, high preovulatory serum E2 Levels and GnRH- agonist down regulatory serum protocol.
There are Several strategies to prevent OHSS, such as coasting, which is the most popular, GnRH antagonist protocol, clomiphen citrate and hMG and cetrorelix (GnRH Antagonist) protocol which recently, there has been a trend to use Such mild stimulation protocol to reduce OHSS and multiple pregnancy and *in vitro* maturation (IVM) of oocytes.
The introduction of GnRH antagonist makes soft Stimulation possible and it is the time to trigger ovulation with GnRH agonist. The last approach to reduce the risk of OHSS is elective freezing of all the embryos.
In IVM protocol, oocytes are collected from follicles in 8-12 mm size from unstimulated or minimally stimulated ovaries, and keep them in culture media until they get matured and then fertilized *in vitro*. There is no risk of OHSS and this clinical approach is patient-friendly.
Clomiphen /hMG/ cetrorelix protocol also is another option for such a patients who had previons OHSS experience in GnRH agonist long protocol.
This protocol can reduce the use of hMG, so it can produce more favorable outcome rather than GnRH agonist long protocol.
In summary each of the approaches has advantages and disadvantages and a prospective randomized study is necessary to prove their efficacy.

I-27: ART Protocols in Endometriosis

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Treatment strategies for the infertile couple with endometriosis must be based on the specific situation.
Traditional medical therapy for endometriosis, such as progestins, danazol, and GnRH agonists, have been shown to have little impact on enhancing spontaneous pregnancy rates despite well described beneficial effects on reducing pain.
For the young women with only minimal or mild endometriosis, expectant management may be the most appropriate treatment modality. However, for women approaching the end of their reproductive age, the chances of conceiving drop precipitously .In these women, intervention, in the form of COH/IUI, or *in vitro* fertilization (IVF) seems more rational.
For women with severe endometriosis, or when male-factor or a combination of etiologies are involved, assisted reproduction is more reasonable.
Considering the optimal stimulation protocol in endometriotic patients, it seems that they respond to ovarian stimulation in a manner that is similar to other infertility etiologies.
Long-termGnRH agonist suppression has been thought to improve IVF outcome for patients with endometriosis .The length of suppression varied from 6 weeks to 7 months. The mechanism of action for this effect has not been established.
Recently aromatase inhibitors are added to therapeutic modalities for the treatment of endometriosis-associated pain. With its efficacy in treating there are studies, to evaluate its utility in ovulation induction in ART cycles.

I-28: Role of Mevalonate-Ras Homology (Rho)/Rho-Associated Coiled-Coil-Forming Protein Kinase-Mediated Signaling Pathway in The Pathogenesis of Endometriosis-Associated Fibrosis

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Background: Endometriosis, a disease affecting 3-10% of women of reproductive age, is characterized by the ectopic growth of endometrial glands and stroma surrounded by dense fibrous tissue. Whereas, normal eutopic endometrium shows scarless tissue repair during menstrual cycles, which suggests that the endometriotic tissues have distinct mechanisms of fibrogenesis. During the development of endometriotic lesions, excess fibrosis may lead to scarring and to alteration of tissue function. It has been suggested that type I collagen is a major contributor to endometriosis-associated fibrosis. Alpha-smooth muscle actin (SMA)-positive myofibroblastic cells were frequently detected in the fibrotic areas of endometriosis lesions. We have previously demonstrated that endometriotic stromal cells can differentiate to alpha-SMA-positive myofibroblasts. One approach to understanding the pathogenesis of endometriosis is to investigate the mechanisms underlying the fibrogenesis associated with this disease. Using 3-dimensional collagen gel culture model, we have evaluated the extracellular matrix contractility and myofibroblastic differentiation of endometriotic stromal cells. Endometriotic stromal cells showed enhanced extracellular matrix contractility in comparison with normal endometrial stromal cells. Activation of the mevalonate-Ras homology (Rho)/Rho-associated coiled-coil-forming protein kinase (ROCK)-mediated signaling pathway with simultaneously enhanced myofibroblastic differentiation is involved in this mechanism. In the present study, we investigated the effect of various agents that target mevalonate-Rho/ROCK mediated signaling pathway for the treatment of endometriosis-associated fibrosis using the 3-dimensional collagen gel culture system.

Materials and Methods: Primary cultures of endometriotic cyst stromal cells were utilized for the experiments. The effects of simvastatin, Y-27632, fasudil, heparin and Decidualization on the contractile profile, morphology, cell density, and contraction-related molecule expression of these cells in the 3-dimensional collagen gel culture were investigated using laser scanning microscopy, collagen gel contraction assay, and Western blot analysis. The effects of these mevalonate-Rho/ROCK pathway-targeting agents on the cell proliferation, apoptosis, and cell cycle of endometriotic cyst stromal cells in 2-dimensional culture were also evaluated by methylthiazoletetrazolium (MTT) assay, 5-bromo-2'-deoxyuridine (BrdU) incorporation assay, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay.
Results: Mevalonate - Rho/ ROCK pathway - targeting agents examined in this study attenuated the contractility of endometriotic stromal cells by inhibiting mevalonate-Rho/ROCK pathway activation, cell proliferation, attachment to surrounding extracellular matrices, and the differentiation into the alpha-smooth muscle actin-positive myofibroblastic phenotype. These agents also induced the apoptosis and cell cycle arrest of cultured endometriotic stromal cells.
Conclusion: Research on endometriotic stromal cell biology using 3-dimensional collagen matrices offers new opportunities to understand the reciprocal and adaptive interactions that occur between cells and surrounding matrix in a tissue-like environment. Such interactions are integrated with the regulation of endometriotic tissue morphogenesis and dynamics that characterizes endometriosis-associated fibrosis. It is suggested that the enhanced extracellular matrix contractility of endometriotic stromal cells in the 3-dimensional collagen gel culture is associated with myofibroblastic differentiation and the activation of mevalonate-Rho/ROCK-mediated signaling pathways, and that modulation of mevalonate-Rho/ROCK pathways seems to be a novel therapeutic target for the treatment and prevention of endometriosis-associated fibrosis.
Keywords: Endometriosis, Contractility, Mevalonate-Rho/ROCK Pathway, Fibrosis, Myofibroblast

I-29: Surgical Management of Endometriosis Related Infertility

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The precise relationship between endometriosis and infertility is debated. Surgery is considered to play a role within the framework of the therapeutic options to cure infertile women with the disease even though its effectiveness is generally modest.
In fact, there is unquestionably the need to improve surgical techniques in this area. Specifically, two main aspects require optimization: 1. preventing the injury to the follicular reserve that follows surgical excision of ovarian endometriomas and 2. preventing postsurgical formation and re-formation of adhesions.
The comparison between the excision/stripping and the vaporization/coagulation techniques represents the main point of debate on what is the best procedure to remove ovarian endometrioma. Randomized controlled trials showed that the excision technique is associated with a higher pregnancy rate and a lower rate of recurrence although it may determine severe injury to the ovarian reserve. Improvements to this latter aspect may be represented by a combined excisional-vaporization technique or by replacing diathermy coagulation with surgical ovarian suture.
Barrier agents reduce but not eliminate the post-surgical adhesion formation in women with endometriosis. However, available studies are mainly based on II look laparoscopies performed few weeks after the intervention and data on fertility is lacking. Clinical trials including pregnancy rate as a specific outcome are warranted.

I-30: Prediction Models in IVF

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Initially, *in vitro* fertilization (IVF) was solely performed in couples with bilateral tubal occlusion. In 1992 intracytoplasmic sperm injection (ICSI) was discovered and initiated in couples with severe male subfertilty. Later on IVF/ICSI was also applied in couples who did not suffer from sterility, such as couples with cervical hostility, failed ovulation induction, endometriosis, unilateral tubal pathology or even unexplained subfertility. The major difference between the original indication and the indications for which IVF is conducted nowadays is that the couples with bilateral tubal pathology or severe male subfertility are sterile and have a (near) zero chance of natural conception and completely depend on IVF/ICSI for a pregnancy, while the other couples are subfertile: they do have chances of natural conception, which may or may not be better than with IVF.
Despite the lack of evidence that IVF is effective in subfertile couples, IVF is often considered as a last resort for all couples regardless of the etiology of their subfertility. Contrary to the perception of many, IVF does not guarantee success; almost 50% of couples that start IVF will remain childless, even if they undergo multiple IVF cycles. Subfertile couples should therefore be well informed about the chances of success with IVF before starting their first or before continuing with a new IVF cycle. Based on a couple's specific probability, one should decide whether the chances of success with IVF outweigh the burden, risks and costs of the treatment.
To do so, prediction models have been developed. Most existing models are of limited use for several reasons. They were developed before current clinical and laboratory protocols were established. They do not include the transfer of frozen-thawed embryos. They calculate pregnancy chances only for the first IVF cycle or after one failed IVF.
We developed a model that would calculate pregnancy chances during the complete IVF procedure, after failed cycles, and that included pregnancies after fresh and frozen-thawed embryo transfer which performed well after internal and external, temporal validation.

If couples start or continue with IVF, the aim should be to achieve optimal pregnancy chances with a low risk multiple pregnancies. The optimal embryo transfer strategy would be a ''individualized embryo transfer strategy'' that takes the woman's prognostic profile and embryo characteristics into account; a prediction model that is able to select which and how many embryo(s) should be transferred to obtain optimal ongoing pregnancy rates with low multiple pregnancy rates. To develop such a model, we first constructed a model that was able to rank embryos on day 3 after oocyte retrieval based on their ongoing implantation potential. We then developed an embryo transfer model that consisted of two variables: one variable being the sum of all coefficients of the IVF model and the second variable being the sum of all coefficients of the embryo implantation model. These models will be presented.
With help of these prediction models a more uniform treatment strategy is possible, and also a more 'patient tailored' treatment.

I-31: The Scientific Underpinning of ART in Unexplained Infertility

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Although intra uterine insemination (IUI) and *in vitro* fertilization (IVF) are widely accepted treatments among doctors and patients and practiced on large scale, it is good to realize that they have rarely been evaluated properly in randomized clinical trials or even in comparative cohort studies.
Although the first pregnancy after IUI was established in 1884, it was not until 2008 that the first and sofar only randomized clinical trial (RCT) comparing IUI versus natural conception was published. This trial found no evidence of a beneficial effect (RR: 1.4, 95%, CI: 0.90-2.0). Also the addition of superovulation by gonadotrophins to IUI -also only studied in one RCT- in the comparison with natural conception did not show any evidence of an effect (RR: 0.85, 95%, CI: 0.55-1.3) both on short and long term. The pooled data from the only two RCTs available on superovulation with IUI compared to superovulation and natural conception show a RR of 1.4, 95%, CI: 0.62-3.2.
Unfortunately, the same lack of evidence applies to IVF. Bob Edwards and Edward Steptoe applied IVF successfully in the 29-year old Lesley Brown who had infertility from blocked tubes, resulting in the birth of Louise Brown, the first ''test tube'' baby in the world. Subsequently, IVF was applied more and more, also for women with unexplained infertility. From a pathofysiologic point of view it makes sense that IVF is an effective treatment in couples in whom the egg and the sperm do not meet - for instance due to tubal blockage- since it allows fertilisation, where this can not be expected after intercourse. In older women with any reproductive abnormalities, there is no biologic plausibility to assume that fertilization *in vitro* would be superior over fertilization *in vivo*. This in itself is worrisome, but even more so in view of the massive epidemic of delayed child wish, since all epidemiologic data available so far pinpoint advanced female age as the most important negative predictive factor for chances of pregnancy after IVF. IVF does not improve the natural decline in female fertility. Most doctors have assumed that expectant management is not acceptable for these women and have offered assisted reproductive technology (ART) as treatment of last resort. As a consequence more and more 'subfertile' couples in whom the woman is of advanced age turn to ART for conception. Indeed, the mean age of women undergoing IVF is increasing year after year.
However, evidence of a beneficial effect of IVF is simply not there. Only two very small and underpowered RCTs compared IVF with natural conception and the pooled RR is 2.7 (95%. CI: 0.97-7.5). IVF was only compared in one trial and found no evidence of an effect (RR: 1.1, 95%, CI: 0.69-1.7) and IVF versus superovulation IUI was studied in two trials with a common RR of 1.1 (95%, CI: 0.75-1.5)
It may be that better patient selection based upon prognosis may provide new data on effectiveness of ART, and provisional data will be given in the presentation. For the time being we can only conclude that we have no evidence whatsoever to justify our practice of ART in couples with unexplained infertility. Until evidence of the contrary becomes available, we should counsel our patients with socalled unexplained infertility that we have found no abnormalities after the fertility workup and that therefore there is no disease and thus no treatment.

I-32: Avoiding OHSS in Ovarian Stimulation

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Multiple pregnancies and ovarian hyper stimulation syndrome (OHSS) are the most serious complications of ovarian stimulation and IVF. Indeed, they are more or less the only ones.
OHSS can effectively be prevented in three stages: Before stimulation, during stimulation and at ET.
Before stimulation patients should be assessed as to age, the ovarian follicular count and/or anti mullerian hormone (AMH). A correct starting dose of FSH should hence be administered.
During stimulation and ET many different strategies can be applied:
1. Low dose stimulation: The disadvantage is fewer oocytes and fewer embryos to freeze.
2. GnRH antagonist protocol with oocyte maturation by administering a GnRH analogue instead of HCG. Disadvantage is the handling of the luteal phase after ET in the fresh cycle.
3. Coasting before HCG is administered. Disadvantage is that it does not always work and requires a lot of experience.
4. No fresh ET, freezing of all appropriate embryos. Disadvantage is that it does not influence the first phase OHSS.
5. Dopamin agonist prescribed before HCG is administered. Disadvantage is that it does not affect OHSS if pregnancy occurs.
6. Transferring no more than one embryo. Disadvantage,

if any, is that it may decrease the pregnancy rate.
7. Cancelling the cycle.
These tools make it perfectly possible to avoid any serious OHSS. Given a good freezing programme, the disadvantages are minor. Serious OHSS should no longer be a part of IVF.

I-33: Transvaginal Ultrasound-Guided Ovarian Interstitial Laser Treatment in Anovulatory Women with Polycystic Ovary Syndrome: A Randomized Clinical Trial on The Effect of Laser Dose Used on The Outcome

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Background: In 2005, we designed and evaluated the effectiveness of the transvaginal ultrasound-guided ovarian interstitial laser treatment in twenty-three anovulatory women with clomifene citrate (CC)-resistant polycystic ovary syndrome (PCOS) as a new method of ovulation induction in infertile PCOS women, with a more than 80% ovulation rate and 36% pregnancy rate during six postoperative months. Objective of this study was to explore an optimal laser dose for this new treatment protocol.
Materials and Methods: Eighty infertile PCOS patients with CC-resistant were enrolled between January 2006 and June 2008. All women presented with oligo-/amenorrhea and anovulation for at least 2 years and were seeking pregnancy. The mean (± SD) age was 29.1 ± 3.1 years and the mean duration of infertility 3.3 ± 2.0 years. The mean body mass index (BMI) was 22.9 ± 3.5 kg/m^2. PCOS was diagnosed referring The Rotterdam criteria. Serum concentrations of FSH (6.5 ± 1.4 IU/L), LH (13.7 ± 4.6IU/L) and T (2.9 ± 0.75nmol/L) were assessed at the third day of progesterone-induced bleeding. TVS examination revealed 10-30 subcapsular follicles of 2-8 mm in diameter in unilateral ovary. Any contraindications to surgery, previous treatment with LOD and the presence of tubal or male factors for infertility were considered as exclusion criteria. All subjects were randomly divided into group A, B, C, D and laser coagulation points were as follows: group A, one coagulation point per ovary; group B, two points; group C, three points; group D, four to five points. The procedure of laser treatment has been detailed previously. Briefly, it was location and puncture, laser coagulation and the fibre-optic withdrawal and relocation. Postoperative monitoring include the serum hormone concentrations, follicle development and ovulation, pregnancy and miscarriage and adverse effects. Statistical significance was set at p<0.05.
Results: The ovulation rates of group C (75%,15/20) and D (80%,16/20) were significantly higher than of group A (5%,1/20) (p<0.001) and B (15%, 3/20) (p<0.001). The conception rates were significantly higher in group C (45%, 9/20) and D (40%, 8/20) than in group A (5.%, 1/20) and B (15%, 3/20) (p<0.05). The mean postoperative serum testosterone levels were significantly lower in group C (2.08 ± 0.62 nmol/l) and D (2.07 ± 0.42 nmol/l) compared with group A (3.10 ± 0.63 nmol/l) (p<0.001) and B (2.98 ± 0.63 nmol/l) (p<0.001). The mean LH value and LH/FSH ratio in group C and D were also significantly lower than in group A and group B. Each increase of dose with one point, would decrease the mean LH level 2.238 IU/l (y=14.175-2.238x, R square=0.918), the mean serum T level 0.671 nmol/l (y=4.55-0.671x, R square=0.925) and LH/FSH ratio value 0.411(y=2.585-0.411x, R square=0.834). There were no adverse events.
Conclusion: Three coagulation points per ovary seems to be the plateau dose sufficient to produce an optimal outcome for ovarian interstitial laser treatment in anovulatory PCOS women. Reducing the laser dose below that level is associated with poorer results and increasing the dose above it does not improve the outcome.
Keywords: Anovulation, Dose Laser, Ovarian Interstitial, Polycystic Ovary Syndrome, Transvaginal, Ultrasound-guided

Genetics

I-34: Interactorme of Human Embryo Implantation: Identification of Gene Expression Pathways, Regulation, and Integrated Regulatory Networks

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Background: A prerequisite for successful embryo implantation is adequate preparation of receptive endometrium and the establishment and maintenance of a viable embryo. The success of implantation further relies upon a two-way dialogue between the embryo and uterus. However, molecular bases of these preimplantation and implantation processes in humans are not well known.
Materials and Methods: We performed genome expression analyses of human embryos (n = 128) and human endometria (n = 8). We integrated these data with protein-protein interactions in order to identify molecular networks within the endometrium and the embryo, and potential embryo-endometrium interactions at the time of implantation. For that we applied a novel network profiling algorithm HyperModules, which combines topological module identification and functional enrichment analysis.
Results: We found a major wave of transcriptional down-regulation in preimplantation embryos. In receptive-stage endometrium, several genes and signalling pathways were identified, including JAK-STAT signalling and inflammatory pathways. The main curated embryo-endometrium interaction network highlighted the importance of cell adhesion molecules in the implantation process. We also identified cytokine-cytokine receptor interactions involved in implantation, where osteopontin, LIF and LEP pathways were intertwining. Further, we identified a number of novel players in human embryo-endometrium interactions, such as APOD, EDN1, FGF7, GAST, KREMEN1, NRP1, SERPINA3, VCAN, and others.
Conclusion: Our findings provide a fundamental resource for better understanding of the genetic network that leads

to successful embryo implantation. We demonstrate the first systems biology approach into the complex molecular network of the implantation process in humans.

Keywords: Implantation, Embryo-Endometrium Interactions, Receptive Endometrium

I-35: Polar Body Analysis by Array CGH Identifies Women with Varying Susceptibility to Aneuploidy and Suggests that Non-disjunction Is Not The Predominant Mechanism Leading to Aneuploidy in Humans

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Background: The maternal age effect for trisomy is well known. However what is less established is whether certain women are more (or less) prone to segregation errors, independent of age. Trisomy arises primarily through maternal meiosis I chromosome segregation errors however the precise mechanism by which these errors occur is unclear. Current dogma attributes the origin of trisomy to malsegregation of a whole chromosome to the same pole as its homologue (non-disjunction). Classical cytogenetic studies however suggest that this model does not fully account for the patterns observed in human oocytes. An alternative model (precocious separation of sister chromatids) has thus been proposed, but recurring criticism of this model purports that technical issues may have led to interpretation errors. The purpose of this study was to determine the relationship between chromosome segregation errors of whole chromosomes and single chromatids in human oocytes to (i) provide a more complete understanding of oogenesis and the origins of aneuploidy, and (ii)help identify the best strategy to avoid aneuploidy in pregnancy.

Materials and Methods: Oocytes from 48 patients (aged 29-50 years) were harvested 43-45 hours after administration of human chorionic gonadotrophin and 297 first polar bodies biopsied by micromanipulation. Polar bodies were subjected to whole genome amplification and array comparative genomic hybridization (24 Sure, Bluegnome, UK). Bluefuse software was used to distinguish between whole chromosome (non-disjunction) and chromatid (precocious separation) errors.

Results: Of the 297 oocytes biopsied, a total of 285 (96%) first polar bodies were successfully amplified and analysed. Of those analysed, 129 (45.3%) had no detectable chromosome segregation error (and were classified as euploid) and 156 (54.7%) has at least one gain or loss of a chromatid/chromosome. The total number of errors was 390, giving a per polar body error rate of 1.37. There was no difference overall between the frequency of losses (184=55.3%) compared with gains (149=44.7%) but whole chromosome losses were much more frequent than gains. Notably single chromatid errors were nearly 11 times more common than whole chromosome errors. A positive association ($p<0.01$) between the frequency of segregation errors and maternal age was established, albeit with apparent inter-individual differences. That is, selected women in the younger age group had relatively high levels of aneuploidy and vice-versa. Women who went on to have an unaffected live birth were those who had low levels of aneuploidy. The preponderance of single chromatid errors is consistent with the notion that precocious separation, rather than non-disjunction is the primary mechanism leading to human trisomy. These findings were consistent with other recently published findings using similar technology on 1st and 2nd PB plus the embryo.

Conclusion: The current study demonstrates clearly that whole chromosome errors are rare in comparison to chromatid errors even in women of advance maternal age. Array CGH is a powerful method of diagnosing aneuploidy in oocytes in women of advanced maternal age enabling them to make informed choices about their future reproductive options. Furthermore, it can also act as a screening tool for women to help them select chromosomally normal oocytes as a strategy to avoid aneuploid pregnancy and live births. It remains to be seen whether interventions or lifestyle changes can improve the rates of chromosome errors in the oocytes of individual women, however, array CGH would accurately monitor this. In addition, SNP genotyping (Karyomapping) on the same samples provides the basis for further elucidating the mechanism of chromosome segregation errors; in particular, the role of recombination patterns in the genesis of trisomy.

Keywords: Aneuploidy, Oogenesis, Polar Body, Non-Disjunction, Precocious Chromatid Separation, Array CGH

I-36: Preimplantation Genetic Diagnosis - Where Have We Been and Where Are We Going?

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Preimplantation genetic diagnosis (PGD) is now considered routine in IVF laboratories with micromanipulation capability and access to genetic diagnostic services. The past two decades have witnessed a dramatic increase in the use of PGD, the number of cycles performed, and the indications for which PGD has been used. This increase has been mirrored by a slow, but steady, increase in the range of new technologies used to facilitate PGD. This lecture will focus on the evolution of these different techniques - focussing on those which have persisted (e.g. Fluorescence in situ hybridization and PCR) as well as the newer, more sophisticated tests (e.g. array CGH) which look set to replace them. Microarray based technologies are already in routine use in some laboratories and will likely become commonplace. From the IVF laboratory samples are simple to prepare and with the availability of centralized commercial testing laboratories, the clinical service is scalable. The use of genome wide SNP approaches has led to the possibility of a universal PGD test which can simultaneously diagnose any known genetic disease (for

which a family history is present) and the presence (and, uniquely, the paternal origin of) chromosomal abnormalities. While these new tests are considerably more expensive than their predecessors they should come down in price with time, produce accurate reliable results, can reduce waiting times for test development considerably, and offer a wealth of potentially useful diagnostic information. As a consequence this raises the question: 'How much information is too much information in the context of PGD?' My objectives are to distinguish between different methods of preimplantation testing, compare the strengths and weaknesses of different laboratory methodologies used to perfrom preimplantation genetic diagnosis, describe the new technique of karyomapping which uses genome-wide SNP analysis and its value in preimplantation testing for inherited disorders and aneuploidy. I will conclude by identifying ethical and practical problems associated with different forms of preimplantation testing.

I-37: Establishing High Resolution Genomic Profiles of Single Cells Using Microarray and Next-Generation Sequencing Technologies

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The nature and pace of genome mutation is largely unknown. Standard methods to investigate DNA-mutation rely on arraying or sequencing DNA from a population of cells, hence the genetic composition of individual cells is lost and de novo mutation in cell(s) is concealed within the bulk signal. We developed methods based on (SNP-) arraying and next-generation sequencing of single-cell whole-genome amplifications that enable the detection of DNA-copy number variants with unprecedented accuracy and reliability, profiling the architecture of structural variants, as well as genotyping a single cell to the DNA-basepair level. These methods will expedite novel applications in basic genome research and clinical practice.

I-38: Chromosome Instability in The Cleavage Stage Embryo

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Recently, we demonstrated chromosome instability (CIN) in human cleavage stage embryogenesis following *in vitro* fertilization (IVF). CIN not necessarily undermines normal human development (i.e. when remaining normal diploid blastomeres develop the embryo proper), however it can spark a spectrum of conditions, including loss of conception, genetic disease and genetic variation development. To study embryonic CIN further we have developed new methods based on high-resolution microarray as well as next-generation sequencing technology that characterize the genome of a single human cell. We delivered proof-of-principle for detecting various types of structural variants, including Mb- to Kb-sized duplications and deletions, in single human (tumor) cells by low coverage sequencing and mapping.
Based on the copy number changes that were detected by single-cell microarray analysis of multiple blastomeres of the same embryo, it was hypothesized that chromosome breakages and fusions occur frequently in human cleavage stage embryos and instigate subsequent breakage-fusion-bridge cycles. In addition, we hypothesized that the DNA breaks present in spermatozoa could trigger this CIN. To test these hypotheses, we genotyped both parents as well as 93 blastomeres from 24 IVF embryos and developed a novel SNP-array based algorithm to determine the parental origin of (aberrant) loci in single cells. Paternal as well as maternal alleles were commonly rearranged in the blastomeres indicating that sperm-specific DNA-breaks do not explain the majority of these structural variants. In addition, single-cell genome sequencing together with parent-of-origin SNP-array and microarray-guided FISH analyses demonstrate that breakage-fusion-bridge cycles as well as more complex rearrangements are sparked in the human cleavage stage embryo.
Our data provide evidence that the human cleavage stage embryo is likely an important source of constitutional chromosomal disorders. The developed single-cell genome analysis methods are generic and will deliver novel insights in embryo and tumor genome research.

Reproductive Imaging

I-39: Acute Female Pelvic Pain

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Acute Female pelvic pain is one the common causes of referral to emergency ward. It has many ethiologies of either Gynaecological or non gynecological causes including normal ovulation, haemorrhagic/ruptured ovarian cyst, Tumour, infarction (leiomyoma), Ovarian torsion, ectopic pregnancy, Tubo-ovarian abscess/PID, Endometriosis, … etc. It also has non-gynaecological causes such as renal stones, appendicitis, diverticulitis ,IBD or trauma. It is important to make the precise diagnosis in proper time as some of them are potentially vital and any delay on management may have irreversible sequellas. In this article we discuss the role of US, CT and MRI in evaluation of acute female pelvis, and how to reach an accurate diagnosis based on patients demographics, Clinical scenario and anatomical abnormality. Also we discuss about the added value of MRI in pregnant patients and pearls and pitfalls of imaging of the acute female pelvis.

I-40: Ultrasound in Multiple Pregnancy

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Ultrasound has revolutioned the care of women carring twins.
First trimester evaluation is the best time to determine chorioniocity and amnioniocity in multiple gestations.
First trimester diagnosis is based on the number of gestational sacs, amnions and yolk sacs.
Growth rate in multiple multiple gestations during the fist and early second trimesters parallels the growth rate of singleton pregnancies, dropping off during the late second and third trimesters.
Most cases of growth discrepancy are diagnosed at the second half of the pregnancy and twin to twin transfusion Syndrome is the main differentional diagnosis.
Ultrasound is very important for evaluating complications of twin pregnancy some Complications are more frequent in twin than singleton gestations such as preterm delivery, IUGR, preeclampsia ,PROM, Placenta previa, Abruptia placenta, post partom hemorrhage, umbilical and accidents and congenital anomalies and some are unique to twins: TTTS, twin emboliesation syndrome, Umbilical cord entanglement, Conjoined twin.

I-41: Imaging Strategy for Diagnosis of Adnexal Masses Including US,CT Scan and MRI

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Adnexal masses are spectrum of diseases from benign non neoplastic and neoplastic masses to malignant neoplasms. Endovaginal ultrasonography (US) is the fisrt and invaluable modality for assessment of adnexal masses because it is available and has a high negative predictive value. Important morphologic features are solid (vascularized) tissue, vascular and thick septations, and papillary projections on gray scale and color doppler US. Spectral Doppler analysis parameters (RI and PI and S/D ratio) correlate well with malignancy but generally add little information.
CT scan can help in staging malignant ovarian cancers but some indeterminate adnexal masses need more detailed imaging by Magnetic resonance (MR) imaging. Using MR imaging for diagnosis of adnexal masses includes morphologic characteristics and signal intensity on T1- and T2-weighted images. Cystic masses are probably benign tumors, whereas complex masses are strongly associated with malignancy. Cysts, mature cystic teratomas, leiomyomas, endometriomas and fibromas, can be accurately determined on the basis of T1, T2 and fat-saturated T1-weighted sequences.
New MRI protocols such as dynamic enhanced MRI (DCE-MRI) and MRspectroscopy can be used as adjuant to simple MRI to increase diagnostic accuracy for characterizing adnexal masses.

I-42: MRI Evaluation of Congenital Uterine Anomalies

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Mullerian duct anomalies are an uncommon but often treatable cause of infertility.
Patients with müllerian duct anomalies are known to have a higher incidence of infertility, repeated first-trimester spontaneous abortions, fetal intrauterine growth retardation, fetal malposition, preterm labor, and retained placenta. The role of imaging is to help detect, diagnose, and distinguish surgically correctable forms of müllerian duct anomalies from inoperable forms (see the images below).
In some correctable lesions, the surgical approach is altered based on imaging findings.
MRI of the uterus (in benign conditions such as congenital anomalies or fibroid evaluation) is performed following administration of 1.0 mg IM of glucagon to decrease motion artifacts associated with bowel peristalsis.
For the diagnosis of most anomalies, 5 main sequences are sufficient:
• Coronal single-shot fast spin-echo (FSE) images of the\ ureters and pelvis provide good localized and survey views of ureters.
• Axial T1 spin-echo (without fat-saturation pulse) images.
• Sagittal T2 FSE (without fat-saturation pulse) images. Fat signal is useful to delineate pelvic structures.
• Oblique long-axis T2 FSE (without fat-saturation pulse) images. This plane is ideal for visualization of the uterine cavity and uterine fundal contour.
• Oblique short-axis T2 FSE (without fat-saturation pulse) images are perpendicular to the long axis and sagittal planes (usually oblique axial), providing short-axis (target) views of the uterine cavity useful for visualizing a transverse septum if present

I-43: Imaging Findings of PCOS and Prediction of OHSS

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Story of collar string of beads, multiple immature follicle, and adverse effect in infertile patients is a concern which can be of child bearing conflict. PCOS is the most common endocrine disorder in women of the reproductive age group.
The new definition required the presence of two from the following three criteria: 1. oligo and or anovulation, 2. clinical and or biochemical hyperandrogenism and 3. poly cystic ovaries.
According to the available literature,the Rotterdom criteria should have at least one of the followings: 12 or more immature follicles 2-9 mm in diameter and increased ovarian volume>10 cc.
By using sonography we can predict one of the most serious complications of infertility treatment: OHSS and with appropriate management we can prevent complication at three levels as described in the following.

In the baseline sonography when the clinician is made aware of PCOD pattern of ovaries with changing stimulation management we can prevent OHSS.
After stimulation when we see honey combing pattern>15 follicle with10-15 mm diameter in ovaries we alarm the clinician to be more cautious.
Finally when OHSS occurs imaging modalities can prevent catastrophic complication such as CRF, DIC, hypovolemic shock and ARDS.
Recent assessment of Doppler study may have some value in predicting the risk of OHSS but further study may be needed for confirmation.

I-44: Color Doppler Imaging in Impotence

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Sexual impotent is very common entity and define as inability to induce or maintaining penile erection during sexual intercourse. Studies reveals about 10% of men aged 40-70 years have complete, 17% have mild and 25% have moderate amount of erectile dysfunction.
The sexual impotence has different etiology including psychogenic, neurogenic, arteriogenic and venogenic causes.
Current studies reveal that organic cause of impotent is a bout 50-90% cases.
Among them vascular reasons including arterial insufficiency or venous incompetence contributed 50-70% cases (arteriogenic impotence 30%, venogenic impotence 15% and 25% mixed Venus and arterial).
Diagnosis of an organic cause of impotence is very important because is could be curable.
The cavernosal arteries are the main feeders to penis and where the erection is performed. Selective angiography with selective internal pudendal is an invasive but the gold standard in evaluation of penile arteries in evaluating arteriogenic impotence.
This technique is invasive and is not suitable as a first step or screening examination.
Color doppler imaging could be a suitable technique inassessement of vasculogenic impotence during injection of an intra cavernosal vasodilating pharmacological agent and observing the response.
By color doppler imaging blood velocity mof cavernosal arteries could be measured before and after intracavernosal injection of vasodilating agents.
Also increase in size of the vessel diameter (0.75%) is an indication of normal arterial flow. A suitable vasodilator could be intrcavernosal injection of 60 mg of Papavarine in a 2 ml solution into right or left corpus cavernosum. Alternative drugs are cocktail of triple agent consisting of Papavarine 4.4 mg, phentolamine 0.15 mg and PG-E1.
Drug induced priapism may occur in 2-3% of the patients.
The patient can be divided into normal, with an average PSV of 47 cm/second, B. Mild to moderate with average peak systolic velocity (PSV) of 35 cm/second and C. Severe arterial insufficiency with an average PSV of 7 cm/second.
Generally peak systolic velocity of 40 cm/second was normal.
The parameters that indicate the presence of arterial disease are a subnormal clinical response to vasoactive agents, a less than 60% increase in the diameter of the cavernosal artery, and a peak systolic velocity of the cavernosal arteries less than 25 cm/second.
In the presence of normal arterial function, doppler findings suggestive of an abnormal venous leak are persistent end diastolic velocity of the cavernosal artery greater than 5 cm/second and demonstration of flow in the deep dorsal vein.
The development of diastolic flow reversal after an injection has been found to be a reliable indicator of venous competence.
In conclusion color duplex doppler ultrasoung with intracavernosla injection of vasodilator. Sonography is an excellent and accurate means of assessing patients with erectile dysfunction. It is a minimal invasive test with low complication rate and can produce good results and should replace angiography and cavernosometry as primary or screening test.

I-45: Advance MRI Sequences in Pelvic Endometriosis

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Background: To assess MRI in diagnosing endometriotic lesions, emphasizing T2*weighted imaging efficacy.
Materials and Methods: This prospective study of 48 females (22-38 years, average 29.6) clinically suspected of endometriosis from September 2009 to April 2012.
MRI was performed with a 1.5 T imager (Siemens) with a body array coil. T1, T2 and T2* weighted (2D-FLASH) sequences were obtained without IV contrast. Using intrarectal and intravaginal aqueous gel. All patients undergo diagnostic laparoscopy and pathologic assessment performed when was necessary.
Results: 55 endometriomas and 65 endometriotric implants were detected on MRI. These lesions have various signal patterns on conventional MRI sequences. Assessment with T2*weighted sequence reveals that most of endometriotic lesions have high signal and no non-endomteriotic lesion have this pattern.
With MRI using T2*weighted sequence sensitivity was 88%, specificity 100% and efficiency 91% negative ratio for low signal lesions was 8.5.
Conclusion: MRI using T2*weighted imaging is a sensitive, specific and accurate method to evaluate endometriotic lesions

I-46: Repeat Transvaginal Ultrasound-Guided Ovarian Interstitial Laser Treatment Improved the Anovulatory Status in Women with Polycystic Ovarian Syndrome

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Background: To assess the effectiveness of repeated transvaginal ultrasound-guided ovarian interstitial laser treatment in the management of anovulatory patients with polycystic ovary syndrome (PCOS).

Materials and Methods: A retrospective study was performed in our department on 27 anovulatory PCOS women who failed to respond to the first ultrasound-guided transvaginal ovarian interstitial laser treatment. Each woman received a repeat procedure and postoperative rates of ovulation and pregnancy were monitored.

Results: Of 27 patients, 18 (66.67%) ovulated spontaneously following the repeated ultrasound-guided transvaginal ovarian interstitial laser treatment Ten women became pregnant within six postoperative months and a cumulative pregnancy rate of 37.04% was achieved. There were no significant operative complications.

Conclusion: Repeated ultrasound-guided transvaginal ovarian interstitial laser treatment is an effective and safe management in anovulatory PCOS patients who fail to respond or have a transient response to the first transvaginal ovarian interstitial laser treatment.

Oral Presentations

Andrology

O-1: Suitability of Epididymal and Testicular Ultrasonography and Computerized Image Analysis for Assessment of Current and Future Semen Quality in The Ram

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Background: Breeding soundness evaluation (BSE) is the primary assessment for determining reproductive potential of male animals. This method, however, cannot be used to evaluate semen frequently or to predict future semen quality. Computerized analysis of ultrasonographic images provides information on histophysiological changes in male reproductive organs.We hypothesized that: 1. semen parameters would correlate with ultrasonographic characteristics of the distal region (cauda) of the epididymis and 2. testicular ultrasound images and/or circulating testosterone (T) concentration would predict future semen quality in the ram.
Materials and Methods: Six adult rams underwent BSE and scrotal ultrasonography approximately 60 days apart (average duration of the spermatogenic cycle) both during the breeding (December and February) and non-breeding (June and August) seasons.
Results: An inverse correlation was found between pixel intensity (numerical pixel values) of the epididymes and percentage of sperm in semen with normal morphology ($r=-0.46$, $p<0.05$). Pixel heterogeneity (standard deviation of pixel values) correlated negatively with percentage of sperm with normal morphology ($r=-0.42$, $p<0.05$) and directly with percentage of spermatozoa with abnormal tails ($r=0.43$, $p<0.05$). Pixel heterogeneity of testicular parenchyma obtained approximately 60 days prior to semen evaluation inversely correlated with percentage of sperm with normal morphology ($r=-0.73$, $p<0.01$) and sperm progressive motility ($r=-0.76$, $p<0.01$), and directly with percentage of sperm with abnormal tails ($r=0.72$, $p<0.01$) and loose heads ($r=0.79$, $p<0.01$).
Conclusion: We concluded that scrotal ultrasonography combined with computer-assisted analyses of epididymal and testicular echotexture in the ram was a valuable method for determining certain current and future semen parameters, respectively.
Keywords: Epididymis, Testis, Ultrasonography, Semen, Breeding Soundness Evaluation, Ram

O-2: Food Group Intakes and Risk of Idiopathic Asthenozoospermia: A Case-Control Study

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Background: Asthenozoospermia, a disorder of sperm motility, is a common cause of human male infertility and is found to play a role in approximately 19% of infertile patients. Epidemiological studies indicated a link between environmental pollutants, lifestyle changes, and dietary habits and infertility. In view of the fact that nutrition is coming to the fore as a major environmental factor, it may affect reproductive health. This study investigated the association of food group intakes and the risk of asthenozoospermia in a hospital-based case-control study in Tehran, Iran.
Materials and Methods: In total, 72 asthenozoospermic men and 169 normozoospermic men underwent face-to-face private interviews in 2011. At the first visit, the primary infertility exams due to the 5th edition of World Health Organization laboratory manual for the examination and processing of human semen were done for couples; according to spermatogram results, cases and controls were selected. Controls were frequency-matched with cases by age. Semen quality was assessed by sperm volume, concentration, motility, morphology and total antioxidant capacity. Usual dietary intakes were assessed through the use of a 168-item semi-quantitative food frequency questionnaire (FFQ). This FFQ has previously shown relative validity and reproducibility for food group intakes among Iranian adults. Odds ratios (OR), 95% confidence intervals (CI), and tests for trends were calculated using logistic regression. The first adjusted model included age, body mass index (BMI), total energy intake, smoking status and heavy traffic near home; the second adjusted model included the first model and other food groups.
Results: By design, cases and controls had the same age distribution. Cases had significantly lower BMI (19.9 vs. 26.1) and total energy intake (1705.3 vs. 1935.8). After adjusting for potential confounders, the risk of asthenozoospermia increased significantly in the highest tertiles of meat processed foods (OR: 2.03, 95%CI: 1.70-2.44) and sweets (OR: 2.05, 95% CI: 1.09-2.26). In contrast, being in the highest tertile of total fruits and vegetables, dark green vegetables, skimmed milk, poultry intake, and sea food intake reduced the asthe-

nozoospermia (p for trend = 0.04, 0.01, 0.02, 0.03 and 0.04 respectively).
Conclusion: The results of the present study suggest that intake of meat processed foods and sweets consumption may directly associate with risk of asthenozoospermia, whereas fruits, vegetables, poultry, skimmed milk and sea foods may have protective role.
Keywords: Food Groups, Asthenozoospermia, Sperm Quality, Male Infertility

O-3: Identification and Characterization of Repopulating Spermatogonial Stem Cells from The Adult Human Testis

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Background: This study was conducted to identify and characterize repopulating spermatogonial stem cells (SSCs) in the adult human testes.
Materials and Methods: Testes biopsies from obstructive azoospermic patients and normal segments of human testicular tissue were used. Flow cytometry, real time PCR and immunohistochemical analysis were performed. Purified human spermatogonia were transplanted into busulfan treated recipient mouse testes and integrated cells were detected by human nuclear protein antibody co-localized with stem cell and germ cell markers.
Results: Testicular biopsies collected from obstructive azoospermic men showed similar morphology and distribution of markers to the normal human testes. Flow cytometry showed distinct populations of SSEA-4, CD49f and CD90 positive cells in the adult human testes. SSEA-4 (+) cells showed high expression levels of SSC specific genes and high levels of telomerase activity. Extensive colonization of human cells in the mouse testes indicates the presence of highly enriched populations of SSCs in the SSEA-4 (+) sorted cells. All the HNP (+) cells in the mouse testes were positive for germ cell marker VASA and only half of them were dimly positive for c-kit. In addition, subpopulations of human spermatogonia that colonized mouse testes were positively stained for CD49f, GPR-125, Nanog and Oct-4 indicating the existence of population of cells among human spermatogonia with SSC and pluripotent characteristics.
Conclusion: This study clearly demonstrates that repopulating human SSCs have phenotypic characteristics of SSEA-4+, CD49f+, GPR-125+and c-Kit neg/low. The results have direct implications for enrichment of human spermatogonia for further culture and germ cell differentiation studies.
Keywords: Surface Markers, Repopulating, Spermatogonial Stem Cells, Human, Testes

O-4: Protective Effect of Silymarin on Sperm Viability and Chromatin Packing in Varicocele-Induced Rats

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Background: Varicocele has been repeatedly implicated as a cause of infertility in selected men. Previous studies have showed high levels of seminal oxidative stress, as evidenced by increased levels of ROS and reduced total antioxidant capacity in varicocele men, suggesting that sperm dysfunction in varicocele patients may be partly related to oxidative stress. Thus current study was designed to evaluate the effect of slymarin on varicocele-induced oxidative stress and to analyze sperm viability and motility.
Materials and Methods: Eighteen Mature male Wister rats divided into 3 groups as; control-sham (left varicocele induced for 42 days), Varicocele+SMN-administrated (50 mg/kg/day for 42 days, orally) group and control group. The testicular malondialdehyde (MDA) was evaluated. Aniline-blue and Eosin-nigrosin staining techniques were performed in order to evaluate sperm nuclear maturity (Chromatin packing) and viability respectively.
Results: Biochemical analyses showed that the MDA level significantly ($p<0.05$) increased in varicocelized rats and slymarin administration remarkably reduced MDA level. Light microscopic observations revealed that, the percentage of viable sperms and as well the percentage of sperms with matured nuclei significantly ($p<0.05$) increased in slymarin-administrated group. Meanwhile in varicocelized rats the sperm viability and nuclear maturity remarkably ($p<0.05$) decreased in comparison to control and slymarin-received groups.
Conclusion: Slymarin as antioxidant compound can fairly protect sperm viability by reducing varicocele-induced oxidative stress. The results also indicated that slymarin decreases free radicals detrimental effects on sperm DNA by increasing sperm nuclear maturity rate.
Keywords: Varicocele, Sperm Viability, Sperm Nuclear Maturity, Oxidative Stress

O-5: The Influence of Genetic Polymorphism On Idiopathic Male Infertility

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Background: Infertility is a multifactorial disorder affecting one in ten couples, and in 50% of infertile couples, the male factor due to deteriorated semen quality is a major cause. In about 30% of infertile men seeking help for their problem, the etiology and pathogenesis are not yet known and their condition is considered idiopathic. Through animal studies conducted recently, hundreds of genes have been found to be related to impaired spermatogenesis and reported as possible pathogenic mechanisms for idiopathic male infertility; however, their human counterparts need to be investigated in infertile patients. This study aims to examine if there is an association between endothelial nitric oxide synthase (eNOS) T-786C, G894T, and 4a/b gene; glutathione-S-transferase (GSTM1, GSTT1, GSTP1) gene; estrogen receptors alpha, and beta (ESR1, ESR2) gene; the (TAAAA)n repeat and Asp327Asn in the sex hormone-binding globulin (SHBG) gene; Methylenetetrahydrofolate reductase (C677T, A1298C, and G1793A) gene; and the follicle stimulating hormone (FSH) receptor gene Thr 307 -Ala and Asn 680 -Ser polymorphisms as risk factors for idiopathic male infertility.
Materials and Methods: Genotypes for all gene polymorphisms were identified by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Reproductive hormones were measured and at least two semen analyses were performed in each subject.
Results: Multivariate regression analysis showed an increase in risk to infertility in the patients with null genotype of GSTM1 (OR: 2.18; 95% CI: 1.64-3.32; p<0.001) or GSTT1 (OR: 1.88; 95% CI:1.12-2.52; p<0.04). There was a significant difference between the group of infertile patients with azoospermia and oligoasthenoteratozoospermia (OAT) when compared by genotype distribution (-786CC vs. 786TT, 894TT vs. 894GG, and 4aa vs. 4bb) (all p<0.01). Significant differences were observed in the frequency distribution of PvuII and XbaI in the ESR-α gene and RsaI and AluI in the ER-β gene between patients and controls. The variant Asp/Asn genotype was associated with a more than 50% reduced risk of infertility (OR: 0.46, 95% CI: 0.25-0.80, p=0.001). Genotype analysis demonstrated six SHBG (TAAAA)n alleles with 6-11 repeats. The 677T allele carriers (TC or TT) had a significantly increased risk of infertility compared with the CC homozygotes (OR:1.60, 95% CI:1.21-2.75, and OR:2.68, 95% CI:1.84-3.44, respectively). No significant association between codon 680 and codon 307 genotypes and infertility was observed (p=0.076 and p=0.073, respectively).
Conclusion: These results support the hypothesis of increased risk of GSTM1 or GSTT1null genotypes, eNOS genotypes, ESR-α, and ER-β variants, SHBG Asp237Asn and (TAAAA) n polymorphisms, MTHFR C677T polymorphism, and for developing infertility. We did not observe any significant association of FSH-R genotype frequencies with any of the sperm characteristics analysed in either group.
Keywords: Genetic, Glutathione-S-Transferase (GST), Infertility, Male Factor, Polymorphism

O-6: Pharmacological and Molecular Evaluation of Herbal Drugs in The Treatment of Sexual Dysfunction and As Stimulating Agents

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Background: Vajikarana herbs from Ayurveda are known to enhance the sexual performance in folklore. The objective is to evaluate their effect by molecular and pharmacological screening.
Materials and Methods: In the present study, the effect of four Vajikaran Rasayana herbs on penile erection, sperm count, seminal fructose content *in vivo* and nitric oxide (NO) release *in vitro* was assessed. Penile erection index and sperm count were determined by visual observation; the seminal fructose concentration was measured spectrophotometrically using resorcinol reagent; and NO release was assessed in a mouse macrophage cell line (RAW264) spectrophotometrically using a commercial Griess reagent kit. Penile erection index, sperm count, seminal fructose concentration and *in vitro* NO release were the parameters measured.
Results: A significant effect on the sperm count, seminal fructose content and penile erection index was observed upon treatment with the extracts. The effect of extracts on inducible NO release *in vitro* directly correlated with the enhanced erectile function *in vivo*. The aphrodisiac claims attributed to the four Vajikaran Rasayana herbs were tested and a distinctive effect of all extracts tested was observed, with C. borivilianum showing a highly significant response for all parameters measured *in vivo* and *in vitro*.
Conclusion: The present study also provides a good correlation between the *in vivo* improvement of penile erection and *in vitro* NO releasing activity of the extracts. Increase in seminal fructose levels and sperm count further validates the role of these herbs in improving reproductive function.
Keywords: Vajikaran, Infertility, Sexual Perormance, Penile Erection Index, Sexual Dysfunction, Oligozoospermia

O-7: Y Chromosome Microdeletions Are Not Associated with Spontaneous Recurrent Pregnancy Loss in A Sinhalese Population in Sri Lanka

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Background: Many advances have been made in reproductive medicine yet the spontaneous loss of a pregnancy remains the most common complication of pregnancy. The aetiology of spontaneous recurrent pregnancy loss (RPL) is multifactorial. Y chromosome microdeletions are found in approximately 7% of men with low sperm counts and, compared to the general population, a higher frequency of spontaneous pregnancy loss occurs in infertile couples. The current study was designed to examine whether Y chromosome microdeletions were associated with RPL in a Sinhalese population in Sri Lanka.
Materials and Methods: The subjects were 76 male partners of couples where the female partner had experienced three or more recurrent pregnancy losses. 120 random males from the general population were also analysed as a control group. DNA extracted from peripheral blood was tested for Y chromosome microdeletions in the AZFa, AZFb, AZFc regions using a multiplex PCR amplification system. Partial deletions within the AZFc region were also tested.
Results: None of the men (76 with RPL, and the 120 controls) had any microdeletions in the AZFa, AZFb, AZFc regions or partial deletions within the AZFc region.
Conclusion: Y chromosome microdeletions do not appear to be important in the aetiology of RPL in this population in Sri Lanka.
Keywords: Y-Chromosome Microdeletions, Recurrent Pregnancy Loss, AZFa, AZFb, AZFc

Animal Biotechnology

O-8: Clinical Application and Evaluation of Autologous Keratinocyte and Fibroblast Cells Culture on Horse Open Wound Healing

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Background: Attempt to treat horse limb wound with KFGS (Keratinocyte fibrin glue suspension) and FKFGS (Fibroblast keratinocyte fibrin glue suspension) methods were performed in this study.
Materials and Methods: Four healthy adult horses were used in this study (350 ± 117). Fibroblast cells have been separated by explant culture method from neck, keratinocyte cells have been separated by enzyme digestion from lib skin samples and Fibrinogen has been obtained by glycine precipitation technique from horses' blood. Four full thickness excision wounds (2×3 cm) were created on the lateral and middle of the metacarpus and metatarsus of each horse aseptically. After five days proper granulation tissue developed and cell grafts were performed. The wounds were divided into four groups in Latin square design; A- Control group just were changed bandage B- Fibrin glue group that fibrinogen mixed with thrombin, calcium gluconate and tranexamic acid that were solved in normal saline, C-Keratinocyte fibrin glue suspension (KFGS) group and D-Fibroblast keratinocyte fibrin glue suspension (FKFGS) group. Treatments were done once in fifth day and bandages were changed three times weekly and photograph were taken twice weekly for macroscopic evaluation. Whole wounds excision biopsies were performed at the 26th day after wounding for histopathological evaluation.
Results: There was no significant difference in epithelialization, contraction and total wound healing between each group. Just a non-significant difference was seen in FKFGS group in reepithelialization rate. Histopathologically, basal cells of epiderm in FKFGS group arrange more regular and have more melanin granules than other groups and stratum spinosum cells are more differentiated than other treatments and control group also collagen fibers in derm of FKFGS group has better amount (expansion), diameter and direction than other groups.
Conclusion: For increase of cell treatment effects significantly, it is better to use this treatment on larger wounds, preparation of wound bed with allogenic skin and repeated treatments.
Keywords: Keratinocyte, Fibroblast Cell, Wound Healing, Horse

Embryology

O-9: The Central Role of Mitochondrial Function in Quality of Human Oocyte

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Background: Mitochondria are the most aboudent and small essential organelles found in eukaryotic cells. These are semiautonomous organelles for the production of cellular ATP that through its various biochemical pathways. The primary pathway for ATP production is OXPHOS via the electron transfer chain (ETC) which is encoded by nuclear DNA and mtdna genomes. Mitochondria consist of double stranded DNA that encodes 13 of the polypeptides that are part of the ETC. Mito-

chondrial DNA are normally inherited exclusively from the mother. Human oocyte maturation (MII Stage) consists of 100000 mitochondrial DNA that arise from during oogenesis. Oocyte quality carchterezied by its abilities to be maturation, fertilization and give rise to normal offspring. Good quality oocytes are essential for fertilization especially for successful *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) which are two major assisted reproductive technologies for female infertility. Despite over 3 decades of experience with human IVF, current practice is formidable challenges to improve outcome fertilization remain. Recently many researchers have been studied on the functions of mitochondria in quality oocytes and outcome fertilization.

Materials and Methods: This article presents result of a systematic review about role of mitochondrial function in quality of human oocyte.

Results: These studies showed that the mitochondrion plays a crucial role in the oocyte maturation cytoplasm, for it can provide adenosine triphosphate (ATP) for fertilization and preimplantation embryo development. Likewise, they can act as stores of intracellular calcium (Ca) and proapoptotic factors. The central impotence of mitochondrial role in evolution oocyte and quality oocyte furthermore they are important for fertilization outcome and embryonic development. However, the specific means by which mitochondrial dysfunction effect on outcome fertilization.

Conclusion: The evalution of mitochondrial function for infertility patients with poor quality oocyte have been reviewed and the suggested methods for their treatment.

Keywords: Quality Oocyte, Mitochondria, Fertilization

O-10: Assessment of Oocyte and Embryo Quality in Different Time between Oocyte Retrieval and hCG Priming

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Background: Abnormal oocyte morphology has been associated with the hormonal environment to which the gametes are exposed. In this study, we evaluated the oocytes morphology, fertilization rate, embryos quality, and implantation rate resulted of retrieved oocytes in different times after human chorionic gonadotrophin (hCG) administration.

Materials and Methods: A total of 985 metaphase II were retrieved 35, 36, 37 and 38 hours after the injection of hCG (groups 1-4 respectively). Oocyte morphology was divided into 1. normal oocytes, 2. oocytes with extracytoplasmic abnormalities (large perivitelline space and dark zona), 3. oocytes with cytoplasmic abnormalities (dark cytoplasm, granular cytoplasm and aggregates of smooth endoplasmic reticulum) and 4. intracytoplasmic vacuoles.

Results: Extracytoplasmic abnormalities were encountered in 17.76% and 31.1% of these oocytes (groups 3 and 4 respectively, p< 0.001) in comparison with 12.23% (group 2). Intracytoplasmic vacuoles rate were 10.52% and 3.12% (group 4 versus group 2, p< 0.01). Cytoplasmic abnormalities differed between the group 4 and other groups. 23.88% (p< 0.01) and 43.25% (p< 0.001) of resulted 2PN (two pronucleus) from groups 3 and 4 showed grade Z3 respectively in comparison to group 2 (16.44%). Normal and various categories of abnormal oocytes did not differ regarding fertilization and cleavage rates (p> 0.05). However, group 4 showed significantly difference in the rate of embryos fragmentation (grade 3 and 4 embryo) in comparison with group 2 (40.96% versus 24.93%, p<0.01). The pregnancy rate was influenced but the implantation rate did not differ.

Conclusion: Furthermore, oocyte retrieval time following hCG priming affected on oocyte morphology, 2PN pattern and embryos qualities subsequently.

Keywords: Oocyte Retrieval Time, hCG, Oocyte Morphology, Embryo Quality

O-11: Dynamics of Flagellar Force Generated by A Hyperactivated Spermatozoon

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Background: To clarify the mechanism of sperm penetration through the zona pellucida, the flagellar force generated by a hyperactivated spermatozoon was evaluated using the resistive force theory applied to the hyperactivated flagellar waves that were obtained from the mammalian spermatozoa.

Materials and Methods: The hydrodynamic calculation of the flagellar force of the activated (non-hyperactivated) and hyperactivated mammalian spermatozoon was carried out by applying resistive force theory to the flagellar waves that were obtained using high-speed video microscopy and digital image processing.

Results: The head of the activated spermatozoon moved in a nearly straight path, suggesting that mainly propulsive force, which was parallel to the longitudinal sperm head axis, was effective. On the other hand, the head of the hyperactivated spermatozoon moved in a small circular or figure-of-eight path, suggesting that the flagellar force had the transverse component, which was perpendicular to the longitudinal sperm head axis, larger than the propulsive force. No difference in the maximum propulsive force was found between the activated and hyperactivated spermatozoa. The maximum transverse force (45 pN) of the hyperactivated monkey spermatozoon was ~2.5 times its propulsive force. As the beat frequency of the flagellar beating remarkably decreased during the hyperactivation, the slowly oscillating transverse force (5 Hz) by the hyperactivated spermatozoon seems to be most effective for sperm penetration through the zona pellucida.

Conclusion: The hyperactivated spermatozoon penetrates through the zona pellucida by the shear force

generated by the slow oscillation of the transverse force while pushing the zona surface by the propulsive force.
Keywords: Digital Image, Flagellar Wave, Hyperactivation, Resistive Force Theory, Zona Pellucida

O-12: Do Dietary L-Carnitine and Omega-3 Fatty Acids Affect on Ram's Sperm Plasma Membrane Integrity and Mitochondrial Membrane Potential?

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Background: Unique omega-3 fatty acids may contribute to the membrane fluidity which is necessary for the motility of sperm tails and also dietary fish oil (FO) can improve sperm quality. The positive effects of L-carnitine (LCAR) on male reproductive performance have been reported in some species. This study has been focused on the plasma membrane integrity and mitochondrial membrane potential in Iranian ram fed FO and/or LCAR, that till now, there is no report this issue.
Materials and Methods: Nineteen Zandi rams were randomly assigned to experimental groups and were offered an isoenergetic and isonitrogenous ration. The treatments consisted of control (CTR; n=4); fish oil (FO); 35 g/d/ram; n=4); L-carnitine (LCAR; 220 ppm/d/ram; n=4) and FO+LCAR (n=4) with constant level of Vitamin E. In addition, three rams were used without Vitamin E. Diets were fed to the rams for 70 days and semen was collected once a week. Sperm routine parameters were analyzed by CASA. Sperm plasma membrane integrity and mitochondrial membrane potential (MMP) assessed by hypo-osmotic swelling test (HOST) and JC-1 respectively. Data were analyzed by SPSS 16.
Results: The results showed that all sperm characteristics increased with FO. HOST-positive spermatozoa were significantly different between control and FO+LCAR (87 vs. 82 vs. 77 and 76% for FO+LCAR, FO, LCAR and CTR, respectively; $p< 0.05$). MMP was affected and increased by inclusion FO+LCAR in diet (85.5 vs. 76 and 75 vs. 64%, FO + LCAR, FO, LCAR and CTR, respectively; $p< 0.05$).
Conclusion: Addition of FO in combination with LCAR could improve sperm functional test significantly. On the other hand, lonely LCAR at this supplemental level did not improve plasma membrane integrity in comparison with CTR, but it is effective on mitochondrial membrane potential. FO and LCAR treatments have similar effects on mitochondrial membrane potential.
Keywords: L-carnitine, Fish Oil, Ram Sperm, MMP, HOST

Ethics and Reproductive Health

O-13: Reproductive Health Options among HIV-Infected Persons in The Low Income Niger Delta of Nigeria

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Background: With the advent and wide spread use of Highly Active Antiretroviral Therapy (HAART) for the treatment of HIV, persons living with HIV/AIDS are living a good quality, longer and healthier lives. Many HIV-affected couples (sero-discordant and sero-concordant) are beginning to consider options for safer reproduction. The aim of this study was to assess the reproductive health concerns among persons living with HIV/AIDS in the Niger Delta of Nigeria.
Materials and Methods: The reproductive health options was investigated among 195 HIV-infected subjects who were aged 18 - 58 years, mean age of 41.25 ± 11.50 years and made up of 88 males (45.1%) and 107 females (54.9).
Results: Out of the 195 subjects studied, 111 (56.9 %) indicated their desire to have children. The main reasons for wanting to procreate included: ensuring lineage continuity and posterity (52.3%), securing relationships (27.0%) and pressure from relatives to reproduce (20.7%). Single subjects were more inclined to have children (76.3%) compared to married (51.5%), widowed (18.2%) and separated/divorced (11.1%) ($p=0.03$). Of the 111 subjects that indicated their desire to have children, women were more inclined to have children (64.5%) compared to men (47.7%). The major concern among the 84 (43.1%) subjects not desiring more children were the fear of infecting sero-discordant partner and baby (57.1%), fear of dying and leaving behind orphans (28.6%) and the fear that they may become too ill and unable to financially support the child (14.3%). Persons with no formal education were more likely to have children irrespective of their positive HIV status (66.7%) compared to persons educated to tertiary education level (37.0%) ($p=0.01$) as can be seen in table 1. Out of the 111 subjects that desired to have more children, only 58% had gone for reproductive health counseling with HIV counselors. Reasons for not seeking advice were anticipated negative reactions and discrimination from the counselors. A significant number of subjects were only aware of some reproductive health options available to reduce risk of infecting their partners and or baby such as artificial vaginal insemination, intrauterine insemination, caesarean section, avoidance of breast feeding and offering prenatal pre-exposure prophylaxis to the fetus. They were unaware of other options such as sperm washing, IVF and ICSI. Of the 43.1% not anticipating more children, 36.9% were anticipating adoption.
Conclusion: Our study has shown that a significant number of HIV-infected in the Niger Delta of Nigeria desire to have more children irrespective of their positive sero status. There is the need to support the sexual and

reproductive rights of HIV-infected individuals. Additional training needs to be offered to HIV counselors on evidence- based best and affordable practices regarding reproductive health issues among persons living with HIV. Policies that support the availability and accessibility to relevant reproductive and sexual health services including contraception and procreation needs to be developed. Public enlightenment programs on HIV are needed to reduce the stigmatization that HIV-infected persons face from family members and their communities.

Keywords: Reproductive Health, HIV-Infected, Low Income Setting, Niger Delta, Nigeria

O-14: Spouse Image of Body and Sexual Function: A Comparison of Fertile and Infertile Women

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Background: One of the body image concepts is spouse image of body. It has an important role in self-esteem, relationship satisfaction and women's sexual health. That is, women with more positive spouse image of body may be more confident that their partners will continue to accept them and thus have more sexual function. This study therefore aimed to compare the relationship of spouse image of body with sexual function in fertile and infertile women in 2010 in Mashhad, Iran.

Materials and Methods: This comparative correlation study was carried out on130 infertile women referred to Montaserieh Infertility Research Center and 130 fertile women referred to urban health clinics, Mashhad who were selected using convenient and cluster sampling, respectively. Research tools were consisted of valid and reliable demographic questionnaires including personal and fertility/infertility-related information, modified Younesi Body Image Questionnaire and ROSEN female sexual function index (FSFI), which were completed by the subjects. Data analysis was carried out by SPSS software using t-test, one way ANOVA, Spearman and Pearson correlation tests and linear regression.

Results: There was no significant difference between the mean score of spouse image of body in fertile and infertile women (51/08 ± 11/5 versus 52/7 ± 10/5). However, the mean score of sexual function in fertile and infertile women was significantly different (25/8 ± 4/3 versus 27/2 ± 3/8) ($p<0.01$). A direct correlation was also found between spouse image of body and sexual function in both fertile ($p=0.002$) and infertile women ($p<0.001$), although the strength of relationship was higher in infertile women.

Conclusion: The higher sexual function of infertile women in comparison to fertile and its direct relationship with spouse image of body should draw the attention of reproductive health care providers to adopt strategies to promote sexual function in fertile women particularly for those who suffer from sexual function.

Keywords: Body Image, Spouse Image of Body, Sexual Function, Fertility, Infertility

O-15: A Qualitative Approach to Understand the Motivators of Reproduction in Couples of Reproductive Age

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Background: There have been considerable changes in childbearing decision-making in Iran over the last 30 years. These changes have affected the understanding and predicting of fertility behavior changes by reproductive health policy makers and program planners. This study was conducted to explore the motivators of reproduction and fertility in couples of reproductive age.

Materials and Methods: In this qualitative research in-depth interviews were conducted with 30 participants including 19 fertile women, three parents, three husbands and five midwives and health care providers selected purposively in urban health centers, homes and workplaces in Mashhad, Iran, 2011. Data analysis was carried out using MAXqda software based on conventional content analysis approach through giving analytical codes and identification of categories. Study rigor was confirmed via prolonged engagement, and member check.

Results: Results of data analysis demonstrated five major categories about motivators of couples' reproductive intentions including bio-drives, affective, spiritual, sociocultural and economic stimulus.

Conclusion: For those who are interested in reproductive behavioral changes, these findings significantly improve knowing of couples' motivators of reproduction beyond that of demographic variables alone and promote the interventions in the field of population fertility.

Keywords: Motivators of Reproduction, Couples of Reproductive Age, Qualitative Study

O-16: The Relationship between Menarche Age and Body Mass Index in High School Girl Students in Mashhad City in The Year 2011

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Background: Menstruation is a nearly universal experience in women's lives that are considered indicators of women's health more generally. The aim of this study was to evaluate the relationship between Menarche age and Body Mass Index in high school girl students in Mashhad city in the year 2011.

Materials and Methods: This study was a cross-sectional study that carried on 407 high-school female in Mashhad city. The sampling was two-stage method. Data was collected using a questionnaire about demographic, socioeconomic and family educational status, age of menarche and BMI. Data was analyzed using SPSS version 14 software and statistical test such as Pearson correlation test.

Results: Results showed the mean (±SD) age of students were 16.26±1.05 years, age of menarche 12.82 ± 1.09 years, BMI 21.16 ± 1.02 kg/m^2. 56.5% age of 14-16y, 66.3% BMI of 18.5-24.99. 90.6% of students had working mothers, 91.7 % (n=354) moderate social status and 97% single (marital status). Also there was a negative significant relationship between age of menarche and BMI (p=0.002, r=-0.09).

Conclusion: Likewise other parts of Iran, a reduction in the mean age of menarche was found in Mashhad, too; which was due to improvement of nutritional and health condition. Also due to relationship between menarche age and BMI, special attention should be paid to ideal weight, proper nutrition and sufficient physical activities in this high-risk group.

Keywords: Menarche, BMI, Student

O-17: Female Genital Mutilation: A Curse or Blessing among Women of Reproductive Age in Nigeria?

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Background: Female genital mutilation (FGM) practice is mostly carried out by traditional circumcisers, who often play other central roles in communities, such as attending childbirths. Increasingly, FGM is also performed by health care providers. However, FGM is recognized internationally as a violation of the human rights of girls and women. The study investigates a broad cross-cultural study on knowledge, attitude and practice of female genital mutilation among women of reproductive age in 20communities in Nigeria, to examine health seeking behavior of women at reproductive age especially in rural communities, to investigate the health blessing or curse of female genital mutilation among women of reproductive age, the study identifies various classifications of FGM, the study examines the reasons for the FGM practice in Nigeria.

Materials and Methods: This paper randomly selected 1500 dwellers in 20 communities in Nigeria. The questionnaire and interviews were recorded verbatim in a bid to elicit information from the respondents. To support the assumptions and perceptions of this study, statistical tools such as; percentages and regression analysis were employed for the purpose of presenting the data in a meaningful way.

Results: The result then shows that knowledge, attitude and practice of FGM is shallow and traditional forgetting the harmful effect, the result also shows there health-seeking behavior which is the visit to herbalist or traditional healers.

Conclusion: The study recommends a proactive approach to addressing the issue through and appropriate health care for girl and women who have already undergone FGM respecting the dignity, identity and culture of all people and communities affected by FGM, recognizing the abandonment of FGM which requires a holistic approach.

Keywords: Female Genital Mutilation, Women, Reproductive Age

O-18: Sexual Behavior, Knowledge and Attitude of Young Adolescent in Nigeria

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Background: Health information on adolescents, by contrast is not widely available in many developing countries apart from indicators on sexual and reproductive health collects by major international health surveys, particularly in the context of HIV/AIDS. Adolescents are a key target group for HIV and pregnancy prevention efforts, yet very little is known about the youngest adolescent: those under age 15. A new survey data from 12-14 years old in Nigeria are used to describe their sexual activity, knowledge about HIV/STIs and pregnancy prevention and attitude towards sexual and reproductive health, including sex education in schools education in schools. The study attempt to investigate adolescent sexual behavior in general.

• To examine adolescent knowledge on STIs and HIV/AIDS.

• To find out adolescent attitude towards sexual activities, (Sexual and Reproductive Health issues).

• To examine knowledge of sexual; and reproductive health among adolescent.

Materials and Methods: The study use data from nationally- representative house-hold based survey from the National Bureau of Statistics (NBS) on 12-19 years old. A first stage systematic selection of enumeration areas was made from a household. All 12-19 years old de facto resident in each sampled household were eligible for inclusion in the survey. Once the parent or caretaker gave consent, separate informed consent was then sought from the eligible under-age adolescent. This paper analyzes data for infrequently studied group of very young adolescent aged 12-14. Interviews were completed with 2500 respondent which fals within the age range of 12-14 years olds in 5 states of the federation in Nigeria namely Lagos, Ibadan, Ekiti, Abuja and Calabar.

Results: The result shows that very young adolescent are already beginning to be sexually active and as such some believe their close friends are sexually active. They have high level of awareness but little in-depth knowledge about pregnancy and HIV prevention. Multiple information sources are used and also preferred by very young adolescents. Given their needs for HIV/STIs and pregnancy prevention information that is specific and practical and considering that the large majority attend school where sex education is preached, school based sex education is particularly promising avenue for reaching adolescent under age 15.

Conclusion: In the findings, it was discovered that the major reasons that many young adolescents have not received sex education is that it is not offered in their schools to this end there is a great need for development of programs and approaches tailored to reach out of school adolescents, given the high rate of schools drop-out in many countries in Africa, and given a situation like Lagos and Ibadan where the proportion of who have ever been to school is still low. Program that provides sexual health information to young adolescent who are not attending schools may be channeled through multiple sources.

Keywords: Sexual Behavior, Young Adolescent

O-19: Relationship between Postpartum Depression and Breastfeeding Outcome in Clients Whom Were Admitted in Clinics of Tehran University of Medical Sciences

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Background: Breastfeeding is a golden standard of infant feeding. One of variables that may effect on breastfeeding is psychological factor and referring to the effect of postpartum depression, which is the most common mood disorder during postpartum period, there is controversy. The aim of this study was to examine relationship between postpartum depression and breastfeeding outcomes.
Materials and Methods: In this cross- sectional study, by consecutive sampling, 597 volunteer postnatal women, whom attended in 6 primary health care clinics of Tehran University of Medical Sciences (TUMS) during fourth months after delivery, after filling in informed consent were involved (Year 2009- 2010). Tools of study had three main parts of personal characteristics, breastfeeding questionnaire and self-reporting Edinburgh postnatal depression scale (EPDS, cut of point 13). Data was analyzed with X^2 test. All ethical points were considered in this study and approved by research committee of TUMS.
Results: Average of participants' age was 27.01± 4.94. According to personal characteristics, just there was relationship between EPDS and planned pregnancy, and there were no relationship between breast feeding outcome and personal characteristics. Also no relationship was found between EPDS and breastfeeding outcomes.
Conclusion: There were no relationship between EPDS and breast feeding outcomes in this study, which may be because of missing some cases before four months after delivery. It is better to follow the same goals by prospective design. Acknowledgments: Authors would like to thank Research chancellor of Tehran University of Medical Sciences for support and grants of this study.

Keywords: Postpartum Depression, Breastfeeding, Edinburgh Postnatal Depression Scale (EPDS)

Female Infertility

O-20: Maternal Age and Congenital Malformation in Yazd, Islamic Republic of Iran

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Background: Congenital malformation (CM) is the major health problems in childhood because treatment and rehabilitation of children with CM is costly and complete recovery is usually impossible. For most of single primary defect the etiology is unknown. The aim of this study was to determine frequency of CM and its association with maternal age.
Materials and Methods: This descriptive-observational study carried on 4800 births delivered at all maternity hospitals. The type of birth defects was classified by the diagnostic standardization of congenital malformation from the International classification of diseases (ICD-10) codes. Data were analyzed by SPSS version 17. The level of significance was $p<0.05$.
Results: In this study the prevalence of CM was 2.83%. Musculoskeletal anomaly, central nervous system and genital system were accounted as the most common. Overall the mean maternal age in group with CM and normal newborns was 25 ± 2.75 years and 22 ± 3.23 years, respectively (p=0.43) .There was not statistically significant difference between prevalence of CM and mother's age.
Conclusion: Prevalence of congenital malformation among the newborn was higher than previous reported from Iran and we noticed that, there is not an association between maternal age and increase risk of CM.

Keywords: Congenital Malformation, Maternal Age

O-21: Stimulation of The Endometrium with High-Grade Blastocyst Culture Supernatant (SEHB) Can Improve Pregnancy Outcome for Couples Undergoing Intracytoplasmic Sperm Injection (ICSI): A Randomized Clinical Trial

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Background: To evaluate the impact of stimulating the endometrium with high-grade blastocyst culture supernatant (SEHB) perfusion before blastocyst transfer (BT) on implantation rate, pregnancy rate, and pregnancy outcome in ICSI cycles
Materials and Methods: Ninety-four infertile couples who were referred to the Valiasr department of Imam Hospital complex between January 2010 and March 2011 enrolled in this randomized clinical trial. They were

randomly divided into only BT or SEHB groups. Implantation rates, pregnancy rates, abortion, preterm and term delivery rates were compared between the two groups.
Results: Implantation rates and pregnancy rates did not differ significantly between the two groups. The abortion rate was significantly higher in the BT only group while term delivery was significantly higher in SEHB group. Odds ratios of term delivery and abortion were 4.5 (p= 0.001) and 0.3 (p= 0.04), respectively.
Conclusion: The SEHB application may improve pregnancy outcome in infertile couples who are candidates for the ICSI reproductive method.
Keywords: ICSI, Blastocyst Transfer, SEHB

O-22: Comparison of Conventional IVF versus ICSI in Non-Male Factor, Norm Responder Patients

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Background: Conventional IVF and ICSI are two common techniques to achieve fertilization. IVF has long been used for treatment of infertility, although it is not an effective treatment in severe male infertility. The use of ICSI has been expanded in severe male factor and fertilization failure after IVF cycle. In spite of the widespread use of ICSI in patients with non-male factor infertility, there is still little evidence to confirm its effectiveness in this population. The purpose of this study was to evaluate assisted reproductive technology outcomes between IVF and ICSI cycles in non-male factor, norm responder patients.
Materials and Methods: A total of 220 non-male factors, norm responder patients who were indicated for ART were enrolled in this study. The patients received standard long GnRH agonist or GnRH antagonist protocols for ovarian stimulation and after oocytes retrieval, the patients were divided into two groups (IVF and ICSI groups). In IVF group (n=112), all of retrieved oocytes were treated by conventional IVF and in ICSI group (n=88), microinjection (ICSI) was done on all of retrieved oocytes.
Results: In IVF group, fertilization and implantation rates were significantly higher than ICSI group (66.22% and 16.67% in IVF group versus 57.46% and 11.17% in ICSI group, respectively). Chemical and clinical pregnancy rates were statistically higher in IVF group as compared with the ICSI group (42.9% vs. 27.3% and 35.7% vs. 21.5%, respectively).
Conclusion: According to our study, the routine use of ICSI is not improved fertilization, implantation and chemical pregnancy rates and is not recommended in non-male factor, normozoospermic patients.
Keywords: Infertility, ICSI, *In Vitro* Fertilization, Fertilization, Pregnancy Rate

O-23: Transfer of Blastocysts Derived from Frozen-Thawed Cleavage Stage Embryos Improved Ongoing Pregnancy

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Background: The aim of our study was to compare the resulting from transfer of embryos that cryopereserved in cleavage stage after thawing and transfer of embryos after thawing and culture in sequentional media until blastocyst formation.
Materials and Methods: In this prospective clinical study, we have evaluated 128 cycles in women undergone *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) with embryo cryopreservation. Exclusion criteria were women with age >35 years; BMI >30; history of Diabetes Mellitus; thyroid disease; history of sever endometriosis. Frozen embryos were thawed and then cultured in sequential media until blastocyst stage in blastocyst group and were compared with thawed embryos in cleavage stage group.
Results: Implantation rate was significantly higher in blastocyst group 30% compared to 17% in cleavage group. No statistically differences were reported in chemical and clinical pregnancy rates. Ongoing pregnancy rate was significantly higher in blastocyst group 42.9% compared to 24.6% in cleavage group (p=0.023).
Conclusion: Our results indicated that blastocyst formation after thawing of cleavage stage embryos is a good predictor for embryo viability and pregnancy outcome.
Keywords: Blastocyst, Cryopreserved Embryo, Cleavage Embry, Pregnancy Outcome

O-24: Increased Homocysteinein PCOS Affects Oocyte and Embryo Quality

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Background: The biochemical characteristics of the follicular fluid (FF) play essential role in oocyte quality, the subsequent fertilization potential and embryo development. The oocyte quality and metabolism largely depends on follicular microenvironment. Present study aimed to investigate the relationship between oocyte quality and FF homocysteine (Hcy) level in women undergoing to IVF treatment.
Materials and Methods: Following standard long protocol, 52 infertile women were selected for IVF process. FF sample was obtained from a single follicle in each patient. FF Hcy, glucose and estradiol (E2) levels

were evaluated at time of oocyte retrieval. Moreover the oocyte and embryo qualities were estimated. Three embryos were transferred to each patient approximately 48hsafter insemination and finally scored based on the shape, number and fragmentation of blastomers.

Results: Observations demonstrated that, there was a negative correlation between FF Hcylevel with oocyte quality. Accordingly patients with increased Hcy revealed with poor oocyte, low fertilization rate and with low embryo quality as well.

Conclusion: Our results indicated that in the case of increased FF Hcy, the fertilizing rate and the embryo quality reduces. Thus the FF Hcy can be considering as a prognostic marker for IVF outcome.

Keywords: Homocysteine, IVF, Oocyte Quality, Embryo Quality

O-25: Relation between Follicular Fluid Levels of 25 Hydroxy Vitamin D and Clinical Pregnancy following Embryo Transfer

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Background: Animal and *in vitro* studies have shown that 25-OH vitamin D plays a significant role in different parts of body. Few data exist about the role of 25-OH vitamin D in reproduction. Insufficient levels during pregnancy are potentially associated with increased risk of preeclampsia, insulin resistance and gestational diabetes mellitus. Recently, few studies have assessed the prognostic role of this vitamin in follicular fluid in infertile cases undergoing assisted reproductive techniques. This study aimed at evaluating possible association between follicular fluid levels of 25-OH vitamin D and clinical pregnancy following embryo transfer.

Materials and Methods: In this analytic - descriptive study, 80 infertile female candidates of the assisted reproductive techniques (*in vitro* fertilization, micro injection) were recruited during a 12-month period in Tabriz Alzahra Teaching Hospital. Upon the puncturing time of the mature follicles, their fluid was aspirated and the follicular fluid level of 25-OH vitamin D was measured. The serum estradiol was measured on the day of HCG administration. Finally, the patients were categorized according to the fertilization outcome (with and without clinical pregnancy).

Results: The mean follicular fluid level of 25-OH vitamin D was 12.5 ± 5.5 (1 - 24) ng/ml and the mean serum estradiol was 2593.8 ± 1407.5 (568 - 4500) pg/ml in the studied population. Overall, there were 18 cases with clinical pregnancy (22.5%). Median follicular fluid 25-OH vitamin D was significantly higher in the group with clinical pregnancy (14.5 vs. 13 ng/ml; p= 0.007). There was not a significant correlation between the follicular fluid 25-OH vitamin D and the serum estradiol levels (rho = 0.067, p= 0.56). There was a significant direct correlation between the follicular fluid 25-OH vitamin D and age (rho = 0.264, p= 0.018) and the implantation rate (rho = 0.301, p= 0.007).

Conclusion: This study proposed a significant positive association between the follicular fluid 25-OH vitamin D level and clinical conception following assisted reproductive techniques. So administration of this vitamin might be appreciated in this group of patients.

Keywords: Assisted Reproductive Techniques, Vitamin D, Pregnancy

O-26: Comparison of Congenital Malformation of Infants Conceived by Assisted Reproductive Techniques versus Infants with Natural Conception in Tehran

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Background: In many countries, 1% to 3% of newborn infants are conceived by Assisted Reproductive Techniques (ART). Despite the success of ART, there is concern about the risk of congenital malformations among these infants. We report our experience to determine whether use of ART is associated with an increase in major congenital malformations or adverse pregnancy outcomes

Materials and Methods: We performed a historical cohort study of major congenital malformations (MCM) in 978 births from January 2008 to December 2010. The data for this analysis were derived from Tehran's ART linked data file. In our study, the risk of congenital malformations was compared in 326 ART infants and 652 naturally conceived (NC) infants.

Results: We found 56 infants with major congenital malformations: these included 29 NC infants (4.4%) and 27 ART infants (8.3%). In comparison with NC infants, ART infants had a significant 1.94-fold increased risk of MCM. After adjustment for sex, maternal age, stillbirth, abortion and type of delivery, we found a significant difference in risk (OR=2.04, 95% CI: 0.92-4.5). The preva-

lence rate for the ICSI group was 6.5% for the IVF group was 15.9% or 2.73-fold higher than ICSI group (p=0.018 and 95% CI: 1.18-6.3).
Conclusion: Our study shows an overall increase in major congenital malformations after ART when compared with naturally conceived births. This is consistent with other similar studies. We also found evidence of a difference in risk of MCM between IVF and ICSI. When examining the type of malformations, musculoskeletal and urogenital abnormalities were the most frequently reported MCMs in ART infants. Musculoskeletal, cardiovascular and endocrine abnormalities have been reported more commonly in ART infants than in NC infants. More visual, nervous and genetic disorders were reported in NC infants than in ART infants.
Keywords: Infants, ART, Assisted Reproductive Technique, Congenital Anomalies, Malformation

O-27: Maternal Age and Low Birth Weight

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Background: The weight of fetus at birth is widely acceptable parameter that can demonstrate the health of mother. Low birth weight (LBW) (birth weight less than 2500g) is significantly related to the neonatal survival and postnatal morbidity. Adolescence pregnancy is an important risk factor of LBW. The main objective of this study was therefore to determine the prevalence of LBW and relationship between maternal age and the birth weight.
Materials and Methods: In a case - control cross-sectional study, during the entire year of 2008 , all births taking place in all maternity hospitals of Yazd central city of Iran evaluated and LBW neonates detected and compared from view of maternal age to control cases (birth weight > 2500g). For each LBW neonate (case group), two control cases were included. Neonate birth weight of 1500 - 2499g, less than 1500 g and less than 1000 g were classified as moderately LBW (MLBW), very LBW (VLBW) and extremely LBW (ELBW), respectively.
Results: During one year, a total of 5897 deliveries took palace. The prevalence of LBW infants was 8.8%(519 neonates); of them, 8(1.5%), 39(7.5%) and 472 (91%) were ELBW, VLBW and MLBW. The incidence of LBW infants in mothers with age <20, 20-35 and 35-40 years old were: 27.5% , 6.6% and 6.5% respectively that show the teenage pregnancy increased risk of having LBW neonates (p=0.02).
Conclusion: The incidence of LBW infants in Yazd, Iran, was 8.8% and teenage pregnancy (maternal age< 20 years old) was significantly associated with Low Birth Weight neonates.
Keywords: Low Birth Weight, Neonate, Maternal Age

O-28: High Maternal Hemoglobin Concentration and Preeclampsia

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Background: High maternal hemoglobin concentration has been increasingly linked to poor pregnancy outcomes in recent years. This study was aimed to determine whether high maternal hemoglobin (Hb) in first trimester is associated with preeclampsia.
Materials and Methods: In 2009 a retrospective study was conducted and demographic and obstetric records of 1513 low risk pregnant mother registered to a state-run hospital in Tehran were studied. The association between first trimester hemoglobin concentration, BMI, Pregnancy weight gain, maternal age, and parity supplementation with calcium and/or multivitamin and preeclampsia was assessed in multiple logistic regression model.
Results: The mean age of the studied mothers was 26.4 ± 5.2 years. 54.1% of sample were nulliparous and 44.1% had a BMI > 25 kg/m^2, mean Hb concentration was 12.5 ± 1.1 g/dl. 5.6% of pregnancies were complicated by Preeclampsia. Results of binary logistic regression analysis showed that Hb concentration greater than 13.2g/dl (OR: 1.73, 95% IC: 1.07-2.81), BMI (OR: 1.58, 95% IC: 1.17-2.12) and calcium supplementation (OR: 0.38, 95% IC: 0.22-0.64) were significantly associated with preeclampsia.
Conclusion: Our findings suggest that high Hb in the first trimester is related to subsequent preeclampsia. Prophylactic iron supplementation may increase the risk of adverse pregnancy outcomes when mother does not have iron deficiency or IDA, therefore routine iron administration to women with high Hb values might be modified.
Keywords: Hemoglobin, Iron Supplementation, Preeclampsia, Pregnancy

O-29: Fertility Sparing Treatments in Young Patients with Gynecological Cancers: A Large Iranian Experience and A Literature Review

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Background: The marriage age has been increasing recently throughout the world and researches have shown an increased rate of gynecological cancers among

young women. Therefore, fertility sparing in these patients is an important point and many works have been done on conservative management in young women with gynecological cancer.

Materials and Methods: In this study, we report our experience in fertility sparing in cervical, endometrial and ovarian cancers and a literature review of the experiences in the world.

Results: In case of cervical cancer, radical trachelectomy with para-aortic and pelvic lymphadenectomy can be done in patients with earlier stages such as stage IA1-IB. Since patients with early stages have had low recurrence rate, we can investigate them for radical vaginal or abdominal trachelectomy. Also there are more experiences about radical vaginal trachelectomy with laparoscopic pelvic and para-aortic lymphadenectomy. The complication of this surgery is lower than radical hysterectomy. Also the complications of pregnancy are abortion, preterm labor and preterm rupture of membrane (PROM). In endometrial cancer, the best treatment is total abdominal hysterectomy + bilateral salpango - oopherectomy (TAH+BSO), but young patients with early stage (stage I), are suggested to use hormonal therapy vs. radical surgery. If we evaluate the patients completely by MRI and the result is the early stage disease without the other site involvement while the grade of tumor is well-differentiated, we can do hormonal therapy for these patients. GNRH analog, oral medroxyprogestrone acetate (MPA), 100-800 mg/day, megestrol acetate 40-160 mg/day and combination of tamoxifen and a progestin are used for treatment. However, it is important to note that they should undergo a repeated curettage for investigating the medical treatment after 3 months of using drug. If they have normal pathology we will follow medical therapy for 3 months after curettage and they can be evaluated for infertility treatment. The best option for patients treated by medical therapy is TAH+BSO after termination of normal term pregnancy. In ovarian cancer, there are many experiences on fertility sparing surgery. The results of studies in Iran have shown that in order to preserve fertility, conservative surgical management could be performed in young patients with stage I (grade 1, 2) of epithelial ovarian tumor, sex cord-stromal tumor and patients with borderline and germ cell ovarian tumors.

Conclusion: Fertility sparing in young women with gynecological cancer is a novel point which should consider before starting the surgical treatment and getting consent inform from the couples is so important.

Keywords: Gynecological Cancers, Fertility Sparing, Complication, Recurrence, Outcome

O-30: Impact of Environment Tobacco Smoke Exposure on Oxidative Stress in Antral Follicle and Assisted Reproduction Outcomes

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Background: Cigarette smoke contains many oxidants and may alter the human reproduction by inducing oxidative stress (OS) in both active and passive smokers. This study was designed to evaluation the effect of environment tobacco smoke (ETS) exposure on oxidative stress in follicular follicle and assisted reproduction outcomes.

Materials and Methods: A prospective study was carried out on 236 infertile women who underwent assisted reproduction cycles. The ETS exposure was assessed using self-reported ETS exposure and cotinine level in follicular fluid. To evaluate the OS in FF malon-di-aldehyde (MDA) and total antioxidant capacity (TAC) were measured. The number of retrieved oocytes, rate of metaphase II stage oocytes, fertilization rate, good cleavage rate and no fragmented embryo rate were considered assisted reproduction outcomes. The results were adjusted for age, body mass index, duration and etiology of infertility. P-values of less than 0.05 were considered significant.

Results: The MDA and TAC levels in FF were not related to both self-report number of weekly ETS exposure and cotinine levels in FF ($p<0.05$). Also, the number of retrieved oocytes, MII stage oocytes, fertilization rate, good cleavage rate and no fragmented embryo rate were not related to cotinine level and weekly ETS exposure. But in women who their cotinine levels in FF were lower and equal/ above 3.5ng/ml, the number of retrieved oocytes was higher (12.63 ±. 71 vs. 9.28 ± 1.11). The relationship between OS and cleavage rate ($p<0.05$) and the relationship between MII stage rate and TAC ($p<0.05$) were positively significant.

Conclusion: The ETS exposure may alter assisted reproduction success influencing on number of available oocytes. Although, the OS in follicular environment affect ability of oocytes to reach specific cleavage stages at appropriate time intervals, it does not mediate poor assisted reproduction outcomes due to ETS exposure.

Keywords: Assisted Reproduction, Environment Tobacco Smoke Exposure, Oxidative Stress, Follicular Fluid

O-31: Follicular Fluid The Best Medium for *In Vitro* Maturation and *In Vitro* Fertilization

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Background: Induction of *in vitro* maturation and development of immature oocyte is one of the methods that has been frequently used recently in artificial reproduction techniques and has drawn attention of many investigators. This method is useful especially in women who are affected by cancer and polycystic ovary syndrome. Despite using many types of *in vitro* media, an appropriate environment has not been reported yet. Present study has been designed to assess the effect of heated human follicular fluid (hHFF), which is similar to *in vivo* environment for oocyte, on the maturation and fertilization potential of mouse immature oocytes.

Materials and Methods: This study was performed by experimental method. Healthy female mice, aged 4-6 weeks, were sacrificed via cervical dislocation and their ovaries were extracted under sterile conditions. After washing, the separated immature oocytes were divided into three groups: In the first group, 209 immature oocytes were placed in culture medium contained MEM-α, HCG, FSC 20%, and rFSH. In the second group, 203 immature oocytes were put in culture medium contained 100%hHFF. In the third group, 205 immature oocytes were placed in culture medium contained MEM-α, HCG, rFSH, and 20% hHFF. Immature oocytes were placed in an incubator for 24 hours. Then, the stages of oocyte maturation were assessed by invert microscope and mature oocytes in each group were transferred to sperm-contained drops. After 24 hours, rate of two-cell embryos was recorded using invert microscope. Data was analyzed by Chi square test.

Results: Maturation rate of oocytes in the second group (83.7%) was significantly higher than first (59.8%) and third (67.3%) groups ($p<0.0005$). The difference between first and third groups was not statistically significant ($p<0.2$). The formation rate of two-cell embryo in the second group (83.1%) was higher than first (47.2%) and third (54/3%) groups ($p<0.002$ and $p<0.01$, respectively). The difference between first and third groups was not statistically significant ($p<0.07$).

Conclusion: It seems hHFF could improve *in vitro* maturation and fertility potential of immature oocytes and consequently the formation rate of two-cell embryos in mice, in comparison with MEM-α even supplemented with 20% hHFF.

Keywords: Follicular Fluid, IVM, IVF

O-32: Nitric Oxide and Asymmetric Dimethylarginine Levels of Serum and Peritoneal Fluid in Women with Endometriosis

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Background: Endometriosis is a common gynecological syndrom. Its etiology is still not fully understand .A great number of studies reported increase NO concentration, with altered peritoneal immune defence reaction, was involved in pathogenesis of endometriosis. Asymmetric Dimethylarginine is an endogenous competitive inhibitor of NO synthase. Elevated ADMA level is associated with reduced systemic NO production. This study was design to compare NO and ADMA concentration in serum and peritoneal fluid of patients with and without endometriosis. Previosly, we reported this result in 9th congress of the european society of Gyn in Denmark with 89 subject. This result according sample size, 189 patients is ready.

Materials and Methods: This study was approved by Ethical Committee of Isfahan University of Medical Sciences. Women who were subject to laparoscopy for the evaluation of infertility or pelvic pain at the Isfahan Fertility and Infertility Center were considered. The patients with hypertension, coronary arterial diseases, diabetes, renal diseases, active pelvic inflammatory disease or polycystic ovarian syndrome were excluded. After laparoscopy. In a case - control study 88 women were allocated to two groups, according to their laparoscopic results. The first group, women with (n=90) and the second group, without (n=89) endometriosis. The venous blood samples were obtained from all patients before induction of anesthesia. The samples were centrifuged and the serums were stored at -20°C until measurement. The peritoneal fluid samples were collected from pelvis before any manipulation. The bloody fluids were excluded. The peritoneal fluid samples also were centrifuged and the supernatant were stored at -20°C until measurement. The serum and peritoneal levels of nitrite (stable NO metabolite) were measured using a colorimetric assay kit that involves the Griess reaction (promega corporation USA). The serum and peritoneal fluid levels of ADMA were measured using enzyme immunoassay kit (DLD Dignostica Gmbh Germany). Then, absorbance was measured by a microreader in 540 nm wavelength. The samples nitrite concentration was determined by comparison to nitrite standard reference curve. Data are expressed as mean ± SEM. Unpaired t tests was applied to compare the parameters between the groups. Value of $p<0.05$ was considered.

Results: There is significant difference in pf level of No between two groups. There is no significant difference in pf level of ADMA between two groups.

Conclusion: The elevated level of No in PF but not in serum implaying a possible role of NO in the pathogenesis of endometriosis. This results support that pelvic inflammation is a consequence of endometriosis.

Keywords: Endometriosis, NO, ADMA, Serum, Peritoneal Fluid, Infertility

O-33: Serum Anti-Müllerian Hormone Predicts Ovarian Response and IVF Outcome

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Background: To evaluate the level Anti-Mullerian hormone (AMH) in serum in response to ovarian stimulation (group 1) in low-response (n=26), moderate-response (n=40), and high-response (n=29) patients and to compare its changes (n=23, group 2) throughout the menstrual cycle between pregnant and nonpregnant patients.
Materials and Methods: Serum was collected from 210 patients on day 3 of menstrual cycle and studied for the level of AMH, FSH and the number of antral follicles (AF). Furthermore serum and follicular fluid (FF) were collected from 95 IVF/ICSI patients on the day of follicular puncture (FP). The aetiology of patients was tubal or male factor infertility. These patients were divided into two groups: In Group 1 we studied the comparison of AMH level in serum in response to ovarian stimulation and pregnancy rate, respectively. In group 2, 25 patients were monitored throughout the menstrual cycle until 4 weeks after embryo transfer.
Results: There was a significant correlation between basal AMH in serum and total AF on day 3 of menstrual cycle ($r= 0.7$, $p<0.001$). We found inverse relations between serum AMH levels, FSH and advanced patients' age. In group 1 on the day of FP, the mean AMH level in FF was significantly higher than in serum ($p<0.001$). We found a significant correlation between AMH levels in serum and FF ($p<0.001$) and also in their correlation to AF. In response to ovarian stimulation AMH levels in serum increased from low, through moderate, to high response patients ($p=0.001$), pregnancy rates were 17%, 25% and 48%, respectively. In group 2 the levels of AMH in serum of pregnant (n=12) and non-pregnant (n=13) patients decreased throughout stimulation phase and reached a minimum on the day of embryo transfer.
Conclusion: Our results demonstrate an association between AMH and ovarian response to gonadotropins. AMH provides a new perspective for clinicians who may now have a reliable serum marker of ovarian reserve.
Keywords: Antral Follicles, Response to Ovarian Stimulation, Follicular Fluid, Pregnancy Rate

O-34: Cell Membrane Toll like Receptors Expression in Follicular Cells of Women with Endometriosis

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Background: Endometriosis is the growth of endometrial cells outside the uterine cavity. It has been suggested that immune system plays important roles in both initiation and progression of the disease. Several studies have been shown that women with endometriosis diverge in their expression of different genes including heat-shock proteins, fibronectin, and neutrophil elastase, which might be involved in the process of Toll-like receptor (TLR)-dependent sterile inflammation. To date, 10 TLRs are identified in human. TLR1,2,4,5,6 are expressed on the cell membrane. TLRs signaling may lead to change in follicular fluid that is the microenvironment for oocyte development. The aim of this study is to investigate TLRs gene expression in follicular cells obtained from women with endometriosis in comparison to normal women.
Materials and Methods: Twenty patients (10 infertile women with endometriosis and 10 normal women with male factor infertility) underwent controlled ovarian stimulation. The follicular fluid was obtained from the largest follicle (>18 mm) then transferred to a sterile Petri dish. After oocytes removal, the fluid was centrifuged at 300g for 5 min. The supernatant was removed. Total RNA was extracted separately from cellular pallet in each group and real time PCR was performed.
Results: TLRs genes were expressed in both groups. Although gene expression of TLR1, 2, 5, 6 was higher in endometriosis than normal women but only TLR5 expression was significant. In contrast, TLR4 gene expression was lower in endometriosis than normal women but it wasn't significant.
Conclusion: Our findings suggested that TLRs may be involved in pathophysiology of endometriosis. It was proposed that inflammatory markers such as IL-6, IL-8, IL-12 and TNF-α are elevated in endometriosis. Since these cytokines could be produced by TLRs signaling, therefore it's possible this alteration is a result of TLRs activation. Our further studies are directed towards understanding TLRs function in endometriosis.
Keywords: Endometriosis, TLRs, Follicular Cells, Oocyte, Innate Immunity

Genetics

O-35: Over-Expression of XRCC1 As Potential Biomarker for Poor Prognosis in Human Preimplantation Embryos: Selection by Study of 84 Genes Involved in DNA Damage Signaling Pathways

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Background: Chromosome abnormalities are associated with poor morphology and development in human preimplantation embryos, all together lead to poor outcomes. This study aimed to explore altered expression of DNA damage pathways in "poor morphological and development embryos with sever aneuploidies".
Materials and Methods: Surplus day-4 embryos of PGD cases were pooled in two groups: Poor prognos-

tic group included grade C to D embryos with abnormal rate of cell division and sever aneuploidies, more than one aneuploid chromosome in FISH based PGD of day-3, this group named as group 1; good prognostic group as control included grade A to B embryos with normal rate of cell division without aneuploidy or mild aneuploid, just for one chromosome in day-3 PGD. Total RNA was isolated and reverse transcribed from each biological replicate included 15-20 embryos. Synthesis of cDNA was done by using of about 2 ng of RNA from each biological replicate. Gene expression of DNA damage signaling pathways was analyzed using commercial real-time PCR-based array including 84 genes of interest after specific preamplification of cDNA by a primer mix included all genes of array plate.

Results: RPL13A was used as housekeeping gene for normalization of data based on the results of experiments. XRCC1 were over-expressed (p=0.001) in group 1. CDK7, DDIT3, FEN1, LIG1, MSH3, RAD50 and ACTB also showed over-expression in group 1($p<0.05$). Some other genes had considerable fold changes while their statistical significance need to more biological replicates.

Conclusion: XRCC1, as a participant in the base excision repair of DNA single strand breaks, could be considered as a biomarker for preimplantation embryos with poor prognosis.

Keywords: Preimplantation, Expression, XRCC1, Poor Prognosis

O-36: Genome Haplotyping and Detection of Meiotic Homologous Recombination Sites in Single Cells, A Generic Method for Preimplantation Genetic Diagnosis

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Background: Haplotyping is invaluable not only to identify genetic variants underlying a disease or trait, but also to study evolution and population history as well as meiotic and mitotic recombination processes. Current genome-wide haplotyping methods rely on genomic DNA that is extracted from a large number of cells. Thus far random allele drop out and preferential amplification artifacts of single-cell whole genome amplifications as well as algorithmic shortcomings have precluded genome-wide haplotyping of one cell.

Materials and Methods: Cells were isolated and lysed. The single-cell genomes were subsequently amplified using MDA- or PicoPlex technology, and SNPs were typed using Affymetrix or Illumina platforms. Genome-wide haplotype reconstruction was performed with the Merlin as well as a newly developed family-based haplotyping algorithm PROSPER (preimplantation optimal screener and proper estimator of recombination). The reconstructed haplotypes were further interpreted using novel interpretation algorithms.

Results: By developing an innovative data extraction and analysis pipeline of genome-wide SNP-data, the haplotype was reconstructed from single EBV-transformed lymphoblastoid cells as well as human blastomeres derived from *in vitro* fertilized (IVF) embryos. The methodology applies an optimized single-cell genotype computation and a mixture of novel algorithmic approaches that compute and interpret single-cell haplotypes. When compared to the reference haplotypes which were determined from DNA-samples extracted from many cells of the corresponding EBV cell lines, this computational pipeline improved genome-wide SNP-haplotype discordance rates from ~25% to less than 5% for single cells, thus enabling the detection of homologous recombination sites in individual cells. Furthermore, when applied to single human blastomeres of an embryo carrying a disease allele identified by conventional preimplantation genetic diagnosis the method was able to confirm the diagnosis.

Conclusion: This generic haplotyping method will revolutionize reproductive genetic options as it not only enables to select pre-implantation embryos for a single trait, but also broadens the selection spectrum to multiple Mendelian traits as well as to qualitative and quantitative traits present in (animal) embryos. In addition, we envision that the method will increase our insights in the mechanisms of meiotic and mitotic recombination processes and enable the analysis of specimen with limited or degraded DNA.

Keywords: Reproductive Genomics, Haplotyping, Preimplantation Genetic Diagnosis, Single Cell

Reproductive Imaging

O-37: Yolk Sac Diameter and Embryonic Heart Rate As Prognostic Factors of First Trimester Spontaneous Miscarriage

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Background: During the past 20 years a lot of information is achieved about the first trimester of pregnancy through ultrasound and intrauterine fertilization. Many researchers tried to use the symptoms of ultrasound in early pregnancy like measuring the gestational sac and yolk sac of the pregnancy to predict the continuation of pregnancy. Aim of the recent study is to predict the risk of spontaneous abortion at 8-6 weeks of pregnancy us-

ing ultrasound markers.

Materials and Methods: This prospective and observational study was performed on 239 pregnant women admitted to Ayatollah Roohani Hospital in the years 2010-2012; pregnant women were enrolled with regular menstrual cycles (28-30 days). For all patients at 6-8 week of gestation vaginal ultrasound was performed and yolk sac diameter (YSD) and embryonic heart rate (HER) were evaluated. Then the patients were followed the end of 20 weeks of pregnancy.

Results: Patients were followed up until 20th weeks, and 18 (7.5%) aborted. We evaluated the variables by receiver operator characteristic curve to predict abortion. The areas under the curve for EHR (0.95) and for YSD (0.80) were statistically significant ($p< 0.023$, $p< 0.039$ respectively). The logistic regression analysis was done to predict the risk of abortion during pregnancy and EHR were statistically significant ($p< 0.003$).

Conclusion: EHR is more capable to predict the risk of abortion than yolk sac diameter in the first trimester.

Keywords: Spontaneous Abortion, Gestational Sac, Yolk Sac

Poster Presentations

Andrology

P-1: Addition of Omega-3 Unsaturated Fatty Acids in Sperm Extender Does Not Improve The Quality of Chilled Bovine Sperm

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Background: The importance of polyunsaturated fatty acids (PUFAs) on chilled sperm quality is under investigation. Addition of fatty acids to the diet can influence sperm quality and male fertilizing capacity. The aim of this study was to investigate if the addition of n-3 PUFAs improves quality of bovine chilled sperm.
Materials and Methods: In order to do experiment, 5 proven bulls were randomly selected and their ejaculates were collected by artificial vagina. Immediately, semen characteristics including volume, pH value, motility, viability, abnormality and concentration were recorded. Only ejaculates with normal characteristics were chosen. Then, each ejaculate was diluted by extender containing different supplementation (in 5 groups: group 1) basic extender (BX) as control, group 2) BX plus Polyethylene glycol (PEG) as a solvent, groups 3-5) BX containing three different concentration of n-3 PUFAs (1, 2.5 and 5%) in combination with PEG. Basic semen extender contains Tris-citrate buffer medium plus egg yolk and glycerol. All samples were maintained in refrigerator (5°C) for 24 and 48 hours.
Results: Motility (by CASA), viability and morphology were investigated in chilled sperm samples after 24 and 48 hours. The results were evaluated by repeated measure ANOVA using SPSS and $p<0.05$ was considered significant. Immotility (D) increased and all other parameters decreased over the liquid preservation period within all groups including control. Although parameters were decreased during the next 24 hours of preservation, they were not significant. Different concentrations of omega-3 supplementation didn't improve morphology and motility parameters significantly during the liquid preservation period. Even more, average of viability in group 5 (BX plus PEG and 5% PUFAs) was significantly decreased in compare with control group ($p=0.001$) which show an adverse effect on viability.
Conclusion: Our results showed that PEG has some detrimental effects on sperm motility and viability during chilling, and the addition of n-3 PUFAs to semen extenders could not attenuate the detrimental effects of PEG and did not significantly improve bovine sperm quality. Nonetheless, diet supplementation of bulls with n-3 PUFAs is advisable to modify the fatty acid compositions of sperm plasma membrane for increasing its resistance to cooling.
Keywords: Bovine, Sperm Quality, Omega-3 Fatty Acids, Chilling

P-2: Evaluation of Apoptosis in Germ Cells, following Treatment with Vincristine and Cetrorelix (GnRH Antagonist)

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Background: Infertility problem affects young couples. One of the known causes of spermatogenic disorder is chemotherapy in patients with cancer. Since dividing cells are mainly affected by anticancer drugs, the aim of the present study is to investigate the preventive effect of GnRH antagonist on spermatogenic cell apoptosis produced by anticancer drug.
Materials and Methods: In the present study 30 adult male mice aging 6-8 weeks were used. The mice were divided into 3 equal groups as: control, vincristine (V) group and vincristine + cetrorelix, (V+C) group. A single dose of Vincristine was injected as ip, at 1.5 mg/kg. In (V+C) group cetrorelix injection was started one week before vincristine treatment and continued for 3 more weeks. Since spermatogenic cycle in mice is 35 days, mice in all groups were sacrificed 35 days after vincristine injection. Half of testicular specimens were fixed in 10% formaldehyde and prepared for histochemical studies. Performed tunnel staining with POD- Kit.
Results: Histochemical studies, showed induce apoptosis in germ cells. The rate of apoptotic cell in control group was 1.54 ± 0.27µm, in (V) group was 5.97±0.70µm, and (V+C) group was 2.42±0.26 µm. Statistical analysis of data showed significant differences in rate of apoptotic cells between control and (V) group ($p<0.05$). But in (V+C) group apoptotic cells were similar to control group. And rate of apoptotic cells in (V) group were significantly upper than (V+C) group ($p<0.001$). Conclusion: According to the result it is concluded that GnRH antagonist administration before cancer treatment could partially prevent the side effect of anticancer drugs.
Conclusion: According to the result it is concluded that GnRH antagonist administration before cancer treatment could partially prevent the side effect of anticancer drugs.
Keywords: POD-Kit, Histochemical Studies, Apoptosis

P-3: Effects of Increased Mast Cells Number-Induced by Sulpiride Atipycal Antipsychotic Drug on Sertoli Cells and Sperm Production in Adult Male Mice

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Background: Male infertility is a widespread problem and increased number of mast cells-induced by some drugs like sulpiride atipycal antipsychotic drug, ara described in the testis of males, exhibiting impaired sper-

matogenesis and fertility disorders. In this study number of mast cells and sertoli cells, thickness of seminiferous tubule's lamina propria and sperm concentration and viability in sulpiride treated mice were investigated.

Materials and Methods: 18 adult male mice were categorized into 3 groups. Treatment group was administered sulpiride 40 mg/kg/day IP for 45 days. Control sham received sesame oil as a vehicle of drug and control group received nothing. 45 days later couda epididymis were removed and placed in HTF medium. After sperm swimming up, the spermatozoal suspention was analyzed for sperm concentration in couda epididymis and viability. Testicular tissue samples were taken for histological studies using Toluidine-blue (mast cells), Iron-weigret (sertoli cells) and Azan-mallory (type IV collagen fibers) staining methods.

Results: Increased number of mast cells, reduced number of sertoli cells and obvious thickness of seminiferous tubule's lamina propria are observed in treated group in comparison with control-sham and ontrol groups. Statistically significant decrease in sperm concentration and viability are found between treated group and 2 other groups ($p<0.05$).

Conclusion: In sulpiride consumers, accumulation of mast cells and fibrotic remodeling of seminiferous tubule's lamina propria cause sertoli cells dysfunction with reduced sperm production that can lead to male infertility.

Keywords: Mast Cell, Sertoli Cell, Lamina Propria, Sperm, Sulpiride, Mice

P-4: Effect of Reduced FSH Serum Concentration- Induced by Sulpiride Atypical Antipsychotic Drug on Repopulation Index and Number of Primery Spermatocyte Cells in Adult Male Mice

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Background: Usage of some atipycal antipsychotic drugs like sulpiride has severed effects on male fertility by impressions on sexual hormones secretion. These hormones regulate male germ cells proliferation and differentiation. In the present study we sought to elucidate the impact of sulpiride on serum prolactin and FSH concentration. Also effect of FSH on spermatogonium A/B (repopulation index) and primery spermatocyte numbers were evaluated.

Materials and Methods: 24 adult male mice were divided in 3 groups as test, control sham and control. Treatment group was injected 40 mg/kg sulpiride solution IP, daily for 45 days. Control sham received placebo. After 45 days all mice were sacrificed by cervical dislocation. Prolactin and FSH in serum samples of all mice were measured. Testicular tissues were fixed in 10% formalin fixative and subsequently embedded in paraffin. Sections were stained with iron-veigert for assessment of spermatogony and primery spermatocyte cells.

Results: Outcome shows a significant increase in serum prolactin levels and decrease of FSH serum concentration in treated mice. No significant differences are found between 2 other groups. Repopulation index and the number of spermatocyte cells significantly reduce in treated group in comparison with control sham and control groups.

Conclusion: Current study proves that sulpiride antipsychotic drug is able to affect on sperm production by indirect reduction of some sexual hormones serum concentration and can cause secondary infertility in some cases.

Keywords: Sulpiride, FSH, RI, Mice, Spermatocyte

P-5: Evaluation of The Effect of Sulpiride As An Atypical Antipsychotic Drug, on The Serum Levels of Prolactin, LH, FSH and Testosterone in Adult Male Mice

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Background: Prescription of the atypical antipsychotic drugs like as Sulpiride for the treatment of schizophrenic patients was increased because of their less side effects. This study was aimed to evaluate the effect of sulpiride on hormonal milieu of adult male mice namely their serum level of prolactin and sexual hormones.

Materials and Methods: 24 adult male mice were divided in three groups as test, control sham and control. The test mice were received 40 mg per kg sulpiride solution, daily for 45 days IP. Mice in the control sham were received the solvent only. Prolactin, LH, FSH and testosterone were measured in the serum samples of all mice and data was analyzed by statistical methods.

Results: A significant increase in serum prolactin levels was found in the treated mice which were along with a significant decrease of LH, FSH and testosterone levels. No significant differences were found between control sham and control mice.

Conclusion: These findings indicate that sulpiride is able to induce reproductive dysfunctions via reduction of sexual hormones levels and induction of prolactin secretion.

Keywords: Sulpiride, Prolactin,Testosterone, Mice, FSH

P-6: Protective Effects of Green Tea Extract on Lead-induced Damage in Mouse Testis

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Background: Lead is a heavy metal that has been known for its adverse effects on many body organs and

systems and. In the present study, the protective effect of green tea (GTE) on the male reproductive system following the administration of lead (pb) was investigated.

Materials and Methods: Forty male NMRI mice were divided into four groups of 10 animals each. Controls, GTE treated, pb treated and pb + GTE treated. The rats received pb (1%) orally in drinking water. GTE (100 mg/kg) was coadministered daily intraperitoneally. After 6 weeks, the animals were sacrificed and testes were removed and processed for light microscopy. Blood samples were evaluated for biochemical parameters. The data has been compared using statistical methods (SPSS, ANOVA, $p<0.05$).

Results: The results showed a significant reduction in the body and testicular weight, Sertoli and Leydig cell population with a significant increase in blood lead level and plasma malondialdehyde (MDA) concentration in Pb treated groups compared to control. GTE, however, significantly reduced these adverse effects of Pb. By histological analysis, germ cell damage was greatly attenuated by green tea extracts coadministration. Additionally, green tea significantly increased the plasma total antioxidant capacity (TAC) in both groups GTE and GTE +pb compared to the control group. Whereas no significant reduction were observed in pb group.

Conclusion: It is concluded that the green tea can has protective effect on lead induced testicular toxicity.

Keywords: Green Tea, Lead, Testis, Malondialdehyde, Total Antioxidant Capacity

P-7: Testosterone and Gonadotrophin Hormones Plasma Concentration-Induced Changes in Sertoli and Leydig Cells Number in Hyperprolactinemic Mature Male Mice

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Background: Hyperprolactinemia is a particular concern in the treatment of schizophrenic patients. The side effects associated with high prolactin levels can have a negative impact on GnRH pulsatile secretion and, consequently LH, FSH and testosterone pulsatility and follows by, on special testicular cells that have these hormon's receptors such as sertoli and leydig cells. Quantification the degree of correlation between sexual hormones (LH, FSH, Tes) biosynthesis and leydig and sertoli cells number in hyperprolactinemic mature male mice is the aim of this research.

Materials and Methods: 18 mature male mice were divided in 2 groups as test and control. For hyperprolactinemia induction, test group received 40 mg/kg sulpiride solution, daily for 45 days IP. Prolactin, LH, FSH and testosterone were measured in the plasma samples of all mice. Testicular tissue samples were fixed, subsequently embedded in paraffin and sections were stained with iron-weigert staining method for assessment of sertoli and leydig cells number.

Results: Higher prolactin and lower LH, FSH and testosterone plasma concentration in treated group than control group were found. Sertoli cells number decrease obviously in hyperprolactinemic group in compare with other group. Compensatory increase of leydig cells/mm2 of test group testicular interstitial tissue were observed.

Conclusion: Results show that high prolactin serum concentration by impacts on gonadotrophin hormones secretion, causes changes in male germ-cells supporting cells (sertoli) and male sex hormone producing cells (leydig) number that induce secondary infertility in hyperprolactinemic patients.

Keywords: Sulpiride, Leydig Cell, LH, Sertoli Cell, Prolactin

P-9: The Effects of Chronic Multiple Sequential Stress on Rat's Prostate

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Background: The increase of stress following the technological improvements appears to be an important factor that causes organs disorders like genital system. Therefore the present study was conducted to investigate the effects of chronic multiple sequential stress on rat prostate.

Materials and Methods: 18 Wistar rats were divided randomly into two equal groups. In animals under stress, the mice were exposed to different multiple sequential stress as Forced swimming, Restraint, Water deprivation, Isolation and Food deprivation for 10 days while the animals in control group were kept in their cages without any disorders. After weighing the animals and anesthesing with xylazine-Ketamine, the prostate of animals were removed and weighed. After fixation with formaldehyde (10%), testis samples were prepared for light microscopic study. The thickness of the prostate epithelium layer determined using Image Tool software in the studied groups. Finally the data were compared statistically.

Results: The present study showed that the mean thickness of epithelal layer in stress group have significantly decreased compared to control group ($p<0.001$). Moreover, the mean of weight in both group didn't have significantly compared to control group.

Conclusion: Our study showed that chronic multiple sequential stress can have negative effects by reducing the thickness of the prostate epithelium layer in rat but more studies are needed to confirm these results.

Keywords: Chronic Multiple Sequential Stress, Prostate, Epithelium, Thickness, Rat

P-9: Protective Effect of Achillea Millefolium Extract Against Cyclosporine - Induced Toxicity on the Fertility Rate in Adult Male Rats

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Background: Cyclosporine is one of the main immunosuppressors used for renal transplant recipients, and is given to prevent transplant rejection. Although the drug increases the survival of patients and grafted organs, it has some side effects independent of its effect on the immune system that are usually ignored. Achillea Millefolium is famous for its medical application as an analgesic and antiseptic in folk medicine. In this study, the effect of Achillea Millefolium extract on the embryo quality in adult male rats which were treated with cyclosporine.

Materials and Methods: This in study, 24 rats were randomly divided into 3 groups as described below according to the treatment they received; control group, Achillea Millefolium + cyclosporine group(100 mg and 45 mg/ kg per day, orally respectively) cyclosporine group (45 mg/ kg per day, orally) .The treatment period was 45 days. Percentage of zygotes, two cell, blastocyct and type of embryos (type I, embryo with low quality type II embryo moderate type II high quality) were evaluated.

Results: Results indicated that the percentage of zygotes or fertilized oocytes, two cell and blastocyct embryoes reduced in cyclosporine -treated rat in compartion with control and Achillea Millefolium + cyclosporine groups, also type III (high quality) embryos increased in Achillea Millefolium + cyclosporine group.

Conclusion: Cyclosporine - induced toxicity on fertility caused to decrease in zygotes and level premental of embryoes. But Achillea Millefolium caused to increased of zygots and improve of development embry to Blastocyts stage.

Keywords: Achillea Millefolium, Cyclosporine, Male Rat, IVF

P-10: Effects of Cadmium on Reproductive Enzymes of Blood and Reproductive Tissues in Adult Male Mice

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Background: Heavy metals such as cadmium (Cd2+) are natural components of the Earth's crust which can not be degraded or destroyed. Industrial, mining and agricultural activities, particularly the excessive use of phosphate fertilizers, have led to high levels of cadmium contamination at many locations worldwide. Some toxic effects of cadmium are due to its inhibition of various enzyme systems such as enzymes containing sulphydryl groups. Chemistry Department of Shiraz University reported the presence of cadmium in water pools of Fars province. So in this study we investigated the effects of cadmium in a concentration similar to that found in Maharloo Lake (Fars, Iran) on activity of the male reproductive system enzymes.

Materials and Methods: Balb/c male mice divided to three groups (C, I and II) which was respectively received 0, 23 and 50 mg/kg of cadmium chloride in 0.5 ml distilled water for 45 days. The activities of prostatic and nonprostatic acid phosphatase in serum, testis and prostate gland were evaluated using acid phosphatase kit.

Results: Nonprostatic acid phosphatase activity decreased while prostatic acid phosphatase activity increased in the serum of group I and II compared to group C. These changes were statistically significant in group III. Prostatic acid phosphatase activity decreased in the prostate of the treated groups compared to group C. In testis of group II and III, acid phosphatase activity increased but prostatic acid phosphatase activity was very low and showed slight decrease compared to group C.

Conclusion: The level of cadmium that finds in underground and pool water around Shiraz, such as Maharloo Lake, and according to its indirect entrance into the food chain, cadmium is a threat to male fertility among mammals in that area, also it requires further study.

Keywords: Cadmium, Reproductive Enzymes, Male, Mice

P-11: The Effects of Kerack Used in Iran on Sperm Parameters and Testis Structure in Adult Mice

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Background: Kerack is emerging illicit substance which its use is rising up in Iran. It has harmful effects on body organs. The aim of this study is to investigate the effects of Iranian Kerack on sperm parameters and testicular structure of mice.

Materials and Methods: In this study, 25 male mice (Balb/C) were divided into five groups (control, sham and three experimental). Experimental groups of Kerack-dependent mice (received ascending dose of Kerack for seven days twice daily) were divided into three categories, experimental I, II and III. Experimental I was given Kerack at a dose of 5 mg/kg, experimental II 35 mg/kg and experimental III 70 mg/kg, intraperitoneally twice a day for a period of 35 days. The sham group received normal saline and lemon juice (2.6 µl/ml) whilst the control group just received water and food. Mice were then scarified and sperm removed from cauda epididymis and analyzed for sperm count, motility, morphology (normal/abnormal) and viability. Testes were also removed, weighed and processed for light microscopic studies.

Results: The results showed that epididymal sperm parameters were significantly decreased in experimental groups (dose-dependent) compared with sham and control groups ($p \leq 0.01$). Gonadosomatic index and diameters of seminiferous tubules were significantly reduced

with high dose Kerack (70 mg/kg) injected in comparison with control testes.

Conclusion: It is concluded that Kerack used in Iran has the destructive effects on reproductive system in male mice.

Keywords: Kerack, Illicit Substance, Sperm Parameters, Testis

P-12: The Improvement of Sperm Parameters and Chromatin Quality by Vitamin E Supplement in Mice

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Background: Vitamin E is believed to be the primary component of the antioxidant system in spermatozoa. There is a relationship between activity of these antioxidant and function of sperm. Vitamins E and C which are belong to non-enzymatic antioxidant are used as a supplemented drug to improve sperm quality and male fertility. The present study was carried out to investigate the positive effects of vitamin E on sperm chromatin quality and sperm parameters (count, motility, viability and morphology) in mice.

Materials and Methods: Totally 16 adult male mice were divided equally into two groups (n=8). The mice of group 1 served as control fed on basal diet, whereas; group 2 received basal diet and vitamin E (100 mg/kg, IP.) for 35 days. Finally, the tail of epididymis was incised and then placed in Ham's F10. Released sperm were used to analyze number, motility, morphology (Pap-staining) and viability (eosin-Y staining) of the spermatozoa. The DNA integrity and chromatin condensation assessments were done by standard cytochemical techniques including acridine orange test (AOT), aniline blue (AB), toluidine blue (TB).

Results: In vitamin-treated mice, a significant Increase was found in sperm number, sperm motility , sperm viability and sperm morphology compared to control animals. A significant increase was also found in the rate of sperm cells with mature nuclei, and spermatozoa with double-stranded DNA in vitamin E group when compared with controls.

Conclusion: Vitamin E not only is able to improve the sperm parameters but also can increase the sperm chromatin quality in mice.

Keywords: Vitamin E, Sperm Parameters, Sperm Chromatin Quality

P-13: Protective Effect of Black Grape Seed Extract on Sperm Quality in Male Rat Adult That Were Treated with Cyclosporine

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Background: Infertility is a problem that now affects many couples that are causing the disintegration of many families and as a psychological crisis, a lot of stress on couples has been entered. Cyclosporine (CsA), a cyclic undecapeptideof fungal origin, is a powerful immunosuppressive agent that has markedly improved graft survival rates in organ transplantation. The drug has adverse side effects such as, hirsutism, and gynecomastia. Black grape extract contain a large amount of phenolic compounds in their skin, pulp and seeds. This study was conducted to investigate the effect of black grape seed extract on sperm quality in male rat adult that were treated with CsA.

Materials and Methods: In this study 24 adult male rat, weighing 200 ± 20 gr were used. The animals were divided into three groups (control, cyclosporine, cyclosporine+ black grape seed extracts). Cyclosporine A and black grape seed extract were given by gavage at the dose of (30 and 150) mg/kg/day respectively for 45 days. After 45 days animals were sacrificed by dislocation of cervical vertebra and cauda epididymis were mince in 1ml HTF + 4mg/ml Bovin serom Albumin and putting for 30 minutes in CO_2 incubator 37°C, after get out sperm in the medium, different parameter such as sperm viability, motility, sperm count and sperm with DNA fragmentation were measurd.

Results: CsA causes both testicular and spermatozoal toxicity by affecting the oxidant/antioxidant balance of testis. This study determined it causes decreaed in sperm viability, motility, sperm count and level of serum testestrone.

Conclusion: Finding have been shown that CsA can causes decreased sperm count and live sperm and increase sperm with damaged DNA and immature sperm. While black grape seed extract is improved relative to this situation.

Keywords: Cyclosporine, Black Grape Seed Extract, Male Rat

P-14: Sperm Quality in Male Adult Rat That Were Treated with Cyclosporine

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Background: Spermatozoa are germ cells that have a pivotal role at fertilisation. Infertility is a problem with a large magnitude. Sperm damages are one of the factors that cause infertility. Reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), superoxide anion (O_2−U) or molecules and/or hydroxyl radical (UOH) affect both male and female gametes. Cyclosporine A (CsA), a neutral lipophilic cyclic undecapeptide (C6H11N1IO12), it is a powerful immunosuppressive agent that has markedly improved graft survival rates in organ transplantation. CsA therapy induces overproduction of reactive oxygen

species (ROS) in hepatocytes and lowers their antioxidant capacity.
Materials and Methods: In this study 16 adult male Rat, weighing 200 ± 20 g were used.The animals were divided into two groups (control, cyclosporine). CsA were given at the dose of 45 mg/kg/day by gavage for 45 days. After 45 days animals were sacrificed by Dislocation of cervical vertebra and cauda epididymis were mince in 1ml HTF+ 4mg/ml Bovin serom Albumin and for 30 minutes put in CO_2 incubator 37°C, After get out sperm in the medium, Different parameters, such as sperm viability, motility, sperm count and sperm DNA fragmentation were measurd.
Results: This study demonstrates that CsA have a Profound effect on the reproductive system of the male rats. CsA arrest the spermatogenic process, and the number of spermatids and spermatozoa were decreased. CsA also reduced motility and the fertilitizing ability of spermatozoa .
Conclusion: That CsA can cause negative effects on sperm and decreased sperm count and live sperm and increase sperm with damaged DNA and immature sperm.
Keywords: Cyclosporine, Male Adult Rat

P-15: Effects of Vitamin E and Cystein on Semen Quality and Biochemical Parameters Related with Lead Toxicity in the Male Rat

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Background: This study was undertaken to investigate whether treatment with vitamin E and N acetyl cystein protects rat sperm by inhibiting reactive oxygen species generation induced by lead (Pb) exposure.
Materials and Methods: After an acclimatization period of one week, 20 Male rats were divided into 4 groups of 5 rats each. Animals within different treatment groups were maintained on their respective diets for 6 weeks as follows: group 1, untreated control; group2, treated orally with lead acetate (1000 ppm); group 3, treated orally with vitamin E (20 ppm) plus lead acetate (1000 ppm); group4 treated orally N-acetyl cystein (800 ppm) plus lead acetate (1000 ppm). The blood and sperm Pb levels were analyzed by graphite furnace atomic absorption spectrophotometer.
Results: Significant decrease in the weights of testes and epididymis were observed in lead treated animals. Exposure to lead acetate significantly increased malondialdehyde levels with a significant decrease in the superoxide dismutase and catalase activities in testes of rats. Epididymal sperm count, viable sperms, motile sperms decreased significantly in lead-exposed rats. Co-administration of vitamin E and N acetyl cystein to lead exposed rats showed a significant increase in the weights of reproductive organs, reduction in lead-induced oxidative stress in the testis tissue and improvement in selected reproductive parameters.
Conclusion: The rats received vitamin E + Pb and cystein + Pb could enhance antioxidant system and semen quality which consequently reduced the oxidative strees and improvement reproduction in rat.
Keywords: Cystein, Lead Toxicity, Rat Testes, Vitamin E

P-16: Effect of Vitamin E and Green Tea Extracts on Semen Quality and Oxidative Stress Parameters in Rat Exposed to Cadmium

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Background: Cadmium is a potential pollutant of the environment. It manifests cyto-toxic effects in different organs in animals. In the testis, changes due to disruption of the blood-testis barrier and oxidative stress have been noted. Therefore, the present study was carried out to investigate the potential protective effects of vitamin E and green tea extract in combination against cadmium (Cd) toxicity.
Materials and Methods: Twenty adult male rats were treated with four different regimens: Animals within different treatment groups were maintained on their respective diets for 5weeks as follows: group 1, untreated control; group 2, treated with Cadmium coloraide (4 g/L); group 3, treated orally with green tea extract (400 mg/kgw) plus Cadmium coloraide (4 g/L); group4, treated orally with vitamin E (100 mg/kg) plus Cadmium coloraide (4 g/L).
Results: Cadmium coloraide produced a reduction in epidymal sperm count, sperm motility and sperm viability. Co-administration of aqueous extracts of green tea and vitamin E with cadmium coloraide prevented the changes in sperm and biochemical parameters. Exposure to Cadmium coloraide significantly increased malondialdehyde levels with a significant decrease in the superoxide dismutase and catalase activities in testes of rats. Supplementation of vitamin E and aqueous extracts of green tea to Cd-induced rat groups declined lipid peroxidation, increased sperm motility and viability and increased the activity of antioxidant enzymes.
Conclusion: These studies suggest that administration of vitamin E and extracts of green tea protects against cadmium - induced and testicular oxidative stress.
Keywords: Cadmium, Vitamin E, Extracts of Green Tea, Semen Quality, Oxidative Stress

P-17: Effects of Garlic (Allium Sativum L), Vitamin E and Cystein on LH and Testosterone Hormone Level Related with Lead Toxicity in The Male Rat

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Background: Lead (Pb) is one of the ubiquitous non-essential haevy metals in the environment. There are

evidences, which show that lead toxicity has many undesired effects, including neurological, behavioral, immunological, renal, hepatic, male reproductive, dysfunctions. The objective of the present study was to investigate the effects of aqueous garlic extracts, vitamin E and N-Acetylcysteine (NAC) on LH and Testosterone Hormone level in male rat.

Materials and Methods: 25 Male rats were divided into five groups of five rats each. Animals within different treatment groups were maintained on their respective diets for 35 days as follows: group 1, rats served as control (C) and received water ad libitum; group 2, received lead acetate by gavage (1000 ppm); group 3 treated with A. sativum extract (400 mg/kg, by gavage) plus lead acetate(1000 ppm); group 4, treated orally with vitamin E (300 mg of α tocopherol/kg of chow)plus lead acetate(1000 ppm); group5 treated orally N-acetyl cysteine (800 ppm)plus lead acetate (1000 ppm).

Results: No significant changes in testosterone levels were observed in rats after lead administration. However, there was a decrease in Serum levels of this hormone in the lead-exposed group. On the other hand, a significant ($p<0.05$) increase was observed in LH level in the rats exposed to lead plus NAC in comparison with lead plus aqueous garlic extract group.

Conclusion: Lead caused to decrease in the serum level of testosterone due to both direct effect on testis and suppression of luteinizing hormone secretion. Co-administration with garlic extract, vitamin E and NAC to lead exposed caused to reduction of lead effect and improvement of reproduction in male rats.

Keywords: Cystein, Garlic, Lead Toxicity, LH, Rat, Testosterone, Vitamin E

P-18: The Effect of Extremly Low Frequency EMF on Epididymis In Rabbit

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Background: Considerable attention is focused on effects of electromagnetic field (EMF) and its increasing use in everyday life. Appliances and various equipments are sources of electromagnetic field with a wide-range of technical characteristics. In this study we investigated the effect of EMF (950 MHz) on epididymis in rabbit.

Materials and Methods: 18 Newzealand rabbits were selected and divided into three groups (control, experimental 1 and experimental 2). While control was not exposed to EMF, the experimental groups were exposed to EMF (950 MHz) 2 hours a day, 6 days per week and for 14 days. After one week rest, the rabbits were sacrificed; the samples were removed and processed for light microscopic studies. The height of epithelium and the mean diameter of epididymis in 3 groups were measured and compared using statistical methods.

Results: The data showed that the height of epithelium in EMF group was significantly decreased compared to the control group ($p<0.001$). Meanwhile and the mean diameter of epididymis. The data showed that the mean diameter of epididymis in EMF group was significantly decreased compared to the control group ($p<0.001$).

Conclusion: It could be concluded that the exposure to EMF leads to detrimental effects on male reproductive system in rabbit by decreasing the height of epithelial and diameter of reproductive ducts.

Keywords: Extremly Low Fequency, EMF, Epididymis, Rabbit

P-19: Association of Poor Chromatin Remodeling with Cytosolic ROS and Mitochondrial ROS in Sperm of Infertile Men

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Background: Cytoplasm and mitochondria are considered as the major origins of sperm ROS production. Sperm is prone to DNA damage by exposure to ROS or due impaired chromatin remodeling or low DNA protamination. Therefore, the aim of this study was to see if there is any association between impaired chromatin packaging and origin of ROS production.

Materials and Methods: Cytosolic ROS, mitochondrial ROS, DNA protamination and apoptosis were evaluated with dichlorofluorescindiacetate (DCFH-DA), dihydrorhodamine 123 (DHR123), chromomycin A3 (CMA3) and YO-Pro-1/propidium iodide respectively, by flow cytometry (FCM) in 40 infertile individuals.

Results: Both percentages of DCF and R123 positive sperm were associated with percentage CMA3-positive sperm and negatively associated with percentage apoptotic sperm. No correlation was observed between CMA3-positive sperm and percentage apoptotic sperm.

Conclusion: Under protamination of sperm is not associated with origin of ROS production, but their relation suggest that they are associated with general physiological dysfunction of sperm. Furthermore, under protamination does not prone sperm to apoptosis.

Keywords: Sperm, ROS, Apoptosis, Protamine, DNA Damage

P-20: Role of Green Tea in Increasing Fertility

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Background: Green tea is a popular type of tea that is made of unfermented tea leaves. Green tea has been used for thousands of years by people in Eastern coun-

tries for a variety of health reasons. One of the primary benefits of Green Tea in regard to fertility is not in what it contains, but rather in what it does not contain.

Materials and Methods: In the present study attempts to investigate the latest articles about green tea effects on fertility was discussed.

Results: Fertility differs from fecundity, which is defined as the potential for reproduction (influenced by gamete production, fertilisation and carrying a pregnancy to term). A lack of fecundity would be called sterility. Human fertility depends on factors of nutrition, sexual behavior, culture, instinct, endocrinology, timing, economics, way of life, and emotions. research shown that green tea can be effect on fertility.because green tea has a much smaller amount of caffeine than regular tea, or even coffee. Caffeine has been shown, in some cases, to negatively impact fertility. So, drinking green tea is a viable alternative to coffee and some other teas. Some research suggests that two of the main ingredients in green tea, hypoxanthine and polyphenols, may lead to a higher percentage of viable embryos. Other research has suggested that these compounds help increase the maturing of eggs, and may even make the eggs more fertile. In addition, polyphenols serve as antioxidants, which help protect your body from various diseases and other conditions. Drinking green tea may even help not only with female fertility, but with male fertility problems as well. Some green tea advocates suggest that green tea may help with low sperm count, and even with sperm motility.

Conclusion: It is hoped with good food and the use of green tea could be tried in order to reduce infertility.

Keywords: Green Tea, Fertiliy, Hypoxanthine, Polyphenols

P-21: The Effect of Silver Nanoparticles (Ag-Nps) Concentration on The Number of Leydig Cells and Sex Hormones in Wistar Rats

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Background: Owing to their special properties, nowadays, nanoparticles are extensively employed in most industries. Immediate effect, greater stability, and antimicrobial properties are among other significant and special factors of silver nanoparticles causing these nanoparticles to be utilized in most industries, including industries manufacturing medical equipment as well as household appliances. Studies have been undertaken since a few years ago to explore the detrimental effects of nanoparticles on humans and such studies are being conducted increasingly. The present research was aimed at examining harms caused by silver nanoparticles to male sex glands and sex hormones in Wistar rats.

Materials and Methods: 75 male rats of approximately one month of age were organized in five groups of 15 rats (1 control group and 4 experimental groups). The rats in the experimental groups were fed silver nanoparticles with concentrations of 25, 50, 100, and 200 mg/kg. After spermatogenesis (35 days), samples of blood were taken from the rats so that testosterone, LH, and FSH may be analyzed. Afterwards, the rats were dissected.

Results: In general, the results obtained demonstrate a significant reduction in the number of Leydig cells in the experimental groups compared to the control one. Too, the results suggest a reduction in testosterone and a rise in LH, particularly, such a reduction and rise are significant in high doses ($p<0.05$); however, FSH showed no significant reduction.

Conclusion: On the whole the results demonstrated that silver nanoparticles disturb the functioning of sex hormones and disrupt the number of Leydig cells. This may affect the fertility potential in the laboratory animal.

Keywords: Silver Nanoparticles (Ag-NPs), Sex Hormones, Fertility Potential, Leydig Cells

P-22: The Effect of Carbon Tetrachloride on Fertility Parameters and Therapeutic Effect of Cornus Mas

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Background: Various investigations have established that carbon tetrachloride (CCl4), a clear, colourless, volatile, heavy and nonflammable liquid, causes generation of free radicals in many tissues such as testis. a wide range of side effects such as reproductive toxicity has been observed following the administration of this liquid in experimental animals. Numerous studies have shown that among horticultural crops, fruits are sources of diverse antioxidant properties, which can protect body against CCl4 induced oxidative stress. In the present study we investigated the effects of Cornus mas fruit extract against carbon tetrachloride induced reproductive toxicity in rat.

Materials and Methods: Thirty male Wistar rats (250-300 g) were selected and randomly divided into 5 groups (n=6):

- Sham group
- Normal control with olive oil (1 ml/kg i.p.) on the 16th day.
- Toxic control
- CCl4 (1 ml/kg i.p.) on the 16th day.
- Treatment group with plant extract (300 and 700 mg/kg i.g.) for 16 days before CCl4 injection on 16th day.

At the end of the treatment, the biochemical changes in lipid peroxidation (LPO) in testis, as well as testosterone concentration were assessed.

Results: Serum testosterone concentration level were significantly decreased in serum of toxic group compared to sham and control animals, whereas malondialdehyde (MDA) level was significantly increased in CCl4 group compared with those in the control and sham groups.

Conclusion: The results suggest that carbon tetrachloride affects fertility parameters and these findings indicate therapeutic effect of C.mas by providing antioxidants components against CCl4 toxicity in the testes of male rats.

Keywords: Carbon Tetrachloride, Cornus Mas, Testostrone, Free radicals, MDA

P-23: Is There An Association between HOST Grades and Sperm Quality?

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Background: Intracytoplasmic sperm injection (ICSI) can be considered the solstice *in vitro* insemination technique because it has powerfully allowed the treatment of male factor infertility, but in this procedure, except visual morphological selection, there is no standardization for sperm selection. Recently, hypo osmotic swelling test (HOST) has been proposed to have the potential to select intact sperm. Therefore, the aim of this study was to evaluate the ability of this technique to select functional sperm in terms of apoptosis, morphology, nuclear and membrane integrity.

Materials and Methods: Twenty semen samples were randomly collected from men attending the andrology unit. Semen samples were washed, exposed to hypotonic condition, then fixed and simultaneously assessed for abnormal morphology, DNA fragmentation and protamine deficiency using papanicoulau, TUNEL and CMA3 staining, in different HOST grades, respectively. For the assessment of membrane integrity or early apoptosis, the remaining semen samples were washed with Ca buffer and stained by Annexin V. Subsequently, Annexin stained samples were exposed to hypo osmotic conditions.

Results: Healthy sperm were observed in HOST graded "d" and "c" while sperm with HOST grade "g" presented the highest degree of anomalies and frequency of apoptosis, morphology, nuclear and membrane integrity.

Conclusion: Addition of HOST into the sperm selection procedure may provide a valuable tool for selection of functional sperm required for ICSI. Furthermore, selection of sperm with HOST grade "g" should be avoided during ICSI.

Keywords: HOST, DNA Integrity, Protamine Deficiency, Apoptosis, Membrane Integrity, Normal Morphology

P-24: DGC-HOST Method: An Approach for Selection of Normal Sperm during ICSI

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Background: During ICSI, motile sperm with normal morphology are selected and injected into oocyte. But integrity of chromatin is not observable. Therefore, researchers are trying for to present novel method of sperm selection with high percentage of normal chromatin. HOST test is a useful assay of functional integrity of the human sperm membrane. We decide to use HOST test after DGC for infertile men undergoing ICSI and compare clinical outcome with control group.

Materials and Methods: Semen samples were obtained from 39 infertile couple with severe male factor. Oocytes of each partner were divided two group. DGC group that sperm selection were only based on concentration gradient; however in DGC-HOST group, sperm selection were based on both concentration gradient and HOST.

Results: Our results showed that percentage of fertilization were higher in DGC-HOST compared to DGC group. But it was insignificant. In addition, percentage good embryo qualities on day 3 were significantly higher in DGC-HOST compare to DGC group.

Conclusion: DGC-HOST procedure is a novel sperm selection method for infertile men with severe sperm parameters.

Keywords: Sperm Selection, HOST, DGC, Fertilization, Embryo Quality

P-25: Royal Jelly Protects from The Paclitaxel-Induced Damages on Sperm Quality in Rats

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Background: Paclitaxel (Taxol, TXL) is a chemotherapy agent which widely used in the treatment of tumors, such as lung cancer, breast and prostatic cancer. Previous reports indicate that taxol damages mainly dividing cells (spermatogonias and spermatocytes) and postmeiotic epitheliocytes (spermatids and spermatozoa). The toxic effect of the drug on microtubules underlies its antiproliferative effect. The present study aimed to clarify the protective effects of royal jelly (RJ: a secretion of the hypopharyngeal and mandibular glands of worker honeybees) on taxol-induced damages on sperm quality in male rats.

Materials and Methods: Wistar rats were divided into six groups (n=8), including: control (C), TXL-treated (TXL, 7.5 mg/kg, i.p. weekly for 4 weeks), TXL- and RJ -treated and RJ -treated animals (RJ, 100 mg/kg, orally). Those groups which received either compounds, were subgrouped based on the various doses of RJ (50, 100 and 150 mg/kg, daily and orally for 4 weeks). The serum level of hepatic functional enzymes and sperm quality were evaluated. The sperm quality was evaluated by means of sperm viability, motility and total count assays.

Results: Co-administration of RJ with TXL attenuated the TXL-elevated alkaline phosphatase activity and recovered the TXL-decreased body weight gain, significantly ($p<0.05$). Sperm viability was reduced by 3- fold in TXL-treated group while the RJ treatment resulted in a significant improvement (86.8 ± 4.3% control group, 24.6 ± 7.1% TXL-treated group and 80.5 ± 5.1% RJ received animals at 150 mg/kg dose level). Additionally, the TXL-reduced sperm motility was improved in the animals which were treated with 100 and 150 mg/kg RJ.

Conclusion: Our data suggest that the TXL-induced damages on sperm quality could be prevented by RJ. Therefore, RJ could be considered as a protective agent for attenuation of the chemotherapy-induced damages in the liver and sperm quality.

Keywords: Chemotherapy-Induced Damages, Paclitaxel, Rat, Sperm Quality

P-26: Histomorphological Study of Rat Prostate Gland

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Background: Rat prostate gland which is used in various investigation has considerable morphological differences with other mammals. There are few reports about its structure. So, this study was carried on for determining of histomorphological structure of rat prostate.

Materials and Methods: Five healthy adult male rats with average weight 20 ± 180 g and approximately 3 - 3.5 months age were studied. Samples were taken from different lobes, and 5- 6 µ sections were made using paraffin embeding method. Sections were stained with H and E and PAS. The histometrical studies were done using digital Dino-Lite lens and Dino- capture software.

Results: The macroscopic results showed that the rat prostate consisted of four lobes including: anterior, ventral, dorsal, and lateral lobes, which lateral lobe divided 2 sublobes types 1 and 2. Microscopicopical observation showed that prostate parenchyma consisted of secretory ducts and secretory alveoli-tubular units. The secretory alveolar units have many folds but these folds are vary in different lobes, so that the anterior lobe has the maximum folded secretory units and they are spread across the entire lob but they were located in peripheral zone of other lobes ($p \geq 0.05$).

Conclusion: The alveoli cells have different staining intensity, so that the alveoli cells of the lateral lobe 1 is more acidophilic than other lobes. The proportion of secretory units to secretory duts varied in different lobes, so that the dorsal lobe has maximum proportion. Tubular units are usually free of folds and mostly are located in the center of the lobes. The results showed that the epithelial secretory units has twice thickness compare to secretory duct.

Keywords: Histology, Micrometry, Prostate, Rat

P-27: The Effects of Combined Docosahexaenoic Acid and Vitamin E Supplementation on Spermatogram in Asthenozoospermic Men

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Background: The motility patterns of spermatozoa correlate with the rate of natural pregnancy. It is not clear which compounds in spermatozoa or seminal plasma may be involved in the regulation of sperm motility. Considering the decrease of omega-3 fatty acids in spermatozoa of asthenozoospermic men, the aim of the present study was to investigate the effects of combined docosahexaenoic acid (DHA) and vitamin E supplements on spermatogram in asthenozoospermic men.

Materials and Methods: In this randomized, double-blind, placebo-controlled clinical trial, 50 asthenozoospermic males, defined as less than 50% sperm motility or less than 25% with rapid progressive motility, were randomly assigned to one of two groups. Participants in the intervention group took daily 465 mg of DHA and 600 IU of vitamin E; and those in the control group took daily two placebos for 12 weeks. Fatty acid analysis of sperm and serum was performed using capillary gas chromatography and gas-liquid chromatography respectively. Sperm characteristics, dietary intakes, anthropometric measurements and physical activity were measured at the baseline and at the end of the study. Statistical analyses were performed using the SPSS software version 20.0, the statistical tests being analysis of covariance, paired-samples t-test and student t test.

Results: Out of 50 participants, 22 men in the intervention group and 20 men in the control group completed the protocol of the study. The baseline characteristics of patients did not differ significantly between the two groups. There were no significant differences in mean dietary intake of total energy, macronutrients and micronutrients between the two groups at baseline or at the end of weeks 6 and 12. In addition, these factors did not significantly change within each group during the study. In serum, DHA and vitamin E supplementation resulted in increase in DHA of serum and the ratio of serum omega-6/omega-3 fatty acids in the intervention group, as compared with the control values ($p=0.02$ and $p=0.03$ respectively). DHA levels in sperm, percentage of motile sperms, percentage of motile sperms with a straight direction and sperm concentration increased in the intervention group respectively, as compared with the control values ($p=0.001$, $p=0.01$, $p=0.03$ and $p=0.007$ respectively).

Conclusion: The results of our study indicated that combined DHA and vitamin E supplements led to increasing sperm motility and DHA levels in sperm in the asthenozoospermic men. DHA and vitamin E, as an antioxidant, may improve sperm motility.

Keywords: Docosahexaenoic Acid, Vitamin E, Asthenozoospermia, Sperm Motility, Infertile Men

P-28: The Protective Effect of Equisetum Arvense Alcoholic Extract on Sperm Quality, DNA Damage and Sperm Abnormalities in Streptozotocin-Induced Diabetic Male Mice

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Background: Diabetes mellitus as a chronic disease can cause fertility problems in male human population. In fact, it exerts sever damages in testicular tissue and subsequently reduces total number of normal sperms and semen volume. Therefore, this study is designed to evaluate the protective effect of Equisetum arvense extract on sperm quality, DNA damage and maturation in diabetic male mice.

Materials and Methods: To follow-up present study, 24 mature male mice were divided equally in four groups: control-sham, diabetic and two diabetic + EE (250 and 500 mg/kg, orally, daily gavage). Diabetes induction was done by injection of the streptozotocin (50 mg/kg, for five days). After 45 days of STZ administration, all animals were sacrificed. Cauda epididymis were removed surgically and placed in 1ml HTF + BSA 4 mg/ml medium (preequilibrated). Then, they were incubated for 30 minute in CO_2 incubator to allow the spermatozoa to swim out. The spermatozoal suspension was analyzed for sperm motility, concentration in the cauda epididymis, and viability. Also, sperm chromatin quality and DNA integrity were assessed by Aniline blue and Acridine Orange staining following sperm sampling.

Results: The obtained data demonstrated that the sperms motility and viability remarkably ($p<0.05$) increased in diabetic + EE groups in comparison to diabetic group of animals. Although the sperm count was decreased in diabetic group, it was not statistically significant ($p>0.05$) with those in diabetic + EE. Moreover, the percentage of sperm with DNA disintegrity, nuclear immaturity and morphologic immaturity, were significantly ($p<0.05$) decreased in diabetic + EE groups in comparison to diabetic group.

Conclusion: Diabetes can impair reproductive function which is associated with sexual impotence and low sperm quality. Our results showed that diabetes caused damages in testicular function with adverse effect on sperm production, sperm DNA integration and nuclear maturation. While EE could decrease these damages.

Keywords: Diabetes, Equisetum Arvense, Sperm, DNA Damage, Mice

P-29: The Effects of Chronic Multiple Sequential Stress on Rat Epididim

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Background: The increase of stress following the technological improvements appears to be an important factor that causes organs disorders like genital system. Therefore the present study was conducted to investigate the effects of chronic multiple sequential stress on rat epididim.

Materials and Methods: 18 Wistar rats were divided randomly into two equal groups. In animals under stress, the mice were exposed to different multiple sequential stress as forced swimming, restraint, water deprivation, isolation and food deprivation for 10 days while the animals in control group were kept in their cages without any disorders. After weighing the animals and anesthesing with xylazine-Ketamine, the epididm of animals were removed and weighed. After fixation with formaldehyde (10%), testis samples were prepared for light microscopic study. The diameter of epididim tube, thickness of the epididm epithelium determined using Image Tool software in the studied groups. Finally the data were compared statistically.

Results: The present study showed that the mean diameter of epididm tube and in stress group have significantly decreased compared to control group ($p<0.001$). Moreover, the mean thickness of the epididim epithelium in stress group have significantly decreased compared to control group ($p<0.003$).

Conclusion: Our study showed that chronic multiple sequential stress can have negative effects by reducing the diameter of epididm tube and thickness of the epididim epithelium in rat epididim but more studies are needed to confirm these results.

Keywords: Chronic Multiple Sequential Stress, Epididim, Diameter, Thickness, Rat

P-30: The Effect of Exogenous Melatonin on Fertility in Rat Oligospermic Induced with Busulfan and Pinealectomy Model

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Background: Although chemotherapy drugs used in treatment of cancer such as lukemia, prostate and ovary cancer and oligospermia is important contraceptive of this drugs therefore this drugs are author of infertility or low fertility in specimen. In the other hand, the studies show that melatonin is secreted from pineal gland has a direct efficacy on the gonads. The aim of this study was to investigate the effect of exogenous melatonin on spermatogenesis and fertility in adult rat oligospermic model under pinealectomy.

Materials and Methods: In this study, 60 rats weigh-

ing 250-300 gr were used. The animals were randomly divided into 6 groups: 1. the control group did not receive any medication, 2. recipient busulfan, 3. recipient of the DMSO(dimethylsulfoxide) as Vehicle busulfan (busulfan sham), 4. Pinealectomized and recipient busulfan and melatonin, 5. surgical sham animal, only skull bones was opened, 6. Pinealectomized, recipient busulfan and ethanol as Vehicle melatonin .Single dose of busulfan and melatonin 60 days that were used by intraperitoneal injection, the dose of busulfan 20 mg/kg and melatonin 0.5 mg/kg were injected. Animals were sacrificed 60 days after treatment and evalutions were made by determining of sperm parameters (sperm motility, count and morphology), infertility study with IVF. The data were analyzed using SPSS version 16 (ANOVA and Tuckey test).

Results: Busulfan significantly reduced sperm count and motility and normal forms in comparision with that of control group ($p<0.05$). However, combined treatment cause of reduced of sperm parameters but in comparison with recipient busulfan group author loss of cavity in germinal cell epithelium and creation a Spermatogenic cell mass in the seminiferous tubules.

Conclusion: Melatonin has a protective effect on spermatogenesis in the busulfan treated rats. Although the mechanism is unknown that may act by reducing the oxidative damage done. Also it did not affect the infertility and the percentage of fertilized eggs, This is perhaps indicative of the various metabolites of melatonin have been implicated in this action.

Keywords: Pineal Gland, Spermatogenesis, Oligospermia, Busulfan, Melatonin

P-31: Chronic Exposure to Imidacloprid Reduced Sperm Content, Motility and Viability

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Background: Imidacloprid (IMD) is a systemic insecticide which acts as an insect neurotoxin and belongs to a class of chemicals called the neonicotinoids which act on the central nervous system of insects. According to previous studies, IMD is rated as "moderately toxic" on an acute oral basis to mammals and according to United States Environmental Protection Agency this compound is classified as class II and III of toxic chemicals and requires warning label. This study was conducted to evaluate the possible adverse effect of chronic exposure to IMD on sperm content, motility and viability.

Materials and Methods: Twenty four mature male rats were used. The animals were divided into control (received corn oil, 0.2 ml, once a day, orally), low dose IMD-administrated (received 112 mg/kg, once a day, orally) and high dose IMD-dosed (received 225 mg/kg, once a day, orally) groups. All animals received mentioned compounds for total of 60 days. The sperm count was performed based on WHO method for sperm count in rats. The sperm motility analyzed and the eosin-nigrosin staining was used for evaluating sperm viability.

Results: The sperm count significantly ($p<0.05$) decreased in IMD-administrated animals in comparison to control group. Accordingly the high-dose-administrated animals were manifested with 34.34 ± 8.12 ($\times 10^6$) sperm content versus low-dose-received 42.12 ± 6.56 ($\times 10^6$) animals. Comparing percentage of immotile sperms between test and control group showed that, in IMD-administrated groups the sperm motility significantly ($p<0.05$) decreased in comparison to control animals. This impairment developed depending on dose. Light microscopic analyses showed remarkable increase in percentage of dead sperms in test groups depending on administrated dosage.

Conclusion: According to present findings, chronic exposure to IMD can remarkably reduce sperm content, motility and viability, in turn it can influence the male fertilizing potential.

Keywords: Imidacloprid, Sperm Motility, Sperm Viability, Fertilizing Potential

P-32: The Effect of Cell Phone Waves (900 MHZ - GSMBand) on Sperm Parameters and Total Antioxidant Capacity in Adult Male Rats

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Background: There is a great concern for the possible adverse effects of cell phones' microwaves. Contradictory results, however, have been reported for the effects of these waves on the body. In the present study, the effect of cell phone microwaves on sperm parameters and total antioxidant capacity was investigated with regard to the duration of exposure and the amount of the frequency of these waves.

Materials and Methods: This experimental study was performed on twenty eight Wistar adult male rats (200-250 g). The animals were randomly assigned to four groups (n=7): control group, two-week exposure to cell phone simulated waves group, three-week exposure to cell phone simulated waves group, and two-week exposure to cell phone antennae waves group. In all groups, sperm analysis was performed based on World Health Organization (WHO) standards and the mean of sperm total antioxidant capacity was determined by Ferric Reducing Ability of Plasma (FRAP). The data were analyzed by one- way ANOVA statistical technique followed by Tukey test using SPSS (version 16) software.

Results: The findings of the study indicated that sperm viability, motility, and total antioxidant capacity in all exposure groups decreased significantly compared to control group ($p<0.05$). Further, increasing the duration of exposure from 2 to 3 weeks caused a statistically significant decrease in the sperm viability and motility ($p> 0.05$).

Conclusion: Exposure to cell phone waves can decrease sperm viability and motility in adult male rats. These waves can also decrease sperm total antioxidant capacity in rats resulting in oxidative stress.

Keywords: Cell Phone, Oxidative Stress, Sperm Parameters, Male Rat

P-33: The Effect of the Pentoxifylline on Human Sperm DNA Fragmentation

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Background: It is stated that pentoxifyline can improve sperm motility and suggested to use in ART for asthenozoospermia. But, probable effect of this drug on sperm DNA in unknown. Our main goal was to evaluate the impact of pentoxifyline on sperm DNA fragmentation.

Materials and Methods: Thirty eight asthenozoospermic patients were selected for this prospective study. After direct swim-up, specimens were aliquot into two groups of treatment and control. In treatment group, 3.6 mM pentoxifyline was added to sperm medium. Both groups were incubated in 37°C for 45 minutes. Then, sperm parameters as well as sperm DNA fragmentation were evaluated and compared between two groups. DNA fragmentation assay was done by sperm chromatin dispersion (SCD) test.

Results: Pentoxifyline improved progressive motility in treatment group compared to control (85.76 ± 5.96 vs. 79.44 ± 9.37, respectively, p=0.02). Also, there was significant difference for viability test between treatment group and control (87.71 ± 8.29 vs. 83.5 ± 9, respectively, p=0.03). Besides, sperm DNA fragmentation was higher in treatment group compared to control (23.36 ± 10.25 and 18.5 ± 8.74, respectively, p<0.0001).

Conclusion: Although pentoxifyline can improve sperm motility but may has some negative effect(s) on sperm quality. Further studies are necessary in order to provement of pentoxifyline safety prior to use in clinic.

Keywords: Pentoxifyline, Sperm Chromatin Dispersion test, Asthenozoospermia

P-34: Evidence for The Influence of Illegal Drug Consumption on Male Fertility

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Background: Exposure to certain drugs and toxins plays an important role in male infertility. Even fancy using of illegal drugs is an important factor in evaluation of male infertility. There is evidence in previous literature that this drug has harmful effects on fertility, but data based on evidence is limited. The purpose of this study was to evaluate the infertile couples in one of the infertility centers in Iran in terms of drug consumption and its adverse effects on male fertility.

Materials and Methods: In a cross-sectional study, 48 infertile couples that illegal drug consumption was reported in their males were investigated.

Results: Signs of infertility were not reported in the females of these couples. 88% of participants have used Meta - amphetaminesand and narcotics drugs. 12% of them had used bodybuilding special drugs and sportsmanship amino acids. Most of them had a history of illegal drugs consumption more than 2 years.

Conclusion: Meta amphetamines and narcotics, all have an adverse effect on male fertility that this effects include effect on the hypothalamus - pituitary axis, testicles, sperm function and testes structure.

Keywords: Illegal Drugs, Male Fertility, Iran

P-35: Anacyclus Pyrethrum and Tribulus Terrestris Simultaneously Improve The Sexual Parameters of Wistar Rats

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Background: Propagation of race has been threatening by infertility which is a widespread impediment for human. About 50% of infertilities accounts for male Sexual dysfunction. Medicinal plants as a traditional treatment play a great role in remedies due to their accessibility, availability and affordability. Anacycluspyrethrum (AP) roots commonly known as pllitorybelongs. Tribulusterrestris (TT) (Zygophyllaceae) is a Mediterranean plant.

Materials and Methods: 32 rats were divided into 4 groups: 1. PBS control group, 2. TT group, 3. AP group and 4. AT (AP and TT) group. They were treated for 25 days and their weight was recorded daily. After, they were anesthetized and sacrificed. Their testis was dissected for histological studies. Their sperms were isolated to measure sperm count, viability and motility. Serum was performed from their blood to carry out the rate of serum testosterone, LH and FSH and nitric oxide.

Results: Body weight didn't show significant increase in AT group compared to none of the groups as well as sexual organs weight. Sperm count, and motility of AT group significantly increased in AT group compared to control group. Viability showed a significant increase in

AT group same results were obsessed in testosterone, LH, and FSH rates. NO increased significantly in this group compared to control group. Histological studies showed significant increase of spermatogonia, leydig, sertoli cell numbers and epithelial diameter in AP group compared to other groups as well as increased concentration of semeniferous spermatozoa.

Conclusion: Significant increase of sperm viability, testosterone, LH, FSH compared to all other groups which were confirmed with histological studies by significant increase of spermatogonia, leydig, sertoli cell numbers and epithelial diameter in AP group compared to all other groups, as well as its efficacy in sperm count, viability and nitric oxide are all reasons for AP to be effectively ameliorative in MSD.

Keywords: Sperm Count, Viability, Motility, Testosterone, LH, FSH

P-36: Administration of L-Carnitine and Homogenized Testis Tissue on The Structure of Testis and Sperm Parameter in Busulfan-Treated Rats

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Background: Busulfan is a chemotherapy drug that is widely used for cancer treatment. However, administration of busulfan to male patients with malignant diseases may cause temporary or permanent sterility. Therefore, research into drugs or Materials to reduce the side effects of busulfan on the male reproductive system is necessary. In the present study the protective effects of L-carnitine and testis-homogenized tissue on the reproductive system of male adult rat following busulfan treatment has been investigated.

Materials and Methods: Stereological technique was used for estimation volumes of testis, seminiferous tubules and interstitial tissue, the length and diameter of seminiferous tubules, germinal epithelium height, length of flagella and total number of some spermatoginic cell lines. Also Sperm Analysis Techniques was used for assessment of sperm parameters.

Results: Based on this research, the length of sperm flagella and sperm count significantly increased in animals treated with L-carnitine Or Testis homogenized tissue in comparison with the busulfan-treated group. Testis homogenized tissue increased testis volume (6.5%) and weight (8.6%), tubule and interstitial tissue volume (6.9% and 11.7% respectively), seminiferous tubule diameter (3.8%), germinal epithelium height (13%) and sperm count (7.5%), and decreased sperm with abnormal morphology (1%) in comparison with the L-carnitine+busulfan treated group

Conclusion: It seems the use of L-carnitine and testis-homogenized tissue with busulfan decreases some side effects of this drug on the male reproductive system. However, in our study, testis-homogenized tissue is more effective than L-carnitine and led to the recovery of both testis and sperm parameters after treatment with busulfan.

Keywords: Stereology, L-Carnitine, Busulfan, Testis Homogenize Tissue, Testis

P-37: Effect of Gibberellic Acid on Sperm DNA Double Strand Integrity, Nuclear Maturity and Motility

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Background: Plant growth regulators gave entry into animals and human cells through diet. Gibberellic acid (GA3) is an extensively prevalent plant growth regulator due to its use in agriculture. Therefore current study was designed to investigate the effect of GA3 compound on sperm DNA integrity, nuclear maturity and motility.

Materials and Methods: To follow-up current study 12 mature male s rats were used. The animals divided into two groups as; control and GA3 administrated (20 mg/kg/day for 45 days, IP) animals. The acridine-orange staining was performed in order to investigate the sperm DNA damage and the aniline-blue staining was conducted to evaluate the percentage of sperms with nuclear maturity. The sperm motility was analyzed accordance to WHO standard method.

Results: Florescent microscopic analyses showed that, the sperm DNA damage significantly ($p<0.05$) increased in GA3-dosed group. Moreover the GA3 administration remarkably ($p<0.05$) elevated the percentage of sperms with immature nuclei and reduced sperm motility as well. Meanwhile no pathological alterations occurred in control animal's sperm content.

Conclusion: Our results clarified that GA3 administration severely reduces sperm nuclear maturity and motility. Moreover GA3 consumption significantly increases sperm DNA damage.

Keywords: Gibberellic Acid, Sperm DNA Damage, Sperm Nuclear Maturity, Sperm Motility

P-38: Protective Effects of Dorema Aucheri (Bilhar) Against Lead Acetate-Induced Reproductive Toxicity in Male Rats

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Background: Lead-induced oxidative damage is the

most serious problem that leads to reproductive system failure in both human and animals. Present study was carried out to investigate whether hydroalcholic extract of Dorema aucheri leaves could protect the male rats against lead-induced reproductive toxicity.

Materials and Methods: Adult wistar rats were treated with 0.1% lead acetate in drinking water with or without 100, 200 and 400 mg/kg Dorema aucheri extract via gavage for 70 days.

Results: Lead acetate treatment resulted in significant ($p<0.05$) reductions in weight of testis, seminiferous tubules diameter, germinal epithelium height, epididymal sperm count and serum level of testosterone. However, no significant changes were observed in selected reproductive parameters in lead exposed rats treated with 100 and 200 mg/kg but not 400 mg/kg of Dorema aucheri extract.

Conclusion: The results indicate hydroalcholic extract of Dorema aucheri leaves have a potential to restore the suppressed reproduction associated with lead exposure and prevented lead-induced reproductive toxicity in male Wistar rats.

Keywords: Dorema Aucheri, Lead Acetate, Reproductive Toxicity, Spermatogenesis, Sperm Parameters

P-39: The Effect of Professional, Environmental and Geographical Factors on Male Infertility

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Background: Infertility with the prevalence of 15% is one of the main problems of the society. Preventing job injuries is one of the priorities of public health. Approximately 50% of total cases of infertility and subfertility is related to male. Some of the factors that affect male infertility are environmental. It is very difficult to ascertain the precise cause, but it seems that apart from genetic factors, environmental factors including environmental contaminants, cigarette smoking, alcohol and drug can have serious effects on infertility and subfertility. Environmental factors and job exposure have an undeniable effect on male infertility, but our knowledge about this subject is poor and controversial.

Materials and Methods: This cross-sectional study was done on infertile men referring to the Paymaniyeh Clinic in Jahrom. Selecting samples was based on census. Data was collected by a questionnaire and was analyzed for frequency, mean, and standard deviation by SPSS11.5.

Results: Mean age of the infertile men was 32.67 ± 6.19 and the age range was 20 to 67 years old. Of the participants, 78.7% were rural and 21.3% were urban, among whom 37 cases (23.1%) were addicts. The result of spermogram showed that 91.5% of cases were normal. 10.6% of the men had experienced the varicocele surgery and 8.1% had used drugs. Most of the infertile men (55.6%) were workers; 21.6%, were farmers; 14.4% clerks;6.8%, drivers;8.6%, mechanics and welders; 4.9%, shop assistants and 2.5% were builders.

Conclusion: Environmental factors can be effective on male infertility and these factors may vary in different areas, hence the great importance of assessing the effects of these factors. According to these findings, most of the infertile men were rural and workers, leading to the conclusion that workers seem to be more prone to infertility due to the effect of the heavy job on the quality of the sperm.

Keywords: Infertility, Environmental Factors, Geographical Factors

P-40: Silymarin Prevents and Protects from the Doxorubicin-induced Oxidative Stress in Testis and Improves Sperm Quality in Rats

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Background: Doxorubicin (DOX) as an antracycline is used antineoplastic agents against tumors. Although DOX has been recognized as a potent and effectiveness anticancer compound, there are however plenty of reports indicating its toxicity against the heart, liver and testis, which hampers its clinical use. This study aimed to investigate the preventive and protective effects of Silymarin (SMN) on DOX induced damages in the testis.

Materials and Methods: Wistar rats were divided into six groups (n=8), including: control (C), DOX-treated (DOX, 15 mg/kg, i.p.), DOX- and SMN -treated and SMN -treated animals (SMN, 50 mg/kg, orally). Those groups which received either compounds, were sub-grouped based on the preventive (PVT), protective (PTT) and/or therapeutic regimens (TPT) of SMN administration. The antioxidant status analyses in the testis and sperm quality measurement were conducted.

Results: Pretreatment and co-treatment with SMN attenuated the DOX-induced malondialdehyde (MDA) production as a biomarker of lipid peroxidation in the testis, while the total thiol molecules depletion due to DOX administration was recovered. The sperm viability was reduced by 2- fold in DOX-treated group while the SMN-pretreatment resulted in a significant improvement (90.16 ± 4.75 % control group, 44.50 ± 7.42 % DOX-treated group and 79.16 ± 5.23 % SMN-pretreated group).

Conclusion: Our data suggest that the DOX-induced oxidative stress could be prevented and/or protected by SMN. Moreover, the SMN protective and preventive effects attribute to its capacity in the reduction of DOX-induced MDA production and recovering the TTM depletion. SMN improved the DOX-induced disturbance in sperm quality.

Keywords: Doxorubicin, Oxidative Stress, Silymarin, Sperm Quality

P-41: Evaluation of CAPZA3 Expression in Infertile Individuals Undergoing Intracytoplasmic Sperm Injection

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Background: CAPZA3 is one of the actin-capping proteins believed to be play an important role in controlling of actin polymerization during spermiogenesis and has been shown to be peresent in mature sperm. Immunohistochemistry showed that human CAPZA3 is localized in the neck region of ejaculated sperm, with moderate and faint signals also detected in the tail and post-acrosome region respectively. For the first time, we evaluated correlation between CAPZA3 expression and fertilization rate in infertile men undergoing ICSI (intracytoplasmic sperm injection).
Materials and Methods: We have measured the expression of CAPZA3 in 25 infertile men candidate ICSI with realtime PCR technique.
Results: We observed a significant correlation between CAPZA3 expression and fertilization rate in these infertile men.
Conclusion: CAPZA3 may play a physiologically role in sperm construction and fertility in human.
Keywords: CAPZA3, Fertilization Rate, Infertility

P-42: Protective Effects of Melatonin on The Testis in Post-Status Epilepticus Rats Following Lithium-Pilocarpin Injection As A Model of Temporal Lobe Epilepsy (TLE)

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Background: Reproductive dysfunction and endocrine disorders are common among men with complex partial seizures of temporal lobe origin. More than 90% of men with epilepsy have abnormal semen analysis, including decreased sperm count, abnormal morphology, and impaired motility. The aim of this study was the assay of chronic treatment with melatonin during the latent phase and chronic phase of evolving TLE on the testis and accessory sex organs weight, sperm parameters and antioxidant enzymes concentrations in the testis.
Materials and Methods: Eight groups of male Wistar rats were treated as follows: epileptic rats without any injection, solvent of melatonin (2% ethanol) as a vehicle (Sham); melatonin (5 mg/kg/daily) for 14 days, melatonin (5 mg/kg/daily) for 60 days, melatonin (20 mg/kg/daily) for 14 days, melatonin (20 mg/kg/daily) for 60 days, sedentary control without any injection (Control) and rats with reverse injection of lithium-pilocarpine.
Results: Induction of Status Epilepticus caused significant decrease ($p \leq 0.05$) in sperm count and parameters. This experiment revealed that melatonin 5 mg for 60 days can significantly improve progressive motility of sperm ($p \leq 0.05$). Moreover, melatonin 20 mg for 14 days and 5 mg for 60 days caused significant improvement in total motility of sperms and reduced immotile sperm. Furthermore, there was a significant reduction in total antioxidant (TA), superoxide dismutase (SOD) and catalaze (CA) levels between epileptic and control groups. Melatonin 20mg for 60 days caused a significant increase in the levels of TA, SOD and CA. Melatonin 20mg for 14 days and 5 mg for 60 days showed a significant increase in SOD and CA, respectively. Testis weight and accessory sex organs were not statistically significant.
Conclusion: It seems that chronic administration of low dose melatonin or semi chronic administration of high dose might be effective in ameliorating some consequences of Status Epilepticus on the sperm quality and antioxidant enzymes which can in turn affect male fertility.
Keywords: Melatonin, Testis, Post- Status Epilepticus, TLE, Rat

P-43: A Comparative Study of The Protective Effects of Metformin and Juglans Regia Leaves on the Alterations of Sperm Population and Motility in Adult Diabetic Rats

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Background: Increase of blood glucose leads to structural and functional changes in various target tissues and organs. Gonadal dysfunction and decrease in testosterone production are the consequence of diabetes and these conditions lead to insufficient production of spermatozoids. Besides the use of chemical agents, herbal medications for treatment and reduction of diabetic side effects have been attended. The aim of this study was to compare the effects of herbal and synthetic drug therapy on fertility in diabetic conditions.
Materials and Methods: Adult male Sprague-Dawley rats with mean body weight 200 ± 20 g were used in this study. The animals were divided into six groups of eight animals: 1. control, 2. control + metformin, 3. control + Juglans regia extract, 4. diabetic, 5. diabetic + metformin and 6. diabetic + juglans regia extract. Metformin and Juglans regia extract were administered with dose of 500 mg/kg of body weight for 56 days after two weeks from induction of diabetes. Diabetes was induced using single intraperitoneal injection of 50 mg/kg of Streptozotocin. At the end of 70 days, the animals were sacrificed, and the sperms of cauda epididymides were collected for analysis.
Results: The administration of metformin and Juglans

extract has not effect on the sperm population and motility in control groups. In diabetic group, the sperm population and motility were decreased significantly in comparison to control rats. As well, the administration of metformin to diabetic rats leads to increment of sperm population significantly and sperm motility insignificantly. In this regard, both indices of sperm analysis were significantly increased in diabetic rats that received Juglans regia extract in comparison to other groups.

Conclusion: The results of this study revealed that in similar circumstances, the herbal medications can be used alone or with glucose lowering drugs due to their good performance and fewer side effects.

Keywords: Diabetes, Fertility, Juglans Regia Leaves, Metformin, Spermatozoids

P-44: Effects of Nano Zinc Oxide Supplementation on Testicular Oxidative Stress in Adult Male Rats Exposed to Endurance Exercise

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Background: Physical activity has a range of effects on male reproductive function depending upon the intensity and duration of the activity.

Materials and Methods: In order to investigate the protective effects of nano zinc oxide on endurance exercise induced testicular oxidative stress, 30 adult male Wistar rats were randomly divided into the following groups: control group (n=10); exercised group (n=10); and exercised supplemented group (n=10). A protocol of 60 minutes exercise per day, five days per week was followed for 8 weeks. In supplemented group nano zinc oxide was supplied intraperitoneally at a dose of 1 mg/kg/day five days per week followed for 8 weeks.

Results: No significant differences were observed in weight of testis and prostate, seminiferous tubules diameter and germinal epithelium height between experimental groups. Weight of epididymis and seminal vesicle, testicular content of superoxide dismutase and glutathione peroxidase were declined significantly in rats of exercised group. Moreover, significant elevation was observed in content of malondialdehyde in exercised group.

Conclusion: Nano zinc oxide supplementation in exposed rats showed less oxidative damage and restored the reproductive parameters to the control level. It can be concluded that endurance exercise induced oxidative stress causes dysfunctions in the male reproductive system, which can be protected by nano zinc oxide supplementation.

Keywords: Nano Zinc Oxide, Oxidative Stress, Endurance Exercise, Spermatogenesis, Male Fertility

P-45: Cell Phones and Male Infertility

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Background: Cell phones have become a vital part of everyday life. However, the health risks associated with their usage are often overlooked.

Materials and Methods: MEDLINE, EMBASE, Cochrane Library and CINAHLPUBMED were searched for relevant trials published from respective database inception dates to Jun 2011.

Results: Recently, evidence from several studies supports a growing claim that cell phone usage may have a detrimental effect on sperm parameters leading to decreased male fertility. Nonetheless, other studies showed no conclusive link between male infertility and cell phone usage.

Conclusion: The ambiguity of such results is attributed to the lack of a centralized assay for measuring inflicted damage caused by cell phones.Study design, ethics, and reproducibility are all aspects which must be standardized before any conclusions can be made.

Keywords: Male, Infertility, Spermatozoa, Cell Phones, Radiation

P-46: The Effect of Oral Antioxidants on Male Infertility

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Background: The use of antioxidants in treatment of infertile men has been suggested, although the evidence base for this practice is unclear. A systematic review of randomized studies was conducted to evaluate the effects of oral antioxidants (vitamins C and E, zinc, selenium, folate, carnitine and carotenoids) on sperm quality and pregnancy rate in infertile men.

Materials and Methods: MEDLINE, EMBASE, Cochrane Library and CINAHL were searched for relevant trials published from respective database inception dates to May 2010. Study selection, quality appraisal and data extraction were performed independently and in duplicate.

Results: Seventeen randomized trials, including a total of 1665 men, were identified, which differed in the populations studied and type, dosage and duration of antioxidants used. Only two-thirds of the studies (11/17) reported using allocation concealment and three studies (18%) used intention-to-treat analysis. Despite the methodological and clinical heterogeneity, 14 of the 17 (82%) trials showed an improvement in either sperm quality or pregnancy rate after antioxidant therapy. Ten trials examined pregnancy rate and six showed a significant improvement after antioxidant therapy.

Conclusion: The use of oral antioxidants in infertile men could improve sperm quality and pregnancy rates. Adequately powered robust trials of individual and combinations of antioxidants are needed to guide clinical practice.

Keywords: Infertility, Male Factore, Antioxidants

P-47: The Effect of Apium graveolens Extract on The Pituitary-Testes Axis in Mice

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Background: Celery (Apium graveolens) has many therapeutic effects.This plant has many phytoestrogens that can affect on the reproductive endocrine system and can reduce fertility .In this study, hydro alcoholic extracts of celery leaves on the pituitary-gonad axis in young male l mice of Balb/C was investigated.
Materials and Methods: The samples were randomly divided into 5 groups (three treatment groups, placebo and control). Celery leaves hydroalcoholic extract in different doses (50, 100,150 mg/kg/2day) was injected (IP) for 20 days. The Placebo group were used for injection of normale saline. After 10 injections, samples bloods were analyzed for FSH, LH and testosterone by RIA method and compared with control groups.
Results: Concentrations of FSH in 100 and 150 mg/kg groups decreased significantly while LH and testosterone concentrations in all experimental groups decreased ($p<0.05$).
Conclusion: This study showed dose-dependent negative effect of celery extracts in the pituitary - gonad axis in male mice.
Keywords: Celery Extract, Male Reproductive Hormones, Mice

P-48: Nicotine Alters Both Somatic and Germ Cells in Adult Mouse Testis

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Background: Nicotine as a toxic agent in cigarette has detrimental effects on reproduction. The aim of this study was to evaluate effects of of nicotine on germ and somatic cells in adult mouse testis.
Materials and Methods: Male mice were divided into four groups. Group A or controls, groups of B, C and D were treated with nicotine intraperitoneally in doses of 0.1, 0.2 and 0.4 mg/100 g body weight once daily for 14 days respectively. Evaluations were made by Transmission Electron Microscopy (TEM), determining of mitotic activity of cells by KI-67 staining, and ELISA for assay of serum testosterone levels
Results: TEM revealed alterations both in germ and somatic cells in nicotine treated groups. Nicotine in groups of C and D caused to deformity in nucleus, reduced numbers of lipid droplets and mitochondria in leydig cells. Myoid cells had deformed and irregular nucleus in groups of C and D. the signs of apoptosis were recognizable in primary spermatocytes and spermatids in groups of C and D. In group D some sertoli cells had apoptotic bodies. Administration of nicotine in group D, significantly reduced KI-67 index compared with control group. Serum testosterone levels and maturity of spermatogenesis were significantly reduced in groups of C and D in compare with controls.
Conclusion: This study indicates that nicotine can affect both on germ and somatic cells in testis. Nicotine induces apoptosis and reduces mitotic activity in male germ cells as a dose dependent manner.
Keywords: Nicotine, Eelectron Microscopy, Apoptosis, Proliferative Activity

P-49: Study of Cetrorelix in Inhibition of Side Effect of Vincristine Used in Chemotherapy on Germinal Cell Population Seminiferous Tubules of Mice

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Background: Infertility problem affects young couples. One of the known causes of spermatogenic disorder is chemotherapy in patients with cancer. Since dividing cells are mainly affected by anticancer drugs, the aim of the present study is to investigate the preventive effect of GnRH antagonist on spermatogenic defect produced by anticancer drug.
Materials and Methods: In the present study 30 adult male mice aging 6-8 weeks were used. The mice were divided into 3 equal groups as: control, exp I group and exp II group. In exp I group, A single dose of Vincristine was injected as ip, at 1.5 mg/kg. In and expII group, cetrorelix injection was started one week before vincristine treatment and continued for 3 more weeks. The mice in all groups were sacrificed 3 weeks after the last dose of Cetrorelix injection. Testicular specimens were prepared for LM studies.
Results: Microscopic study revealed that in the control group the mean number of sertoli and spermatogonium, were 13.20 ± 2.20 µm, and 47.42 ± 1.96 µm, respectively. In exp I group the mean number of above mentioned cells were 16.8 ± 1.14 µm and 16.53 ± 2.37 µm. In exp II group the mean number of above mentioned cells 10.20 ± 1.13 µm and 36.28 ± 7.98 µm. The data showed the difference between control and exp I group, was significant, but difference between control and exp II group was not.
Conclusion: It is concluded that GnRH antagonist administration before cancer treatment could partially prevent the side effect of anticancer drugs.
Keywords: Anticancer Drug, GnRH Antagonist, Chemotherapy, Cetrorelix

P-50: Effect of Antioxidants (E, C, Astaxanthin Vitamins) on Sperm Parameters with Restricted High Fat Diet in Rats

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Background: Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer and infertility and dub fertility. Some studies have associated the body mass index (BMI) with reproductive parameters in men, showing that increased BMI is related to poor semen quality, decreased sperm concentration, decreased normal-motile sperm cells and increased DNA fragmentation index. In this study we studied the effects of Antioxidants (E, C, Astaxanthin vitamins) on sperm parameters with restricted high fat diet in rats.

Materials and Methods: Matured wistar albino male rats weighting approximately 200 - 250 g were divided to 3 groups; control with access to standard diet (group I), group with high-fat diet (group II) and animals received high-fat diet and Antioxidants such as vitamins E and C, Astaxanthin (group III). After at three months, the rats were sacrificed and sperm parameters such as sperm count, motility, morphology and viability were analyzed.

Results: The mean values of sperm parameters showed that progressive motility of spermatozoa in group II and III (13% and 16.81%, respectively) were significant lower than group I (35.36%) as well as normal morphology in group II and III (95.11% and 93.3%, respectively) was decreased rather than group I (99%). But there were no significant difference between sperm count and viability of three groups.

Conclusion: The present results suggest that restricted high fat diet can lead to reduction sperm motility and normal morphology, but treatment with antioxidants such as E, C and Astaxanthin vitamins could not compensated and improvement this deficiency.

Keywords: Restricted High Fat Diet, Sperm Parameters, Vitamin E, Vitamin C, Astaxanthin

P-51: Effects of Garlic on Sperm Parameters in Rats with High Fat Diet

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Background: Obesity and raised body mass index (BMI) is a major risk factor for several diseases such as cardiovascular disease, type 2 diabetes, as well as gynecological problems. In recent years, some studies have associated the BMI with reproductive parameters in men, showing that increased BMI is related to poor semen quality, decreased sperm concentration, decreased and normal-motile sperm cells. Therefore, in this study effect of garlic on sperm parameters in high fat diet rats has been evaluated.

Materials and Methods: Matured wistar albino male rats were divided to 3 groups, control with standard diet, high-fat diet group and high-fat diet with garlic. After three months, the rats were sacrificed and sperm parameters such as sperm count, motility, morphology and viability were analyzed.

Results: Our results revealed that sperm motility in rats with high fat diet decreased compare to control sperms. Progressive stage of sperm motility was lower in group II (30%) rather than group I and III (35.36% and 49%, respectively). Sperm number of group II (55.75×10^6 per ml) was lower than group I (57×10^6 per ml) and group III (61.60×10^6 per ml) as well, but, there was not observed any significant differences in other sperm parameters such as sperm viability and normal morphology among three groups.

Conclusion: The results of this study show that high fat diet result in sperm motility reduction significantly and feeding with garlic could increase and improve sperm motility in fatty rats.

Keywords: Garlic, High Fat Diet, Sperm Parameters

P-52: Effects of Curcuma Longa Extract on Sperm Parameters with High Fructose Diet Rats

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Background: Obesity and increased body mass index (BMI) affect the reproductive parameters in men such as poor semen quality, decreased sperm concentration, decreased normal-motile sperm cells and increased DNA fragmentation index. Lipid peroxidation contributes to the damage of the sperm plasma membrane. The Curcuma Longa is a resource of antioxidants, hence in this study also was evaluated the effect of Curcuma Longa extract on sperm parameters in rats with diet containing fructose pay.

Materials and Methods: Wistar albino male rats, approximately 150-200 g divided into 3 groups: control diet during 10 weeks of study (group I), diet with high fructose (group II) and diet with high fructose treated by Curcuma Longa extract. After 10 weeks, animals were killed and sperm parameters analysis was done.

Results: The results of this study revealed the significant increase in sperm count in group III (49.4×10^6 per ml) rather than group II (38.2×10^6 per ml), and sperm count in group II (37.8×10^6 per ml) was reduced rather than groups I and II, significantly. But progressive motility of sperm in groups II and III (28.5% and 29.7%, respectively) was lower than group I (35.8%), but there was no significant differences between sperm morphology and viability of three groups.

Conclusion: The results of this study suggested that sperm count is affected by diet with high fructose, so that

decreases with high fructose diet and Curcuma Longa extract could increase the sperm count. But Curcuma Longa extract consumption can cause to decrease of sperm motility.

Keywords: Curcuma Longa, High Fructose Diet, Sperm Parameters

P-53: Protective Effect of Alcoholic Extract of Grape Seeds on Spermatogenesis Defects Induced by Dexamehasone

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Background: Exposure to glucocorticoids such as dexamethasone (Dex) leads to numerous changes in various biological systems including the reproductive system. The present study investigates the efficacy of Alcoholic extract of grape seeds, an active component of turmeric, to attenuate Dex-induced spetmatogenic defects in the mouse testicles.

Materials and Methods: A total of 32 male mice were divided into four groups (8 mice each). The first group served as control and received corn oil only for 25 days. Mice of the second group received Dex at the dose of 7 mg/kg body weight by i.p. injection for 7 days. Mice of the third group received Alcoholic extract of grape seeds at a dose of 40 ml/kg body weight. The fourth group received Alcoholic extract of grape seeds at the dose of 40 mg/kg for 25 days and Dex at the same dose of the second group was injected during the last 7 (18 to 25) days. Statistical significance was determined using one-way analysis of variance (ANOVA) followedby Tukey-Kramer multiple comparison tests. A $p<0.05$ denoted the presence of a statistically significant difference.

Results: Testicular histopathology, morphometric analysis and immunohistochemistry assessments were performed for evaluation of the Dex and Alcoholic extract of grape seeds effects on testicular germ cells. There was marked staining for bcl-2, an important antiapoptotic factor, expression with Alcoholic extract of grape seeds +Dex compared with the Dex groups. Dex caused epithelial vacuolization, sloughing of germ cells, reduction of seminiferous tubule diameter, and significant maturation arrest ($p<0.05$). Alcoholic extract of grape seeds +Dex treatments showed significantly prevented these histopathologic changes ($p<0.05$).

Conclusion: It is concluded that, Alcoholic extract of grape seeds may improve the adverse effects of Dex in the mouse testicular tissue by inducing anti-apoptotic mechanism.

Keywords: Alcoholic Extract of Grape Seeds, Dexamethasone, Apoptosis, Spermatogenic Cells

P-54: Toxic effect of Carthamus Tinctorius L. (Safflower) Extract in the Mouse Testis

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Background: Natural products of plant origin are still a major part of traditional medicinal systems in many countries. Carthamus tinctorius L. (CT), also named safflower, is one of the popular herbs traditionally consumed raw amongst people in Iran. The main objective of the present study was to investigate the effects of aqueous extract of CT on the mouse testis.

Materials and Methods: Experimental group received CT extract at the dose of 40 mg/kg for 25 consecutive days and control group received only distilled water. Statistical significance was determined using one-way analysis of variance (ANOVA)followedby Tukey-Kramer multiple comparison tests. A $p<0.05$ denoted the presence of a statistically significant difference.

Results: Testicular histopathology, morphometric analysis and immunohistochemistry assessments were performed for evaluation of the CT effects on testis. There was a significant reduction for bcl-2, an important anti-apoptotic factor, expression with CT treatment ($p<0.01$). Histopathological criteria such as epithelial vacuolization, sloughing of germ and detachment were significantly decreased in CT treated mice ($p<0.01$). Moephometric assessments showed that seminiferous epithelium height and seminiferous tubule diameter were markedly reduced in CT group ($p<0.01$). There was a significant reduction in Johnsen's scoreing with CT treatment ($p<0.01$).

Conclusion: It is concluded that, CT may have adverse effects in male fertility.

Keywords: Spermatogenesis, Carthamus Tinctorius L. (safflower) Extract, Apoptosis, Mice

P-55: Effect of Tempol on Sperm Parameters of Diabetic Rats

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Background: Diabetes mellitus is an endocrine disorder of carbohydrate metabolism resulting primarily from inadequate insulin release (type I) or insulin insensitivity coupled with inadequate compensatory insulin release (type II). Oxidative stress is one of the complications of diabetes that increases in both types of diabetes and treatment with antioxidants can reduce the complications of diabetes is caused by oxidative. Lipid peroxidation contributes to the damage of the sperm plasma membrane. Tempol is an antioxidant that was applied in studies associated with oxidative stress.

Materials and Methods: Wistar albino male rats, 2 months aged and 150-200 g were divided to 4 groups, randomly: group I (control rats without Tempol and STZ), group II (diabetic rats), group III (diabetic rats with Tempol) and group IV (none diabetic rats with Tempol). Rats were done diabetic by STZ injection. After 45 days, the rats were killed and sperm parameters were analyzed.

Results: The results obtained in the study revealed that sperm parameters such as count, progressive motility and normal morphology in group II (34 × 10^6 per ml, 10% and 83.27%, respectively) were reduced rather than group I (57 × 10^6 per ml, 40.4% and 98.5%, respectively), group III (46 × 10^6 per ml, 24.6% and 95.34%, respectively) and group IV (60.31 × 10^6 per ml, 46.5% and 95.8%, respectively), significantly. There were no significant differences between these groups at sperm viability.

Conclusion: The present study showed that count, progressive motility and normal morphology in diabetic subjects were reduced, greatly and supplementation diabetic and non-diabetic subjects with tempol antioxidant improved sperm parameters.

Keywords: Diabetes Mellitus, Tempol, Sperm Parameters

P-56: Silymarin Protected The Sperm Nuclear Maturity and DNA Integrity in Varicocelized Rats

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Background: Varicoceles are the abnormal tortuosity and dilatation of the veins of the pampiniform plexus that drain the testis. Recently, studies evaluating the role of oxidative stress in male infertility have shown that infertile men with varicocele have elevated levels of sperm and testicular tissue-derived reactive oxygen species (ROS). Therefore current study was designed to evaluate protective effect of slymarin (a potent antioxidant compound) on varicocele-induced sperm nuclear DNA damage and motility.

Materials and Methods: Eighteen Mature male Wister rats divided into 3 groups as; control-sham (left varicocele induced for 42 days), Varicocele+SMN-administrated (50 mg/kg/day for 42 days, orally) group and control group. The serum total antioxidant capacity (TAC) and total thiol molecules (TTM) were evaluated. The sperm DNA double strand fragmentation assessed by acridine-orange staining and sperm motility was analyzed accordance to WHO standard method.

Results: The serum levels of TAC and TTM significantly ($p<0.05$) reduced in varicocelized rats, meanwhile the slymarin-administrated group showed remarkable increase in TAC and TTM level. The sperm DNA damage and motility significantly ($p<0.01$) decreased in varicocele-induced animals, while the slymarin-received group showed elevated motility with remarkably decreased DNA damage.

Conclusion: Our data indicate that slymarin at last partly by up regulating the testicular antioxidant system exerts its protective effect on varicocele-induced DNA damage and immobility in sperms.

Keywords: Varicocele, Slymarin, Sperm DNA Damage, Sperm Motility, Oxidative Stress

P-57: Evaluation of HSPA2 in Fertile and Infertile Individuals

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Background: Heat-shock protein A2 (HspA2) is a testis-specific member of the HSP70 family known to play a critical role in spermatogenic cell differentiation. HspA2 is correlated with sperm maturity, function and fertility, and diminished expression of HspA2 results in abnormal sperm maturity and infertility. The aim of this study was to compare expression of HspA2 in fertile and infertile individuals using a recently marketed highly purified polyclonal antibody raised against human HspA2 which only reacts with HspA2 in human.

Materials and Methods: This study included 92 fertile and infertile individuals. Percentage of HspA2 expression was compared by Western blot, microscope florescence, flow cytometry and RT-PCR.

Results: Percentage of sperm expressing HspA2 in anterior and equatorial regions in fertile individuals was significantly higher than infertile individuals. Percentage of positive HspA2 in fixed permeabelized and also capacitated samples was significantly diffrenet between fertile and infertile individuals. Western blot analysis of HspA2 in semen samples revealed high variation within the fertile and infertile population. The results of RT-PCR revealed higher expression of HspA2 in the fertile individuals compared to infertile individuals but this difference was not significant.

Conclusion: In this study for the first time, we show that HspA2 expression has an important role in sperm capacitation and it externalized during the process of capacitation and its abnormal expression effect semen parameters, especially sperm morphology and concentration.

Keywords: HspA2, Fertile, Infertile, Western Blot, Flow Cytometry

P-58: Mast Cells in Interstitial Tissue in The Testis of Male Rats Treated with Acyclovir

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Background: Acyclovir (9-(2-hydroxyethoxymethyl) guanine) (ACV) is an acyclic nucleoside analogue that has shown a potent antiviral activity, and it is known to inhibit the replication of herpesviruses including herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV) in cell cultures and in animals. ACV is khown to be toxic to gonads,then the aim of the present study was to evaluate effect of ACV on the testis tissue in rat.
Materials and Methods: In this study Thirty-two adult male wistar rats (220 ± 20 g) were randomly divided into four groups (n=8 each) with one group serving as control sham (distilled water i.p.), in the drug treated groups Acyclovir administered (4,16,48 mg/kg/day i.p.) for 15 days. All rats were sacrificed by CO_2 inhalation 18 days after the last injection and the testis was removed and fixed in 10% neutural buffered formalin and processed for paraffin embedding. Five µm thick sections were stained with Toluidine Blue. Light microscopical analysis was made to evaluate the structural changes induced in the testis.
Results: Histomorphometrical study confirmed that Acyclovir induced histopatological changes in the rats testis. The mean number of mast cells in peritubular or interstitial tissue in the testis was increased at tow higher dose-levels in compared to control sham group.
Conclusion: Taking collectively, the present results highly support the idea that Acyclovir has adverse effect on testicular morphology in rats.
Keywords: Acyclovir, Testes, Mast Cells, Antiviral Drugs, Rat

P-59: Effect of GnRH on Vincristine - Induced Spermatogenic Defects on Sertoli Cell

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Background: Male factors, mainly spermatogenesis disorder, are responsible for 20-30% of infertility occurs in different societies. One of the known causes of spermatogenesis disorder is chemotherapy in patients with cancer. The side effect of chemotherapic agents may last from 10 years up to the end of the life. Since dividing cells are mainly affected by anticancer drugs, the aim of the present study is to investigate the preventive effect of GnRH antagonist, on sertoli cell defect produced by anticancer drug (vincristine).
Materials and Methods: In the present study 30 adult male mice aging 6-8 weeks were used. The mice were divided into 3 equal groups as: control, vincristine (V) group and vincristine + cetrorelix, a GnRH antagonist, (V+C) group. A single dose of Vincristine was injected as ip at 1.5 mg/kg. In V+C group cetrorelix injection was started one week before vincristine treatment and continued for 3 more weeks. Since spermatogenic cycle in mice is 35 days, mice in all groups were sacrificed 35 days after vincristine injection. Half of testicular specimens were fixed in 2% glutaraldehyde and prepared for EM studies. The thin sections were studied with LEO 906 TEM.
Results: Electron microscopic study showed that in control group sertoli cells had euchromatic nucleus with obvious nucleolus that usually showed one or two invagination in it and their cytoplasm lie on BL and there is BTB between them. In (V) group study showed that there were large spaces between sertoli and BL and also there were vacuoles in and between sertoli cells. Blood testis barrier were so irregular. In (V+C) group sertoli cells were similar to control group.
Conclusion: According to the result it is concluded that GnRH antagonist administration before cancer treatment could partially prevent the side effect of anticancer drugs.
Keywords: Anticancer Drug, GnRH Antagonist, Sertoli Cells, Ultra Structural, Spermatogenesis

P-60: Relationship between Varicocele Surgery and Males' Infertility Based on Semen Analysis

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Background: Semen quality is affected by environmental and genetically factors. The present study aimed to determine the association of varicocele surgery and males' infertility based on semen analysis.
Materials and Methods: In this case-control study, 188 men with infertility disorder in Ilam province were enrolled between May 2008 and May 2009. Semen analysis was done using the Weili Dynamic Sperm Analysis software adapted to WHO classification. This software, categories the sperm activities into four groups A-D based on speed and direction. In group A, sperms have normal speed and direct movement, while in group D, the sperms are motionless. Participants were stratified into two groups of cases with history of varicocele surgery (n=59) and controls without history of varicocele surgery (n=129). Collecting data were analyzed using SPSS software (version 16). The Kolmogorov-Smirnov test was used for distribution normality of data.
Results: Mean age ± SD for cases and controls were 28.0 ± 3.4 years and 30.9 ± 5.5 years respectively (p>0.05). There was no significant relationship between class A, class A+B and live ration (p>0.05). Overall,

72.9%, 74.6% and 62.7% of cases had difficulties in class A, class A+B and live ratio, respectively. Whereas, the corresponding rates for control group were 66.7%, 63.6% and 45%, respectively. However, this difference was significant only for live ratio (p<0.02).

Conclusion: The quality of semen was reduced in men with history of varicocele surgery leading to infertility.

Keywords: Men Infertility, Semen Analysis, Weili Dynamic Sperm Analysis Software, Ilam

P-61: Effects of Acrylamid on Sperm Parameters and Chromatin Quality in Mice

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Background: Acrylamid (AA) is found in carbohydrate-rich foods that are prepared at high temperatures, such as French fries and potato chips. The toxic effects of AA in male animals include degeneration of epithelial cells of seminiferous tubules, decreased number of sperm and elevation in abnormal spermatozoa. The aim of this study was to evaluate the detrimental effects of AA on sperm parameters (count, motility, viability and morphology) and chromatin quality in mice.

Materials and Methods: 16 adult male mice were divided equally into two groups each conaining 8 animals .The mice of group 1 served as controls and fed on basal diet, whereas, group 2 received basal diet and acrylamide (10 mg/kg, water solution) for 35 days. Then the tail of epididymis was incised and placed in Ham's F10 under aseptic condirion. Released spermatozoa were used to analyze number, motility, morphology (Pap-staining) and viability (eosin-Y staining). For sperm chromatin condensation and DNA integrity assessments, the smears were stained by standard cytochemical techniques including acridine orange (AO), aniline blue (AB), toluidine blue (TB).

Results: In AA-treated mice, a statistically significant decrease was found in sperm concentration, sperm motility, sperm viability and sperm morphology when compared with controls. In addition, a significant increase was found in sperm AB-reacted sperm cells (immature sperm), and AO-reacted spermatozoa (single-stranded DNA) in AA-treated mice compared to control animals

Conclusion: Acrylamide reduced sperm parameters as well as sperm chromatin quality and DNA integrity in mice.

Keywords: Acrylamid, Sperm Parameters, Chromatin Quality, Mice

P-62: Aloe Vera Decreases Male Rat Fertility *In Vivo*

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Background: Interests in natural products are being subjected to scientific method of testing. The use of Aloe Vera cuts across barriers of time and culture in the treatment of a broad range of illnesses. The basis of its reputation resides mainly with steadfast belief in claims of its curative properties, but without hard scientific evidence.

Materials and Methods: Thirty three adult male rats were divided into three groups. Experimental groups received Aloe Vera aqueous leaf extract orally for 60 days in two different sublethal doses; of 60 mg/kg, and 120 mg/kg body weight of aqueous extract of Aloe Vera respectively; while, the control received equal volume of distilled water for the duration of a complete spermatogenic cycle.

Results: The administration of the aqueous extract of of Aloe Vera aqueous leaf extract resulted in a significant decrease in testis weight in the two experimental groups in comparison to the control group but had no effect on body or organ weight. The extract of this plant caused a decrease of the following in the two experimental groups, compared to the control group: sperm count, motility and normal morphology, pregnancy rate and diameter and wall thickness of seminiferous tubules. Also, distortion of morphology of the seminiferous tubules and arrest in spermatogenesis was observed in the experimental groups. In addition, the percentage of sperm with damaged chromatin integrity was significantly higher in the two experimental groups.

Conclusion: From the present study, we can conclude that Aloe Vera aqueous leaf extract acts as an anti-fertility agent rather than other herbalists.

Keywords: Aloe Vera, Testicular Weight, Sperm Count, Sperm Motility

P-63: Chronic Exposure to Gly-Phosate; Evidence for Testicular Toxicity and Sperm Parameters

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Background: Glyphosate (GPT) is a broad-spectrum systemic herbicide used to kill weeds, especially annual broadleaf and grasses known to compete with commercial crops grown around the globe. According to United States environmental protection agency the chemical has been reported as a third class toxin. Present study was designed in order to evaluate GPT-induced detrimental effects on testicular tissue and as well to analyze sperm parameters.

Materials and Methods: To follow-up present study 18 mature male rats were used. The animals were divided in to three groups as; control (no administration), con-

trol-sham (2 mg/kg normal saline, orally) and GPT (1250 mg/kg, orally) for 40 days continuously. Tubular differentiation index (TDI) and leydig clls number/one mm^2 of the interstitial connective tissue investigated. The sperm count, motility, abnormality and morphological maturity were evaluated.

Results: More than 50% of the seminiferous tubules were manifested with negative TDI in GPT-administrated group. The number of leydig cells/ one mm^2 of the interstitial connective tissue significantly ($p<0.05$) decreased. Light microscopic analyses for sperm parameters revealed that the percentage of dead, immotile and abnormal sperms remarkably increased in GPT-received animals. Moreover the percentage of morphologically immature sperms significantly ($p<0.05$) elevated in GPT-induced group, while there were no pathological changes in control and control-sham animals.

Conclusion: Our data suggests that GPT exerts its pathological effects by degenerating seminiferous tubules. Moreover chronic exposure to this compound leads to remarkable reduction in semen quality.

Keywords: Clyphosate, Testis, Sperm, Seminiferous Tubules, Tubular Differentiation Index

P-64: Germinal Cells Intracytoplasmic Carbohydrate, Lipid and Lipase levels Alter in Longtime Varicocele-Induced Rats

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Background: Despite several studies many questions remain regarding how varicocele develops its adverse effect on testicular tissue. Spermatogenesis cell lineage needs carbohydrates as a main source of energy for cell division. Any disruption in glucose transporting system in seminiferous tubules results in remarkable decrease in germinal cells biological function. Therefore present study was conducted to evaluate the germinal cells intracytoplasmic carbohydrates, lipid foci and lipase enzyme levels in longtime varicocele-induced rats.

Materials and Methods: To follow-up present study left varicocele was induced in test group (n=18). Non varicocelized rats were considered as control-sham. The test group further sub-divided into three groups based on the termination of the study and sample collecting date (4, 6 and 8 months after varicocele induction). The periodic acid schiff, Sudan-black B were performed in order to analyze intracytoplasmic carbohydrate and lipids accumulation, respectively. The lipase enzyme activity was evaluated by special lipase staining.

Results: Observations demonstrated that in varicocele-induced rats, the first three layers of germial cell lineage exhibited lower number of cells with periodic acid shift positive cytoplasm, higher number of cells with lipid positive stained cytoplasm in comparison to control animals. The lipase activity increased in 4 and 6 months varicoceles, while after 8 months the lipase activity significantly decreased. In contrast to test animals, the control-sham animals exhibited higher lipid accumulation in spermiogenesis cells lineage.

Conclusion: Our data showed that after varicocele induction the germinal cells loss their major energy source (glucose) and switch the energy origins of carbohydrates to lipids. By decreased energy the lipase enzyme synthesis reduces, which in turn results in disruption of biological activity in germinal cells.

Keywords: Varicocele, Germinal Epithelium, Carbohydrates, Lipid Foci, Lipase Activity

P-65: Nitrosative Stress, Lipid and Carbonyl Groups Peroxidations in Long Time-Induced Varicocele; Association with Sperms DNA Damage in Rats

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Background: The pathophysiology of the varicocele has received considerable study, both in humans and in animal models. The sperm DNA damage and related oxidative stress have been identified as significant cause of male infertility. Present study was designed to evaluate the testicular nitric oxide (NO) generation, lipid and carbonyl groups (CGs) peroxidation and to analyze related sperm DNA damage in long time-experimentally varicocelized rats.

Materials and Methods: To follow-up this study left varicocele was induced in test group (n=18). Non-varicocelized rats were considered as control-sham (n=6). The test group further sub-divided into three groups based on the termination of the study and sample collecting date (4, 6 and 8 months after varicocele induction). Levels of malondialdehyde (MDA), NO and CGs were evaluated in testicular tissue. The sperm DNA fragmentation was assessed by commet assay and DNA laddering test was performed for DNA damage.

Results: The NO, CGs and MDA levels significantly ($p<0.05$) increased in varicocele-induced rats in time-dependent manner. Accordingly the 8 months varicocelized rats showed remarkably elevated testicular NO, CGs and MDA levels. The sperm DNA fragmentation (delivered from commet assay) and double strand damage (From Laddering test) significantly ($p<0.05$) increased after 8 months following varicocele induction.

Conclusion: The severe DNA damage of sperms in varicoceles relates to significant elevation in NO, CGs and MDA levels which impairs spermiogenesis. The results also indicate that the varicocele-induced nitrosative stress, lipid peroxidation and CGs significantly enhance depending on time. Ultimately the biochemical changes result in the varicocele-induced sperm DNA damage by the time.

Keywords: Varicocele, Nitrostaive Stress, Lipid Peroxida-

tion, Carbonyl Groups, DNA Damage

P-66: Protective Effect of Testosterone and Vitamin E on Atrazine-Induced Toxicity in Testis of Rats

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Background: Atrazine (ATR) is herbicide which is known to induce endocrine disruption. According to previous reports, long-time exposure to ATR leads to severe seminiferous tubules degeneration and significant reduction in sperm content. Moreover this chemical exerts its pathological impact partly by inducing oxidative stress. Therefore present study was designed in order to evaluate protective effect of Testosterone and vitamin E on ATR-induced detrimental effects in testicular tissue.
Materials and Methods: Thirty mature male rats were used. The animals divided into five groups as; control-sham (corn oil, 0.2 mg/kg, by gavages), ATR - administrated (200 mg/kg, daily, by gavages), ATR + vitamin E (150 mg/kg, every 48 hoursrs.), ATR+testosterone (250mg/kg, per week, ip), ATR + vitamin E + testosterone groups. After 48 days the testicles were dissected and underwent to histological analyses. The sertolli cells index, leydig cells distribution, tubular differentiation (TDI) and repopulation indexes (RI) were evaluated. The serum level of testosterone was evaluated using immunoradioassay method.
Results: The animals in ATR group were manifested with negative indexes for sertolli cells, TDI and RI, while vitamin E and Testosterone-administrated groups were revealed with increased percentage of tubules with positive sertolli cells, TDI and SPI. Accordingly the ATR+vitamin E+testosterone group showed significantly ($p<0.05$) higher percentage of tubules with mentioned indexes. The number of leydig cells per one mm2 of the connective tissue decreased in ATR-induced group. Meanwhile the vitamin E and testosterone administration resulted in remarkably ($p<0.05$) higher survived leydig cells number/one mm^2 of the interstitial connective tissue. Hematological analyses showed that the serum level of testosterone decreased in ATR group, while the highest level of testosterone demonstrated in ATR + vitamin E + testosterone group.
Conclusion: Our data suggest that chronic exposure to ATR could cause reproductive abnormalities. Moreover present findings indicate that ATR, at least partly by interfering in oxidative stress system and by influencing testicular endocrine function, exerts its toxic effects on testes whereas vitamin E and testosterone could fairly protect testicles against ATR toxic effects.
Keywords: Atrazine, Vitamin E, Testosterone, Testis, Sertolli Cells, Tubular Differentiation, Repopulation Index

P-67: Nano-Selenium Protects Cisplatin-Induced Spermatotoxicty and Chromatin Abnormality Associated with Free Radicals Toxic Stress

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Background: Cisplatin (CIS), an anticancer alkylating agent, induces DNA adducts and effectively cross links the DNA strands and so affects spermatozoa as a male reproductive tract toxicant.
Materials and Methods: The present study investigated the cellular/biochemical mechanisms underlying possible protective effect of selenium nano-particles (Nano-Se) as an established strong antioxidant with more bioavailability and less toxicity, on reproductive toxicity of CIS by assessment of sperm characteristics, sperm DNA integrity and chromatin quality. Histopathological analysis of testis and epididymis were performed for assessment of spermatogenic disorders. To determine the role of oxidative stress (OS) in the pathogenesis of CIS gonadotoxicvity, the level of lipid peroxidation (LPO), antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) and peroxynitrite (ONOO) as a marker of nitrosative stress (NS) and testosterone (T) concentration as a biomarkers of testicular function were measured in the blood and testes. Twenty-eight rats were equally divided into four groups. A single IP dose of CIS (7 mg/kg) and protective OP dose of Nano-Se (2 mg/kg/day) were administered alone or in combination.
Results: The CIS-exposed rats showed a significant increase in testicular and serum LPO and peroxynitrite level, along with a significant decrease in enzymatic antioxidants levels, diminished serum testosterone concentration and abnormal histologic findings with impaired sperm quality associated with increased DNA damage and decreased chromatin quality. Coadministration of Nano-Se significantly improved the serum testosterone, sperm quality, spermatogenesis and reduced CIS induced free radicals toxic stress and sperm DNA damage.
Conclusion: The current study demonstrated that Nano-Se may be useful to prevent CIS-induced gonadotoxicity through its antioxidant potential.
Keywords: Cisplatin, Selenium Nano-particles, Oxidative Stress, Reproductive Toxicity, Sperm DNA Damage, Antioxidants

P-68: The Effect of Gundilia Tournefortii Leaf on Reproductive Physiology in Adult Male Rats

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Background: The Gundilia tournefortii leaf contains the chemical compound such as saturated and unsaturated fatty acids. Saturated fatty acid includes stearic and palmitic acid and unsaturated include linoleic and oleic acid, that, these compounds inhibit 5 reductase enzyme. The present study was carried out with the aim of determining the effect of Gundilia tournefortii leaf on spermatogenesis and testosterone level in adult male rats.
Materials and Methods: In this experimental research 45 male rats Wistar strain were divided into 5 group of nine including the control group received nothing, the sham group received an equal volume of ethanol as a solvent and the treatment groups received 200 , 400 and 600 mg/kg B.W of Gundilia tournefortii leaf alcoholic extract for 21 days orally. The results were analyzed through Excel, one- way analysis variance and t test.
Results: The results showed 400 and 600 mg/kg of the extract increased testosterone level and sperm condense in seminiferous tubules and dihydro testosterone level reduced in comparison to the control and saline groups, while it had no significant effect on serum FSH and LH levels.
Conclusion: According to the research results it can be stated that the extract of Gundilia tournefortii leaf has probably caused increasing testosterone level and decreasing dihydro testosterone level via inhibiting 5 reductase enzymes that effect by palmitic, stearic, linoleic and oleic acid of the pit.
Keywords: Gundilia Tournefortii Leaf, Testis, Gonadotropin, Testosterone, Rat

P-69: Cellular and Molecular Mechanism of Male Infertility in The Athletes That Abuse Anabolic Androgenic Steroids: Apoptosis in Spermatogenic Cells, Caspase 3 Activity and The Generation of Reactive Oxygen Species (ROS) in The Rat Model

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Background: Anabolic-androgenic steroids are used at high doses by athletes for improving athletic ability, physical appearance and muscle mass. Unfortunately, the abuse of these agents has significantly increased. It has been established that exercise and high doses of anabolic-androgenic steroids may influence the hypothalamic-pituitary-testis axis, which can in turn affect testicular apoptosis. However, the effects of the combination of exercise and high dose of anabolic-androgenic steroids on testicular apoptosis and the generation of ROS are not known.
Materials and Methods: Five groups of male Wistar rats were treated as follows for 8 weeks: solvent of nandrolone decanoate (peanut oil) as a vehicle (Sham); nandrolone decanoate (10 mg/kg/weekly) (ND); running exercise by treadmill (1 hour/day, 5 days a week) (EX); combination of nandrolone decanoate and running exercise (ND-EX) and sedentary control without any injection or exercise (CO). Apoptosis in germ cells was characterized by TUNEL, caspase-3 assay and the expression of Fas, FasL, Bax and Bcl2 by RT-PCR.
Results: Testis weight significantly decreased in ND-EX but sperm count reduced in ND and ND-EX and sperm motility decreased in all experimental groups relative to the sham and control groups ($p \leq 0.05$). Germ cell apoptosis and Caspase 3 activity was increased in all experimental groups but it was so much in the ND and ND-EX groups. The expression of FAS and FasL was not significant but the expression of Bax was increased and Bcl2 was decreased in the all experimental groups. It shows that germ cell apoptosis in this experiment is from mitochondrial pathway. The generation of ROS was elevated in all experimental groups especially in the ND group ($p \leq 0.05$).Total Antioxidant was decreased in all experimental groups in comparison with CO and Sham groups.
Conclusion: We concluded that running exercise seems to increase the extent of apoptotic changes caused by supraphysiological dose of nandrolone decanoate in rats, which in turn affects fertility.
Keywords: Nandrolone Decanoate, Exercise, Spermatogenic Cells, Apoptosis, ROS, Rat

P-70: Study of GTn-Repeat Expansion in Heme Oxygenase-1 Gene Promoter As Genetic Cause of Male Infertility

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Background: The length of GT-repeats polymorphic region in the promoter of human Heme oxygenase-1 gene (HO-1) alters the level of its transcriptional activity in response to oxidative stresses. Decreased level of HO-1 protein in the seminal plasma has been reported to be associated with oligospermia and azoospermia in male infertility. This is the first study to investigate the association between GT-repeats expansion in promoter of HO-1 and male infertility.
Materials and Methods: The allelic frequencies for different GT-repeats in the promoter of HO-1 gene were determined in 100 case and 100 normal control groups using PCR-PAGE, ABI fragment analysis genotyping and sequencing analysis.
Results: All alleles were classified into S and L alleles.

S alleles with < 27 specified as allele number 0 to 3 and L alleles > 27 repeats were specified as 4 to 6 alleles. The L allele frequency was significantly higher (54.5%) among case group than that in the normal controls (37.5%). Statistical analysis provide significant relationship between L alleles and male infertility ($p<0.001$).

Conclusion: This study shows for the first time that GT-repeats expansion in promoter of the HO-1 gene is associated with oligospermia and azoospermia in male infertility among Iranian infertile cases.

Keywords: Male Infertility, Heme Oxygenase-1 Gene, Polymorphism, Oxidative Stress

P-71: Effects of Cinnamomum Verum on Sperm Parameters with High Fructose Diet Rats

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Background: Obesity is a leading preventable cause of death worldwide, with increasing prevalence in adults and children. Some studies have shown that increased body mass index is related to poor semen quality, decreased sperm concentration, decreased normal-motile sperm cells and increased DNA fragmentation index. Lipid peroxidation contributes to the damage of the sperm plasma membrane. Cinnamomum verum has been used to increase sex recovery. This study also will test the effect of Cinnamomum verum extract on sperm parameters in rats with diet containing fructose pay.

Materials and Methods: Wistar albino male rats, 150-200 g divided into 3 groups: control diet during 10 weeks of study (group I), diet with high fructose (group II) and diet with high fructose treated by Cinnamomum verum extract. After 10 weeks, animals were killed and sperm parameters analysis was done.

Results: The comparative evaluation between three groups revealed significant increase in sperm count in group III (49.7×10^6 per ml) rather than group II (37.8×10^6 per ml), and sperm count in group II (37.8×10^6 per ml) was reduced rather than groups I and II, significantly. as well as progressive motility of sperm in group III (44%) was higher than group I (28.5%) and group II (26.7%), but There was no significant difference between sperm morphology and viability of three groups.

Conclusion: The results of this study suggested that sperm count decreases with high fructose diet and Cinnamomum verum extract could increase the sperm count and motility.

Keywords: Cinnamomum Verum, High Fructose Diet, Sperm Parameters

P-72: Effect of Subinguinal Varicocelectomy on Human Sperm DNA Integrity and Semen Parameters

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Background: Varicocele occurs in approximately 15% to 20% of the male population and it is the most common cause of poor semen parameters. It has been demonstrated that patients with varicocele have poor chromatin packing, DNA damage and nuclear anomalies than the healthy men. Therefore, the aim of this study was to evaluate sperm chromatin integrity and semen parameters in these patients after varicocelectomy.

Materials and Methods: This study evaluated semen parameters and sperm DNA integrity before and 4 month after microsurgical varicocelectomy from 30 men with varicocele in center of infertility in Ahvaz Jundishapour University Medical Sciences. To evaluate parameters of semen was used WHO criteria and sperm DNA integrity, after fixation, sperm smears were stained by aniline blue and toluidine blue.The slides were analyzed by light microscopy and to determine the percentage of mature or immature spermatozoa.

Results: The percentage of positive aniline blue (AB+) and toluidine blue (TB+) significantly decreased following varicocelectomy (59.98% vs. 57.08%, $p=0.047$ for TB+) and (57.08%5 vs. 43.91%, $p=0.039$ for AB+). Morphology, count and motility of sperm showed improving after varicocelectomy but motility of sperm was not considered statistically significant.

Conclusion: This study suggests that microsurgical varicocelectomy can improve human sperm DNA integrity and semen parameters in men with varicocele.

Keywords: DNA Integrity, Varicocelectomy, Semen Parameters

P-73: The Effects of Manganese on Testis Structure and Sperm Parameters in Adult Mice Exposed to Formaldehyde

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Background: Formaldehyde (FA) as a ubiquitous environmental pollutant is extensively used in hospitals, laboratories and many industrial settings. FA exerts adverse effects on testicular structure and sperm param-

eters through increasing oxidative stress. Manganese, a well-known antioxidant, can inhibit oxidative stress damages. The aim of this study was to investigate the influences manganese on testis structure and sperm parameters in adult mice exposed to FA.

Materials and Methods: Thirty five of adult male NMRI mice (age of 6-8 weeks) were selected and randomly divided into four groups: 1. control, 2. sham, 3. FA exposed group and 4. FA and Manganese chloride exposed group. The FA exposed groups were inter peritoneally received 10 mg/kg formaldehyde daily for 14 days and Manganese chloride was just injected 5mg/kg on second weeks. Mice were then scarified and sperms were collected from cauda of right epididymis and analyzed for count, motility, morphology, and viability according to WHO criteria. The other testes tissues were also removed weighed and prepared for histological examination. Seminiferous tubules and lumens diameters, epithelium thickness were measured.

Results: The findings of this study revealed that formaldehyde significantly reduced the testicular weight, sperm count, motility, viability and normal morphology in comparison with control group ($p \leq 0.05$). In addition, seminiferous tubules atrophied and seminiferous epithelial cells disintegrated in Formaldehyde group in comparison with control group ($p \leq 0.05$). Manganese could improve testicular structure and sperm parameters in FA + $MnCl_2$ group in comparison with control group ($p \leq 0.05$).

Conclusion: Manganese can improve formaldehyde damage effects.

Keywords: Formaldehyde, Manganese, Testicular Structure, Sperm Parameters

P-74: Cytochemical Evaluation of Sperm Chromatin and DNA Integrity in Couples with 3 Unexplained Recurrent Spontaneous Abortion

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Background: The aim of this study was to examine the possible relationship between sperm DNA integrity and chromatin packaging evaluated by cytochemical assays, traditional sperm parameters and recurrent spontaneous abortion (RSA) of unknown origin.

Materials and Methods: In this cohort study, 40 couples with a history of RSA and 40 couples with proven fertility were considered as case and control groups respectively. The semen samples of all husbands were analysed for sperm parameters and also sperm chromatin and DNA integrity assessed using cytochemical tests including aniline blue (AB), chromomycin A3 (CMA3), toluidine blue (TB), acridine orange (AOT) and nuclear chromatin stability assay.

Results: Among different sperm parameters, only slow motility was significantly different between the two groups. In sperm chromatin evaluations, there were significant differences between the two groups in all of the tests. In addition, the majority of semen samples in RSA patients exhibited upper percentages of abnormal spermatozoa than the cut-off values regarding different cytochemical assays.

Conclusion: Our study showed that in the cases of RSA, slow motility had a significant reduction in comparison with controls and also spermatozoa of men from RSA group had less chromatin condensation and poorer DNA integrity than spermatozoa that obtained from fertile men with no history of RSA.

Keywords: Recurrent Spontaneous Abortion, Sperm DNA Integrity, Sperm Chromatin 65 Condensation, Cytochemical Tests

P-75: The Protective Effect of Vitamin C on Sperm Parameters and Chromatin Condensation in Diabetic Mice

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Background: Diabetes mellitus (DM), primary or idiopathic, is a chronic disorder of the carbohydrate, lipid and protein metabolism, characterized through hyperglycemia and glycosuria. DM can involve male reproductive function at many levels. Vitamin C (ascorbic acid) is one of necessary elements in human and mammalian diet that is very important in fertility. Vitamin C prevent from gamets damage with free radicals along gametogenesis and fertilization. The aim of this study is to observe the protective effects of vitamin C on sperm parameters (viability, count, morphology and motility) and evaluation of DNA integrity in diabetic mice.

Materials and Methods: Totally twenty eight adult male Syrian mice were divided randomly into 4 groups (n=7). The animals of group 1 were considered as controls, group 2 were diabetic that received a single dose (200 mg/kg) streptozotocin (STZ) intra peritoneally, group 3 received vitamin C (10 mg/kg/daily , intra peritoneal) and group 4 were diabetic mice that received vitamin C (10 mg/kg/daily , intra peritoneal). After 35 days, the cauda epididymis of each mouse was dissected and placed in 1 mL of pre-warm Ham's F10 culture medium for 30 min. The swim-out spermatozoa were analyzed for count, motility, morphology and viability. To evaluate the sperm chromatin quality and DNA integrity, the air-dried smears were fixed with specific fixative and then stained with cytochemical assays before microscopic examinations.

Results: In sperm analysis, all of the sperm parameters were significantly differences between 4 groups ($p<0.001$). Regard to sperm DNA/chromatin quality, although, the results of toluidine blue (TB) and acridine orange (AO) tests showed statically significant differences between groups, but in aniline blue (AB) and chromomycin A3 (CMA3) staining, we didn't see any significant differences ($p>0.001$) between them.

Conclusion: Vitamin C, as a power antioxidant is able to balance the ROS effects of diabet on sperm parameters and DNA integrity.

Keywords: Sperm, Diabetes, Mice, Vitamin C

P-76: Effects of Concurrent Ethanol and Saccharin Consumption on Sperm Parameters in Adult Mice

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Background: Since sperm parameters have a critical role in reproductive function, the objective of this study was to evaluate the effects of concomitant ethanol and saccharin consumption on sperm parameters in adult mice. Chronic ethanol consumption suppresses male reproductive function and sexual behavior in laboratory animals and humans. Furthermore, alcohol abuse by males has been correlated with hypogonadism, feminization and alterations in reproductive hormonal homeostasis, reduced libido, ejaculation problems and impotence. Although, saccharin is an artificial sweetener, there is evidence for the carcinogenicity of sodium saccharin in experimental animals but not in humans.
Materials and Methods: Totally 28 Adult male mice (10 weeks old, 35 g) were divided to 4 group, mice of group 1 served as control fed on basal diet, group 2 received water containing saccharin (0.2% w/v), group 3 received ethanol (10% v/v) containing saccharin (0.2% w/v) and group 4 received ethanol (5% v/v) containing saccharin (0.1% w/v) for 35 days. Finally, left cauda epididymis was cut in Ham's F10. Retrieved spermatozoa were used to analyze count, motility, morphology (Pap-staining) and viability (eosin-Y staining).
Results: Following ethanol and saccharin consumption, the sperm count diminished significantly. Also, a decrease in sperm motility and increase in rates of morphologic abnormalities (coiled and broken tails) were seen in case groups (groups 3, 4, 2 respectively) in comparison with controls. In spite of effect on sperm parameters, in saccharin group, the external genitalia showed morphological alterations. We saw inflammation in 4 animals, and atrophy in external genitalia and urinary bladder tumor in one mouse.
Conclusion: Saccharin consumption affects sperm parameters but concurrent ethanol and saccharin consumption may have more detrimental effects than saccharin consumption alone.
Keywords: Ethanol, Saccharin, Sperm, Mice

P-77: Protective Role of GnRH Antagonist (Cetrorelix) on Side Effect of Vincristine on Diameter of Seminiferous Tubules of Mice

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Background: Infertility problem affects young couples. One of the known causes of spermatogenic disorder is chemotherapy in patients with cancer. Since dividing cells are mainly affected by anticancer drugs, the aim of the present study is to investigate the preventive effect of GnRH antagonist on spermatogenic defect produced by anticancer drug.
Materials and Methods: In the present study 30 adult male mice aging 6-8 weeks were used. The mice were divided into 3 equal groups as: control, vincristine (V) group and vincristine + cetrorelix, (V+C) group. A single dose of Vincristine was injected as ip, at 1.5 mg/kg. In (V+C) group cetrorelix injection was started one week before vincristine treatment and continued for 3 more weeks. Since spermatogenic cycle in mice is 35 days, mice in all groups were sacrificed 35 days after vincristine injection and testis specimens were prepared for Light Microscopic studies.
Results: LM study showed that mean diameter of seminiferous tubules was 190.16 ± 8.34 µm in control group, 135.52 ± 5.59 µm in V group and 178.27 ± 3.93 µm in V+C group. The rate spermatogonia in control group was 47.42 ± 1.96 µm, in V group was 16.53 ± 2.37 µm, and in V+C group was 36.28 ± 7.98 µm. Statistical analysis of data showed significant difference in rate diameter of somniferous tubules and spermatogonia, between control group and V group ($p<0.05$) and also between V group and V+C group ($p<0.05$) but V+C group was similar to control group.
Conclusion: According to the result it is concluded that GnRH antagonist administration before cancer treatment could partially prevent the side effect of anticancer drugs.
Keywords: Vincristin, Cetrorelix, Diameter of Somniferous Tubules

P-78: Effects of Endurance Exercise on Reproductive and Oxidative Parameters in Adult Male Rats

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Background: In order to investigate the effects of endurance exercise on male reproductive function in relation to oxidative stress.
Materials and Methods: a total of 20 male wistar rats were randomly divided into the following groups: control group (n=10) and exercised group (n=10). A protocol of 60 minutes exercise per day, five days per week was followed for 8 weeks. At the end of experiment reproductive organs weight was recorded and following tissue processing morphometric and oxidative parameters of testis evaluated.
Results: There were no significant differences in weight of testis and prostate, seminiferous tubules diameter and germinal epithelium height between experimental groups. Significant decreases were observed in weight of epididymis and seminal vesicle in rats of exercised group.

Conclusion: Endurance exercise significantly increased malondialdehyde (MDA) levels with a significant decrease in the superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the testis of male rats. It can be concluded that endurance exercise induced oxidative stress causes dysfunctions in the male reproductive system.

Keywords: Endurance Exercise, Oxidative Stress, Antioxidant Enzymes, Spermatogenesis, Male Fertility

P-79: The Correlation between Nitric Oxide Levels in Seminal Plasma with Human Sperm Parameters

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Background: Defective sperm quality is a significant cause of infertility in men. It is known that nitric oxide (NO) is a highly reactive free radical gas which has a dual function, being both a cytotoxic and necessary molecule for normal sperm production.
Materials and Methods: To investigate correlation between NO levels with sperm parameters, a case-control study were designed with total of 65 semen samples including, 33 healthy donors as the control group and 32 infertile patients as the case group. After routine semen analysis according to World Health Organization (WHO, 1999) guidelines, the stable metabolites of nitric oxide (nitrite and nitrate) was measured in the seminal plasma of both fertile and infertile men by Griess reagent and compared with sperm parameters.
Results: The mean NO levels in the seminal plasma of infertile men was significantly higher than that in healthy men (p= 0.026) and a negative correlation observed between NO levels and volume of semen (p= 0.005), sperm total count (p= 0.013) and morphology (p= 0.041).
Conclusion: It is suggested that high levels of nitric oxide in seminal plasma may be a risk factor for increased oxidative stress and associate with poor sperm quality in infertile men.

Keywords: Nitric Oxide, Sperm Quality, Infertile Men

P-80: Influence of Formaldehyde Vapor on Male Reproductive Function in Mice

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Background: Formaldehyde (FA, H_2CO), a member of the aldehyde family and one of the simplest organic molecules, is a flammable, pungent, irritating and colorless gas. This study aimed to investigate the effect of formaldehyde (FA) vapor on sperm parameters and testicular tissue concern about the duration of analysis and the amount of the formaldehyde vapor concentrations.
Materials and Methods: In this experimental study, 36 adult male mice were randomly assigned to one control and two experimental groups (n=12): control group (C), group F1 (low concentration) and F2 (high concentration) were exposed to formaldehyde vapor (10 and 20 ppm) respectively for 10 days (8 hours a day). At the end of the exposure period, half of the animals in each group were sacrificed 24 hours after exposure to detect short-term effects and the rest of the mice were sacrificed 35 days after the end of exposure (long-term effects). In all groups, sperm analysis was performed based on World Health Organization (WHO) standards by Computer Assisted Sperm Analyzer (CASA) and histological changes in the testis were determined. Changes in testosterone hormone were also studied. The data were analyzed by one way ANOVA statistical technique followed by scheffe test using SPSS software.
Results: Long-term effects of FA in the experimental groups significantly reduced sperm cell numbers and sperm viability. A drastic reduction in progressive motility and increased percentage of abnormal sperm ($p<0.001$) in comparison with the control group was also noted. Histological study of testes specimens in the experimental group revealed displacement of germinal cells and degeneration of leydig cells and seminiferous tubules.
Conclusion: Exposure to formaldehyde vapor can destroy testicular structure and decrease percentages of sperm count, viability, normal morphology, progressive motility and increase the percentage of immotile sperm.

Keywords: Formaldehyde, Mouse, Sperm Parameters, Testis Histology

P-81: Evaluation of DNA Fragmentation in Patients with Total Globozoospermia

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Background: Infertility is estimated to affect up to 15% of couples of reproductive age, and male factors contribute to 50% of these cases. Globozoospermia syndrome is a severe form of teratozoospermia, with an incidence of less than 0.1%. It is characterized by round sperm head. The most prominent feature of globozoospermia is the malformation of the acrosome, which is totally absent in the total globozoospermia. The aim of this study

was to evaluate the levels of DNA fragmentation in ejaculated sperm of men with total globozoospermia.
Materials and Methods: Sperm samples were obtained from 15 patients with total globozoospermia and 30 men with normozoospermia (normal morphology >4% and motility > 40%) referred to Royan institute, Tehran. Semen analysis was carried out to WHO criteria. DNA fragmentation was assessed using sperm chromatin structure assay (SCSA).
Results: The patients with total globozoospermia had a significantly greater percentage of sperm with DNA fragmentation compared with that found in normozoospermic control ($p<0.001$).
Conclusion: Several studies have attempted to correlate DNA fragmentation rate with sperm characteristics. However, there is just some case reports about DNA fragmentation rate in globozoosprmic men. We have found an association between globozoospermia and DNA fragmentation that because of remarkable patient's number can be reliable.
Keywords: DNA Fragmentation, Globozoospermia, Male Infertility, Round-Headed Sperm Cells

P-82: Investigation of Relationship between Acrosomal Reaction Rate and Sperm DNA Quality in The Fertilization Rate of IVF Undergoing Men

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Background: It is estimated that around 15% of the couple at reproductive age are infertile couples, and approximately the half of infertilities are caused by male factor. Defective spermatogenesis is the result of several different disorders, such as endocrinological disorders, disease, malnutrition, genetic defects and maybe environmental condition.
Materials and Methods: In this study 60 IVF cycles were investigated in total. In couples undergoing IVF treatment, the sperm and blood were collected. Sperm samples were collected after 2-7 days abstinence. For study of acrosomal reaction (AR), the sperm samples were washed whit Hamsf10 culture media and after incubation in 37°C incubator for 2 hours, the samples were divided into 3 tubes. The first one was control, DMSO was added to the second one and follicular fluid was added to the third one. The sample was kept for 18 hours in 37°C incubator with 5% CO_2 and 95% humidity. The samples were stained with double staining method after fixation and then percentage of sperms involved in AR recorded. Finally, the AR of 2nd and 3rd was calculated by extracting the AR of control tube from them. For each of ejaculation two smears of sperm was prepared for DNA analysis. Staining done with Acridine orang and viewed bye fluorescent microscope and the percentage of green colored sperm (single strand) and red (double strand) were recorded.
Results: There was a correlation between induced acrosome reaction and follicular fluid as well as fertilization rate. The results also showed that the difference in the mean of acrosome reaction in group with rate <or=50> 50% were significant ($p<0.05$). Using ROC analysis with cut-off value of 45% for fertilization, a cut-off value of 10.5% for AR was achieved. Correlation coefficient between rate of normal DNA and fertilization were significant ($r=0.041$ $p<0.05$). The mean of normal DNA in cases with rate <OR=50> 50% showed a significant with p value of 0.05.
Conclusion: The sperms functional test such as acrosomal reaction could help us to know the real cause of infertility as well as to predict the result of IVF more accurately.
Keywords: Male Infertility, Semen Analysis

P-83: Which Novel Sperm Selection Method Is Functional? MACS-DGC or DGC-Zeta

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Background: Selection of human spermatozoa prior to assisted reproduction is currently based on motility and morphology. Recently, Zeta method has been introduced for normal sperm selection based on sperm surface charges. In addition, one of the features of apoptosis is the externalization of phosphatidylserine (PS), which are normally present on the inner leaflet of the sperm plasma membrane. MACS method could separate apoptotic sperm from non-apoptotic. Therefore, we aim to compare chromatin structure and morphology in two sperm selection procedure; MACS-DGC and DGC-Zeta.
Materials and Methods: DNA damage, protamine deficiency and morphology were assessed in 20 infertile individuals by TUNEL, CMA3 and Papanicolaou staining respectively.
Results: Both of MACS-DGC and DGC-Zeta procedures can separate normal sperm morphology, and intact chromatin compared to native semen. However, MACS-DGC was significantly better than DGC-Zeta in chromatin integrity
Conclusion: Sperm selection based on apoptotic marker could use as a novel sperm selection method for infertile men undergoing ICSI in future.
Keywords: MACS-DGC, DGC-Zeta, DNA Fragmentation, Protamine Deficiency, ICSI

P-84: Characterization of Androgen Receptor Structure and Nucleocytoplasmic Shuttling of the Rice Field Eel

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Background: Androgen receptor (AR) plays a critical role in prostate cancer and male sexual differentiation.

Mechanisms by which AR acts and regulations of AR nucleocytoplasmic shuttling are not understood well.

Materials and Methods: Degenerate PCR and RACE Cloning of AR Gene; Phylogenetic Analysis and Molecular Modeling;Real-time Fluorescent Quantitative RT-PCR; Northern Blot Hybridization;In Situ Hybridization Analysis;Cell Preparation, Transfection, and DHT/leptomycin B (LMB) Treatments; Transcriptional Activity Analysis; GST Pull-down; DHT Binding Assay.

Results: We have identified AR from a primitive vertebrate with a sex reversal characteristic, the rice field eel. AR of this species (eAR) is distinct from human AR, especially in the ligand binding domain (LBD), and its expression in gonads shows an increasing tendency during gonadal transformation from ovary via ovotestis to testis. eAR has a restricted androgen-dependent transactivation function after a nuclear translocation upon dihydrotestosterone exposure. A functional nuclear localization signal was further identified in the DNA binding domain and hinge region. Although nuclear export is CRM1-independent, eAR has a novel nuclear export signal, which is negatively charged, indicating that a nuclear export pathway may be mediated by electrostatic interaction. Further, our studies have identified critical sequences for ligand binding in the C terminus. A structure of three -helices in the LBD has been conserved from eels to humans during vertebrate evolution, despite a distinct amino acid sequence. Mutation analysis confirmed that the LBD is essential for dihydrotestosterone-induced nuclear import of eAR and following transactivation function in the nucleus. In addition, eAR interacts with both Sox9a1 and Sox9a2, and their interaction regulates transactivation of eAR.

Conclusion: Our data suggest that the primitive species conserves and especially acquires key novel domains, the nuclear export signal and LBD, for the eAR function in spite of a rapid sequence evolution.

Keywords: AR, Nucleocytoplasmic Shuttling, Sexual Development

Animal Biotechnology

P-85: Epididymal Sperm Parameters following Sperm Mediated Gene Transfer of EDFP-pIRES Vector in Rat

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Background: Sperm mediated gene transfer was approved for producing transgenic animals. There are many studies which reported different success rate by this procedure. Failure in this project mostly is returned to transfection and stability of transferred genes. The other problem that may contribute in project failure may return to the testicular damages which occur at the time of transfection, that have impact on male fertility. In this part of the study we followed the impact of gene injection on epididymal sperm parameters in rat.

Materials and Methods: Total numbers of 55 head Rats were assigned in 5 groups as follow; Control (n=10) were the animals which did not receive any treatment, DOTAP (n=15) received intratesticular 100 µL of cationic DOTAP solution, D-Plasmid (n=15) that received 50 µg of EGFP-pIRES gene plasmid in 100µL DOTAP solution, Plasmid (n=15) that received 50 µg of EGFP-pIRES gene plasmid. Days 2, 5, 10, 30 and 60 after gene injection the rats were euthanized with ethical considerations and their cauda epididymis was separated and minced in TALP medium for 15 min. Then sperm parameters include sperm motility, viability and any morphological abnormalities were analyzed using conventional procedures which approved by WHO. Data were analysed using GLM procedure in SAS.

Results: The results showed that sperm count and sperm motility were not affected by testicular microinjection ($p>0.05$). There was significant changes in percentages of sperm abnormalities and sperm viability following injection of different Materials ($p<0.05$). Sperm abnormalities increased at days 10 and 30 in DOTAP group ($p<0.05$). Injection of Plasmid with or without DOTAP cause to significant increase in the percentage of sperm abnormalities at days 2, 5 and 10 ($p<0.05$), then decrease at days 30 to 60. Sperm viability significantly decreased after injection of plasmid with or without DOTAP at day 2 of sperm collection ($p<0.05$). However, the values of sperm viability returned to normal condition at day 10 and over.

Conclusion: Some sperm fertility potentials following intra-testicular microinjection are affected and may contribute in failure of intra-testicular gene transfer procedure. Optimizing the favorite time for breeding following intra-testicular microinjection may improve the procedure outcome.

Keywords: Epididymal Sperm, Rat, SMGT, EDFP-pIRES Vector

P-86: Production of Cloned Mice by Somatic Cell Nuclear Transfer

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Background: For several years, mammalian cloning by splitting an early embryo or nuclear transfer into oocytes method has been successfully performed. Cloning is now also possible using adult somatic cells. Although it has now been 15 years since the first cloned mammals were generated from somatic cells using nuclear transfer (NT), the success rate for producing live offspring by cloning is low, regardless of the cell type and animal species used. Nevertheless, the techniques have potential

as important tools for future research in basic biology.

Materials and Methods: In this study we have been used a stable NT method in the mouse, in which donor nuclei are directly injected into the oocyte using a piezo-actuated micromanipulator. Female B6D2F1 induced to superovulate by consecutive injection of PMSG and HCG. 14-15 hours after HCG injection, cumulus-oocyte complexes were collected from oviducts and treated in M2+Hy medium. Then After enucleation of metaphase II oocytes, nucleus donor cells (Cumulus cells) were injected into separate enucleated oocytes. Following somatic-cell nucleus injection, groups of oocytes were placed in activation medium and incubation was continued at 37.5°C under 5% CO_2. Where appropriate, 2-cell embryos were respectively transferred into oviducts of Pseudo-pregnant mice. Finally at 19 day after transfer embryos, live Pups were raised. The embryos for better nutrition were transferred in mouse cage, which coincided with the birth of cloned embryos.

Results: Since the origin of isolated cumulus cells was black mice B6D2F1, the cloned mice were also black, confirming that the process of cloning was performed correctly.

Conclusion: A somatic cell nuclear transfer technique could have applications in of cloning therapy, regenerative medicine and creation individual specific embryonic stem cells.

Keywords: Nuclear Transfer, Cloning, Cumulus Cell

Embryology

P-87: Effects of Bovine Cumulus Cell Co-Cultire on Post Fertilization Development of Buffalo (Bubalus bubalis) Oocyte

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Background: There is a considerable numbers of buffalo (Bubalus bubalis) in Iran which have an important role in rural life. The reproductive performance of buffalo is lower than cow. Co-culture with somatice cells improved the maturation rate of most of the species. The aim of the present study was to evaluate the effect of bovine cumulus co-culture on post fertilization development of buffalo oocyte.

Materials and Methods: Ovaries were collected from local slaughterhouse. The cumulus oocyte complexes (COC) were picked up form follicles (2-8 mm). The grade A and B COCs were randomly divided into 3 groups. Group 1: COCs were subjected to oocyte maturation medium containing TCM-199+ 5%FCS + 5%FF + 10mg/ml LH + 10 mg/ml FSH. Group 2: the COCs were cultured on a monolayer culture of bovine cumulus cells in a medium contained TCM199 with 5% FCS. Group 3: the denuded COCs were subjected on a monolayer culture of bovine cumulus cells in a medium similar to group 2. After 24 hours of maturation in an atmosphere with 5% CO_2 and 95% relative humidity, oocytes subjected to *in vitro* fertilization using buffalo epididymal sperm. Three and six days after insemination (in BO medium) cleavage and blastocyst rates were evaluated.

Results: Mean percentage of embryos cleaved for each were 41.3, 48.2 and 32.9 in groups 1, 2 and 3 ($p>0.05$), respectively. There was no developed embryo to blastocyst in all groups.

Conclusion: The bovine cumulus co-culture with buffalo oocytes was effective as a completed in developmental potential of buffalo oocytes.

Keywords: Cumulus Monolayer Co-Culture, Buffalo Oocyte, Fertilization

P-88: Mast Cells Number and Distribution and Myoid Cells Population Are Impressed by Sulpiride Antipsychotic Drug

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Background: Spermatogenesis is under the control of variety of factors, which include mast cells. Any changes in testicular mast cells' number and distribution resulting in deterioration of comlex regulation and fine-turning of spermatogenesis. Increased number of mast cells and their abnormal spread pattern have been described in the testis with fertility disturbances. Mast cells' tryptase enzyme is a potent mitogen for myoid cells and can enhance the chemotaxis and activation resulting in seminiferous tubular fibrosis. Also these cells may be involved in testicular inflammatory disorders. In present study the effect of sulpiride antipsychotic drug on testicular mast cells number and distribution and, consequently, myoid cells population changes in mature male mice were investigated.

Materials and Methods: 18 adult male mice were categorized in to 3 groups. Treatment group was administered sulpiride 40 mg/kg/day IP for 45 days. Control sham received sesame oil as a vehicle of drug and control group received nothing. 45 days later testicular tissue samples were taken for histological studies using Toluidine-blue (mast cells) and Iron-weigret (myoid cells) staining methods.

Results: Increased number of mast cells and their wide distribution around seminiferous tubules and in interstitial tissue and, in some cases, in tubular lumens were observed in test group in comparison with control-sham and control groups. Increase in the interstitial fluid showed inflammation in test group samples. Additionally, myoid cells populated significantly in test group in compare with 2 other groups ($p<0.05$).

Conclusion: Mast cells high and wide distribution because of sulpiride atypical antipsychotic drug administration, resulting in proliferation and activation of myoid cells and edema induction, cause pathological changes

in testicular structure that increase the risk of fertility problems in some cases.

Keywords: Mast Cell, Myoid Cell, Mice, Suloiride, Male

P-89: The Developmental Rates of Early Mouse Embryos Derived from *In Vitro* Fertilization of Mature Oocytes Obtained from Ovarian Stimulation Using A Combination of HMG and Estradiol Valerate

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Background: In recent years, a combination of hormones and agents in assisted reproductive technology (ART) cycles as a means of stimulating the ovary and developing more follicles. With respect to the positive effects of Estradiol Valerate on reproductive tissues and the impact on native genes of the egg that transferred to the embryo after fertilization, it seems that this agent can also effective on embryo developmental stages in combination of gonadotropin human menopausal (HMG).

Materials and Methods: 40 NMRI female mice were divided into two groups. Control group received HMG alone (10 IU/mouse) and the estradiol-treated group receiving the combination of HMG and estradiol valerate (1 µg/mouse). After induction of ovulation by HCG, MII oocytes collected from the above groups and then were transferred to fertilization medium for IVF. Finally, the resulting development rates of embryos to the blastocyst stage were evaluated.

Results: The results showed that the mean number of eggs obtained in the control and estradiol-treated groups were 20.6 ± 12.46 and 19.56 ± 8.84 and also the mean percentage of healthy mature oocytes were 57 ± 26 and 60 ± 17 respectively that the difference between the two groups was not statistically significant in these respects(p=0.698, p=0.614). Fertilized egg and blastocyst rates obtained in the control group and estradiol-treated respectively were 30% ± 21 vs. 36% ± 21 and 49% ± 26 vs. 61% ± 31 that also the difference was not significant in these cases (p=0.713 and p=0.232).

Conclusion: This study showed that administration of estradiol in combination of HMG as ovarian stimulator has no any significant effect on oocyte quality and embryo development.

Keywords: Estradiol Valerate, HMG, Ovarian Stimulator, Fertilization, Blastocyst

P-90: The Effect of Nitric Oxide on Mouse Oocyte *in Vitro* Maturation in Two and Three Dimensional Conditions

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Background: *In vitro* culture of ovarian follicles may preserve fertility in women with premature ovarian failure due to cancer .It seems that creation a condition that could maintain cellular communications and supports growth of follicles to produce mature oocytes appear to be essential. Nitric oxide (NO) has been recently shown to act with a dual action in mouse oocyte meiotic maturation depending on its concentration. The propose of this study is investigating on the effect of nitric oxide in oocyte maturation in two dimensional and three dimensional conditions.

Materials and Methods: Germinal stage mouse oocytes were *in vitro* matured with the NOS inhibitor L-NAME (5-10 µm) and NO donor SNP (10, 100 µm) in two and three dimensional culture medium. Metaphase II stage, following *in vitro* fertilization and embryo development rates were assessed subsequently.

Results: The results indicated that three-dimensional culture medium is better than two-dimensional environment for growth of follicles and maintaining of their meiosis capability. Low concentrations of nitric oxide stimulate meiosis maturation of oocytes and embryo development but high concentrations of nitric oxide inhibit oocyte maturation and embryo development.

Conclusion: The effects of nitric oxide on *in vitro* maturation of oocytes and development of embryos and follicle growth are totally concentration dependent. Also the three-dimensional culture environment is more appropriate for *in vitro* maturation of oocytes.

Keywords: In Vitro Maturation, Mouse Oocyte, Nitric Oxide

P-91: Protective Effect of Black Grape Seed and Hawthorn Extracts on Cyclosporine A-Induced on *In Vitro* Fertilization Damge in Male Rat

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Background: Infertility is a disease of the reproductive system that inhibits the ability of a couple to have a Baby. Cyclosporine A (CsA) is a powerful immunosuppressant drug most widely used in management of organ transplantation and autoimmune diseases. The drug has adverse side effects such, hirsutism, and gynecomastia. Hawthorn is one of the oldest herbal medicines that are widely used in medicine. Some crataegus constituents are predicted to be good antioxidants. Black grape extract contain a large amount of phenolic compounds in their skin, pulp and seeds. This study was conducted to investigate the effect of black grape seed and hawthorn extracts on CsA induced on *in vitro* fertilization (IVF) damge in male rat.

Materials and Methods: In this study 32 adult male Rat, weighing 200 ± 20 g were used. The animals were divided into four groups (control, cyclosporine, cyclosporine+ Black grape seed extract, cyclosporine+ hawthorn ex-

tract). CsA were given at the dose of 30 mg/kg/day, hawthorn and black grape seed extracts at dose of 150 mg/kg/day by gavage for 45 days. After 45 day the animals were sacrificed by dislocation of cervical vertebra, and fertility value from each group were studied

Results: Cyclosporine caused decreased in sperm viability, motility, sperm count and level of serum testestrone .In our study, 2-cell embryos were stopped division and did not continue to blastocyct stage in cyclosporine group. Positive effect of Black grape seed and hawthorn extracts improvement of development embryo to blastocyct stage in treatment rats were considered.

Conclusion: This investigation revealed that cyclosporine can decrease fertility in Rat but black grape seed and hawthorn extracts have improved embryo quality and increase zygotes, two celle embryo, and blactocycte.

Keywords: Cyclosporine, Hawthorn Extract, Black Grape Seed Extract, Male Rat

P-92: Specific Activation Requirements of *In Vitro* Matured Sheep Oocytes following Vitrification-Warming

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Background: Oocyte vitrification and assisted oocyte activation have increasingly important implications in assisted reproductive technology. However, an important area of concern with matured oocyte cryobiology is that elements of oocytes intimately involved in metaphase-II arrest may be modified by cryopreservation. In a comparison between different cellular characteristics of unvitrified, vitrified-warmed and unvitrified-activated oocytes, the present study investigated how vitrification-warming process may affect developmental competence of *in vitro* matured sheep oocytes following parthenogenetic activation.

Materials and Methods: At 20-22 hours post-maturation (hpm), sheep oocytes were randomly divided for use as follows: 1. unvitrified, 2. vitrified and 3. unvitrified-activated. At half an hour after vitrification or parthenogenetic activation, the categorized oocytes were used for assessment of: 1. meiotic spindle and chromosomal organization, 2. zona-dissolution time, 3. DNA-fragmentation, 4. ultrastructural organization and 5. quantitative assessment of gene expression. Based on the results obtained during the first part of this study, further experiments were carried out to investigate if, and so how, different activation protocols affect *in vitro* development of vitrified-warmed vs. unvitrified oocytes. Accordingly, unvitrified (group i) and vitrified (group ii) oocytes were activated with nine different activation protocols, and then cultured for 8 days for assessment of embryonic development.

Results: Structural, ultrastructural and molecular analyses indicated that the characteristics of vitrified-warmed oocytes vastly differed from fresh oocytes, yet resembled unvitrified-activated oocytes. For unvitrified oocytes, the highest blastocyst yield (41.8 ± 0.6%) was achieved using maximum ionomycin concentration (5 µM), and importantly, the duration of ionomycin treatment was not of utmost importance at this concentration. In contrast, the maximum blastocyst yield of vitrified-warmed oocytes (28.4 ± 1.4%) was achieved at minimal duration of ionomycin treatment (1 minute), and further increase in duration dramatically reduced developmental potential of vitrified-warmed oocyte.

Conclusion: The obtained results suggested that vitrified-warmed oocytes may need a specific activation protocol different from unvitrified oocytes. In this respect, unvitrified oocytes were more sensitive to the concentration rather than the duration of ionomycin treatment when compared with vitrified oocytes, which were sensitive to the treatment duration. These results may provide a platform to improve the potential applications of vitrified oocytes in medicine and agriculture.

Keywords: Oocyte, Vitrification, Assisted Activation, Lonomycin, Embryo Development

P-93: The Effect of Hypothyroidism on Testis Characteristics in Lamb

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Background: This study was conducted to investigate the effect of inducing hypothyroidism by propyl-2-thiouracyl (PTU) on lambs testicular histomorphology and plasma testosterone concentration.

Materials and Methods: Eighteen Lori-Bakhtari male lambs were divided to 3 groups (n=6) and each received one of the treatments as Control (C: 0 mg PTU/kg BW), Low (L: 10 mg PTU/kg BW) and High (H: 20 mg PTU/kg BW) by gavages, during a 60d experimental period. At the end of the trial, lambs were slaughtered and testes were removed to evaluate their histomorphological characteristics.

Results: Mean concentration of T4 and T3 decreased significantly in L and H groups compared with C group ($p<0.05$). Hyphothyroidism increased testis weight, number of sertoli and lydig cells, diameter of Seminiferous tubules, diameter of seminiferous lumen, total spermatogonia, number of primary spermatocyte, and total spermatids ($p<0.05$). However, PTU had no significant effect on plasma concentration of testosterone ($p>0.05$).

Conclusion: In the present study, PTU increased testicular weight and the number of cells involved in sperm and testosterone production. Probably sheep breeders can consider hypothyroidism as a potential approach to

increase sperm production capacity in rams before puberty. However, further investigation should be carried on the sperm quality and quantity of rams.

Keywords: Hypothyroidism, Testis Histology, Leydig Cell, Sertoli Cell

P-94: Mouse Embryo Vitrification Cannot Effect on Global DNA Methylation in Preimplantation Stage

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Background: Embryo vitrification was effectively used for assisted reproductive techniques. Despite the undeniable benefits of vitrification, cooling and warming stress, and cytotoxicity of cryoprotectant may affect the DNA methylation that have an important role in gene activation and silencing. In the present study effects of 2-cell embryo vitrification on DNA methylation in hatched blastocyst was evaluated.

Materials and Methods: Six-to-eight week-old female mouse was superovulated by 10 IU PMSG, followed 46-48 hours later with 10 IU of hCG and mated with NMRI male. Female mice were sacrificed in day 1.5 after hCG injection for collection of the 2-cell embryos. These embryos were vitrified by using cryotop as mentioned by Kuwayama et al. After warming, survival rate were evaluated. The recovered embryos were cultured in G1/G2 medium. Immunofluorescence staining on hatched blastocysts was done by a mouse monoclonal antibody against 5-methylcytosine (5 MC). Intensity of 5 MC was analyzed by Image J software.

Results: 359 and 357 embryos randomly inserted to control and vitrified groups, respectively. The survival rate for vitrified group was 97.2%. In the vitrified group blastocyst (81.3%) and hatched blastocyst (65.4%) formation rates were significantly lower than the control group (90.8% and 78.3%, respectively) ($p<0.01$). This decrease suggests that vitrification may negatively affect the embryo development. Although that the lowest level of fluorescent intensity for 5mC was related to vitrified group, this intensity was not significantly different from control group ($p>0.05$). This numerically reduction may be related to DMSO which can affect the DNA methyltransfereases. *In vitro* culture also may make environmental stress and have related to hypermethylation. Thus, it may be reason of have no significant difference between two treatments for 5 MC.

Conclusion: Numerical reduction of 5mC in hatched blastocyst of vitrified group may restrict to specific region of DNA such as DMRs or region that related to gene expression.

Keywords: ART, Epigenetic Modification

P-95: Flow Cytometry Analysis of Bovine Semen: A Qualitative Study

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Background: Although AI practices have been introduced little over 60 years, the success rate remains relatively low. This might be due to the exclusive selection of semen based on motility analysis. Recent advancement in sperm sexing using flow cytometry with an increased throughput from next generation cell sorters, made use of this technology in studding sperm qualitative aspects other than motility become highly feasible. This study was carried out to assess the viability of bovine sperm diluted in Bioxcell® and Andromed using fluorescent assisted cell sorting (FACS) in conjunction with motility assessment using computer assisted sperm analysis (CASA).

Materials and Methods: Semen was collected via electro ejaculation twice a week from local breed bulls (n= 7, 2-4 years old) during the period from February to April. Ejaculates were divided in two aliquots and extended (1: 2) in Bioxcell® and Andromed at 37°C. Diluted semen was evaluated for motility using CASA (Sperm Vision®, Minitube). Sperm was liquefied (37°C/10 minutes), washed with 0.9% NaCl, centrifuged (2000 rpm for 3 minutes) and filtered using 40 µm nylon mesh (BD, USA). Semen viability was assessed using LIVE/DEAD® sperm viability kit (Invitrogen, USA). Samples were analyzed using FACSAria™III (FACSDiva software, BD) with an average flow of 20000 sperm/events for each sample.

Results: Our data shows different correlation between sperm motility by CASA and viability via FACS analysis on selected bulls. Interestingly, while some semen samples extended with Bioxcell® exhibited high motility, the viability was low; (71.9, 80.8 and 68.8, 18.9% respectively). This observation was not restricted to Bioxcell® as similar result was found using Andromed (91.3 vs. 49.9%). Conversely however, using both extenders Bioxcell® and Andromed; low semen motility with high viability (69.4, 76.8% and 50, 57.7; 66.6, 71.2% respectively) was observed.

Conclusion: These results demonstrate that this phenomenon can occur regardless of the extender being used. Moreover, our results suggest that motility analysis using CASA alone is insufficient in determining semen suitability for ART; thereby suggesting that FACS analysis should be integrated in AI centers to maximize the selection of elite bulls.

Keywords: Motility, Viability, Bioxcell®, CASA, Flow Cytometry

P-96: Mechanical Activation of Parthenogenesis in Mouse Oocytes Using Hydrostatic Pressure

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Background: Effective protocols are introduced for par-

thenogenesis activation in oocytes. Hydrostatic pressure can act as a mechanical stimulator that rearranges egg contents, leading to new structural or molecular combination. Alternatively, mechanical stimulation could stimulate a mechanically-gated process, such as opening or closing of stretch activated ion channels. This study, investigated the use of hydrostatic pressure in the activation of parthenogenesis in mouse oocytes matured *in vitro*.

Materials and Methods: Immature oocytes were isolated from 8-week-old female NMRI mice. Oocytes were cultured in α- MEM culture medium containing 7.5 IU HCG under mineral oil for 24 hours. *In vitro*-matured oocytes with a polar body were subjected to 10 mmHg pressure for 10, 20, 30 minutes (treatments I, II and III). Oocytes without exposure to pressure were considered as control. Oocytes from two groups were culture for 72 hours and embryo development rate was assessed.

Results: After 72 hours, cleavage rate in treatments I, II, III and control was 18.27%, 41.92%, 24.72% and 6.44% respectively. The result showed that, oocyte activation rate in all treatments was higher than control ($p<0.05$). The highest cleavage rate associated with treatment II which was significantly different from treatmentsI, III and control ($p<0.05$).

Conclusion: We inferred that the exposures to hydrostatic pressure in the parthenogenetic activation can improve the development of *in vitro* matured oocytes.

Keywords: Parthenogenetic Activation, Hydrostatic Pressure, Oocyte, Mouse

P-97: Parthenogenetic Activation of Mouse Oocyte Using Calcium Ionophore in The Presence of Different Concentrations of Extracellular Calcium

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Background: Parthenogenetic activation is a possible way to produce homogeneous embryos with the some ploidy. Probably such embryos could be used in other areas of biotechnology. Calcium signals are known as important regulators of oocyte activation. Extracellular calcium is required for initiation of meiotic resumption and development. Calcium ionophore A23187 is known to elevate intracellular Ca^{2+} levels in the cytoplasm of oocytes through the influx of calcium from extracellular spaces. This study investigated the role of extracellular calcium on calcium ionophore inducing oocyte parthenogenetic activation in mouse.

Materials and Methods: 6 to 8-week-old female NMRI mouse were superovulated by an injection of 10 IU of PMSG, followed by 10 IU HCG 48 hours later. Metaphase II oocytes enclosed in cumulus masses were collected from oviduct 14 hours after HCG injection. The group in which oocytes were cultured in media alone was the control, those cultured was in the presence of 5μM calcium ionophore A23187 for 5 minutes designated as experiment group. Consequently, the three treatments I, II and III are referred to culture in T6 medium containing 0, 1.7, 3.4 mM calcium. Oocytes were cultured for 72 hours and embryo development was assessed.

Results: After 72 hours, in treatments I, II, III from experiment and control groups the percentage of cleavage rate was 0%, 10.1%, 27.2% and 11.3%, 44.2%, 61.3%; respectively. Cleavage rate in experiment group was higher than control group ($p<0.05$).

Conclusion: After 72 hours, in treatments I, II, III from experiment and control groups the percentage of cleavage rate was 0%, 10.1%, 27.2% and 11.3%, 44.2%, 61.3%; respectively. Cleavage rate in experiment group was higher than control group ($p<0.05$).

Keywords: Extracellular Calcium, Calcium Ionophore A23187, Oocyte Activation, Mouse

P-98: Effect of Mouse Embryo Vitrification on Histone Modifications

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Background: Vitrification has been usually used as an assisted reproductive technology in animals and humans. This method needs high concentrations of cryoprotectants that can be toxic with high cooling degrees. Then, vitrification could be change histone modifications such as methylation and acetylation can performance as regulatory controls of gene transcription. So, the purpose of the present study was to assess the effects of embryo vitrification and warming in mouse 2-cell embryo on blastocyst histone modifications. Facts found from the present study using this animal embryo model provide a vision into the epigenetic events that happen in human embryos after vitrification.

Materials and Methods: Six-to-eight week-old female mouse was superovulated by 10 IU PMSG, followed 46-48 hours later with 10 IU of hCG and mated with NMRI male. Female mice were sacrificed in day 1.5 after hCG injection for collection of the 2-cell embryos. These embryos were vitrified by using cryotop as mentioned by Kuwayama et al. After warming, survival rate were evaluated. The recovered embryos were cultured in G1/G2 medium. Immunofluorescence staining on hatched blastocysts was done by mouse monoclonal anti-H3K9ac, rabbit polyclonal anti-H4K12ac and mouse monoclonal anti-H3K4me3. Intensity of these epigenetic modifications was analyzed by Image J software.

Results: 359 and 357 embryos randomly inserted to control and vitrified groups, respectively. The survival rate for vitrified group was 97.2%. In the vitrified group blastocyst (81.3%) and hatched blastocyst (65.4%) formation rates were significantly lower than the control group (90.8% and 78.3%, respectively) ($p<0.01$). This decrease suggests that vitrification may negatively affect the embryo development. Although results show that, the vitrification procedure in mouse embryos did not affect the acetylation level of H4K12 and H3K9 biomarks, while this procedure promotes

the tri-methylation level of H3K4 significantly ($p<0.05$).
Conclusion: Vitrification procedure can increase H3K4me3 significantly that related to gene expression.
Keywords: ART, Histone Modification

P-99: Evaluation of Pluripotency Markers of Mouse Endometrial Tissue in Different Stages of Estrous Cycle

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Background: It is assumed that adult stem/progenitor cells are responsible for cycling remodeling of the uterus endometrium throughout the reproductive life of the female. This study aimed to identify and localize stem/progenitor cells in the mice uterus using immunohistochemistry.
Materials and Methods: 6-8 weeks old virgin female NMRI mice were submitted to the vaginal smear examination to determine the estrous stages and those which were in estrous and di-estrous stage were selected. Immunohistochemical staining of pluripotent markers on middle region of uterus horn was performed to evaluate the association between expression of NANOG and OCT4 markers in the endometrial tissue and estrous stages.
Results: Results showed that NANOG AND OCT4 markers were detectable in the both estrous and di-estrous phase in the endometrial tissue, while in the estrous phase showed a greater extent.
Conclusion: Mice endometrial tissue contains stem cells and appears their status is coordinate with the hormonal changes associated with estrus phases.
Keywords: Pluripotency Markers, Endometrial Tissue, Estrous Cycle

P-100: Interpretation of Mice Vaginal Smears to Determine Estrous Cycle Based on Uterine and Ovary Tissue Sections

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Background: Rodents with short reproductive cycle length are the best model for investigation of physiology and molecular aspects of reproductive biology. This study aimed to establish a correlation between histological changes of uterus and ovaries with the vaginal smear.
Materials and Methods: 6-8 weeks old virgin Female NMRI mice were submitted to the vaginal smear collection and observed using the light microscope. Histological sections were prepared from their uterus and ovaries simultaneously. Correlation analysis between epithelial and stromal parameters of uterine and ovaries were performed, in parallel, these parameters were analyzed in relation to the vaginal smear classification of estrous stages.
Results: Vaginal smears of 4 stages of mouse estrous cycles (proestrus, estrus, metestrus, and diestrus) can predict many concurrent cellular and stromal events in the mouse uterus and ovaries.
Conclusion: Vaginal smear is a reliable method to demonstrate the changes in reproductive organs of mouse during the estrous cycle.
Keywords: Vaginal Smear, Estrous Cycle, Ovaries, Uterine

P-101: Effects of Ethanol and Strontium on Growth and Development of Two-Cell Arrested Mouse Embryos

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Background: Arresting in a certain step of development like two-cell stage could be one of the reasons of infertility. The aim of this study is evaluating the effects of ethanol and strontium on growth and development of two-cell arrested mouse embryos.
Materials and Methods: The females were coupled with male following superovulation. Positive vaginal plug mice were killed 48 hours after hCG injection. Two-cell embryos were transferred to M16 medium and divided to four groups. The first group is incubated without any exposure to low temperature. Groups 2, 3 and 4 were exposed to 4°C for 24 hours. The second group was incubated immediately, while the third and fourth groups were exposed to strontium and ethanol for five minutes respectively.
Results: The data analysis by one-way ANOVA shows that developmental rate is decreased significantly by 4°C exposure. The mean of degenerated embryo was significantly different between groups but the mean cleavage rate hasn't any significant differences. The mean percent of morula, blastocyst and hatched blastocyst formation were significantly different between groups in 120 hours study.
Conclusion: The effect of strontium and ethanol on arrested two-cell embryos hasn't any significant differences on the mean percent of morula, but ethanol increase the mean percent of blastocyst and hatched blastocyst formation significantly more than strontium.
Keywords: Strontium, Ethanol, Two-Cell Embryo, Arresting, Development

P-102: The Effect of Strontium on Growth and Development of Two-Cell Arrested NMRI Mouse Embryos

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Background: Decreasing the growth and developmental rate of embryo and arresting in certain step of development like two cell block could be the reason of infertility in some couples referring to fertility and infertility center. The aim of this study is the effect of strontium on growth and development of two-cell arrested NMRI mouse embryo.
Materials and Methods: The female mice were coupled with males following superovulation and positive vaginal plaque mice were killed 48 hour after HCG injection by cervical dislocation. Subsequently two-cell embryos were collected in Roswell Park Memorial Institute (RPMI) and divided and cultured (in M16 medium) in three groups. The 1st group were washed and incubated without any exposure. The 2nd and 3rd groups were exposed to 4°C for 24 hours in order to delay and arrest for cleavage rate. The 2nd group was incubated immediately, while the 3rd group was exposed to 10 mM strontium for 5 minutes.
Results: The data analysis by one-way Anova shows that the rate of degenerated embryos have significantly differences (p=0.006) between groups by low temperature (4°C) exposure. Our results shows that the mean percent of blastocyst formation (33.4%) and cleavage rate (45.2%) in 2nd group were decreased related to 1st group (%77.7 and 90.9% respectively). With effect of strontium in 3rd group, the cleavage rate hasn't any significant differences with 2nd group (45.2% related to 45.5% respectively), while the blastocyst formation rate were significantly (p=0.019) increased (61%) related to 2nd group.
Conclusion: Developmental rate of mouse arrested two-cell embryos were significantly increased by effect of strontium related to control group.
Keywords: Strontium, Mouse, Two-Cell Embryo, Arrest

P-103: Effects of *In Vitro* Selenium Supplementation on Buffalo (Bubalus bubalis) Sperm Quality following Cryopreservation

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Background: Artificial insemination is a valuable tool in genetic improvement programs and widely used breeding technique in farm animals. Semen processing and cryopreservation causes considerable damage to sperm DNA, motility apparatus, plasma membrane, acrosomal cap and thus, reduced fertility. Selenium is an integral part of glutathione peroxidase (GSH-Px), an enzyme which protects cell internal structures against free radicals. GSH-Px activity has been reported in the semen of several species including human, ram and bull. This experimental study was conducted to determine the effects of *in vitro* supplementation of sodium selenite (Na2Se03) on sperm parameters of semen.
Materials and Methods: In the study, a total number of 25 semen ejaculates were collected from five healthy buffalo breeding bulls (2-4 years age) kept at Buffalo Breeding Center of north- west of Iran. Each ejaculate was split into 6 portions and diluted with tris-egg yolk-glycerol extender containing 0 (control), 0.5, 1, 2, 4 and 8 µg/mL sodium selenite. After equilibration, semen parameters (motility, viability, membrane integrity and DNA fragmentation) were estimated. Then, semen was freezed in liquid nitrogen and the same parameters, as well as total antioxidant capacity (TAC) of the frozen-thawed semen, were estimated.
Results: The results showed that adding 1 and 2 µg/mL selenium to the extender significantly ($p<0.05$) increased the sperm quality of equilibrated and frozen semen of buffalo bulls as compared to the non-supplemented control group; with higher values obtained after adding 2 µg /ml selenium. Selenium at concentrations of 4 and 8 µg/mL had a deleterious effect on sperm parameters.
Conclusion: This study indicates that *in vitro* selenium supplementation may help to ameliorate negative effects of semen storage on characteristics of sperm quality. However, selenium exerts its effects in a dose-dependent manner and exposure to high levels can cause deleterious effects.
Keywords: Sperm, Selenium, Cryopreservation, Buffalo

P-104: *In Vivo* Study of Follicogenesis and Estradiol Level in Mouse Exposed To Forced Swimming

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Background: Strenuous exercise and temperature outside the optimum are associated with hypothalamic dysfunction and therefore disruption of fertility in females. The aim of this study was to evaluate serum Estradiol level and follicogenesis in adult mice exposed to forced swimming in different temperature waters.
Materials and Methods: Adult female mice were randomly divided into four groups as: A. control, without exercise, B. swim group in 23°C water, C. swim group in 12°C water and D. swim group in 36°C water. All animals were swimming for 6 minutes daily for 5 days/week for 14 days, except controls. The mean values of the serum Estradiol level were measured by Elisa and follicogenesis was studied histologically.
Results: Swimming in groups of B and D caused to reduction in serum estradiol levels in compare with controls ($p<0.05$). The numbers of Primary, Secondary and Tertiary follicules were significantly reduced in all swimming groups in compare with controls ($p<0.05$). Reduced follicogenesis and regression of ovary in group D (36°C) were more obvious.
Conclusion: These data suggests that forced swimming as a physical stress disrupt HPG Axis and follicogenesis. Water temperature is also effective in ovarian function.
Keywords: Ovary, Follicogenesis, Swimming, *In Vivo*

P-105: Study of The Protective Effect of Equisetum Arvense Alcoholic Extract on *In Vitro* Fertilization and Embryo Development in Diabetic Male Mice

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Background: Diabetes is a metabolic disorder that in long period can damage the testis and reduce the semen quality. Therefore, it can impair the male fertility potential. Recently, different herbal therapeutic treatment for protection of diabetes and its pathologic effects have been used. The aim of current study is to evaluate the protective effect of Equisetum arvense extract (EE) on *in vitro* fertilization and embryo development in diabetic male mice.

Materials and Methods: Twenty four mature male mice were divided equally in control-sham, diabetic, diabetic + EE250 (250 mg/kg, orally, daily gavage) and diabetic + EE500 (500 mg/kg, orally, daily gavage) groups. In order to induce diabetes, the streptozotocin (STZ) with dose of 50 mg/kg was injected for five days. After 45 days of STZ administration, all animals were sacrificed. Cauda epididymis was removed surgically and placed in pre-equilibrated medium containing 1 ml HTF + BSA 4mg/ml. Then, in order to swim out the spermatozoa, they were incubated for 30 minute in CO_2 incubator. For each male mouse, oocytes of 3 superovulated mice were collected and fertilized by fresh sperms of each groups. The rate of fertilization, two cell embryos, arrested embryos and blastocysts rate examined in period of 120 hours.

Results: The animals in diabetic+EE groups (especially those in diabetic + EE500) showed remarkably ($p<0.05$) higher fertilizing success rate. In diabetic + EE groups compared to diabetic group, significantly ($p<0.05$) higher percentage of fertilized oocytes developed toward blastocyst stage and remarkably lower percentage of embryos blocked in different stages of embryo development.

Conclusion: Reproductive dysfunction is one of the important secondary effects of diabetes, which is associated with sexual impotence and by decreasing the semen quality reduces fertilizing success rate. The treated animals with EE showed higher fertilization rate and embryo development compared to diabetic group.

Keywords: Diabetes, Equisetum Arvense Extract, Spermatozoa, *In Vitro* Fertilization, Mice

P-106: Effect of Cilostamide on Meiotic Arrestof Ovine Oocytes

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Background: *in vitro* maturation (IVM) of oocytes has increasing potential applications in assisted reproductive technology (ART), during which germinal vesicle (GV) oocytes cultured *in vitro* to produce mature (MII arrested) ones. Although functional, IVM oocytes have low developmental competence compared to in vivo matured oocytes; possibly because IVM comprises asynchrony between nuclear and cytoplasmic maturation in which nuclear maturation precedes cytoplasmic maturation. Therefore, considering the central role of cAMP as the regulator of nuclear maturation, a number of studies have attempted to develop biphasic IVM strategies in which nuclear maturation is transiently delayed through inhibition of several pathways of cAMP production in oocytes. In this study, we investigated the effect of cilostameide, inhibitor of phosphodiesterase 3, on induction of meiotic arrest in ovine oocytes.

Materials and Methods: Immature ovine oocytes derived from abattoir ovaries were immediately cultured in presence of three concentration of cilostamide (1,10 and 20 µm) for 22 hours (prematuration culture medium, PMC). Nuclear status of treated oocyte and non-treated oocytes after culture in PMC were assessed using H33342 staning for investigation of meiotic arrest ability of cilostamide.

Results: GVBD occurs 4 hours after IVM and at 8 hours post IVM approximately %100 of ovine oocytes undergoes GVBD. In oocytes treated with 1, 10 and 20 µm for 22 hours, respectively 55, 65 and 72 percent of oocytes are arrested at GV stage while only 4% of control oocytes are arrested at GV stage after 22 hours ($p< 0.05$). 1 µm cilostamide cause lowest meiotic arrest and 20 µm cilostamide cause highest meiotic arrest. futher studies are needed to investigate cytoplasmic maturation markers of prematured oocytes.

Conclusion: Cilostamide cause nuclear arrest in ovine oocytes by a dose dependant pathway.

Keywords: *In Vitro* Maturation, Nuclear Maturation, Cytoplasmic Maturation, Cilostamide

P-107: The Effects of Cryotop Vitrification on Heat Shock Protein 72 Expression in Mouse 2-Cell Embryos by Nested Quantitative PCR

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Background: The aim of the study was to compare the effects of two different concentrations of cryoprotectants by Cryotop vitrification on survival and Heat shock protein 72 (Hspa1a) expression of two-cell mouse embryos.

Materials and Methods: Different cryoprotectants' concentrations of the combination of dimethyl sulfoxide (DMSO)

and ethylene glycol (EG) were used and compared with each other and the non-vitrified 2-cell mouse embryos. The combinations were 15% (vit1: 7.5% of each EG and DMSO), 30% (vit2: 15% EG + 15% DMSO), and control group with no cryoprotectants. Cryotop was the instrument of choice for vitrification. Vitrified and fresh 2-cell embryos were incubated under mineral oil for 1 hour at 37ºC to obtain survival rate. Embryos were defined "morphologically survived", if the cells possessed an intact zona pellucida, blastomeres and refractive cytoplasm. Transcript analysis of Hspa1a gene was performed on survived non-vitrified and vitrified embryos via a nested quantitative polymerase chain reaction (PCR) approach subsequent to normalization with Hprt1 as the reference gene.

Results: The relative expression of Hspa1a in vit2 (30% v/v) was significantly higher than vit1 (15% v/v). Survival rates were the same for both vitrification treatments and significantly lower than control group ($p<0.05$).

Conclusion: Our data demonstrated that vit1 treatment (7.5% EG and 7.5% DMSO) was more efficient than vit2 (15% EG and 15% DMSO) in mouse 2-cell embryos. The Cryotop vitrification with two concentrations of cryoprotectants caused the relative changes of Hpsa1a transcript level, but the stability of the gene in vit1 was significantly higher than vit2 and closer to the fresh 2-cell embryos.

Keywords: Cryotop, Gene Expression, Nested Quantitative PCR, Vitrification, 2-Cell Embryo

P-108: Reproductive Toxic Effect of Carbon Tetrachloride and Therapeutic Effect of Cornus Mas Fruit Extract on Morphology and Histology of Testis in Rats

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Background: Carbon tetrachloride (CCl4), an industrial solvent, is a well-established hepatotoxin. Studies demonstrated that liver is not the only target organ of CCl4 since it causes free radical generation in other tissues such as testis. It has also been reported that exposure to CCl4 induces acute and chronic reproductive toxicity. Diverse medicinal plants have been screened and assessed for properties in antagonism to free-radical-induced oxidative stress. Cornelian cherry (cornus mas), a medicinal plant has been mentioned for the treatment of inflammation in an ancient system of medicine in Iran. The aim of the present study was to investigate the reproductive toxic effect of CCl4 and therapeutic effect of C. mas fruit extract on morphology and histology of testis in rats.

Materials and Methods: Thirty male Wistar rats (250-300 g) were selected and randomly divided into 5 groups (n=6): -Sham group -Normal control with olive oil (1 ml/kg i.p.) on the 16th day. -Toxic control - CCl4 (1 ml/kg i.p.) on the 16th day. -Treatment group with plant extract (300 and 700 mg/kg i.g.) for 16 days before CCl4 injection on 16th day. At the end of the treatment, changes in histological parameters in testis were assessed.

Results: Histological studies of testis structure showed that seminiferous tubules of testis were severely damaged in the toxic group. CCl4 increased the number of sloughing tubules and interstitial space, while it decreased seminiferous tubular diameter (STD), seminiferous epithelial height (SE) and tubule differentiation index (TDI).

Conclusion: The results suggested that carbon tetrachloride affects fertility parameters and cause testis atrophy after administration and these findings indicated that therapeutic potential of cornus mas.

Keywords: Carbon Tetrachloride, Cornus Mas, Testis Structure, Free Radicals

P-109: Effect of Chaste Tree Essential Oil on Sperm *In Vitro* Fertilization (IVF) Index in Mice

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Background: Chaste tree is a phytoestrogenic herb and has a long tradition as a herbal remedy and was used in ancient times as an aphrodisiac. This study was conducted to evaluate *in vitro* fertilization ability and cleavage rate of sperm in chaste tree essential oil treated male mice.

Materials and Methods: In this study, 10 adult male mice were divided in to two groups: group 1 as control and group 2 which received 75 mg/kg essential oil via gastric gavage for seven consecutive days. Animals were housted at a controlled condition in accordance with humane care and animal welfare. After this time, animals sacrificed by cervical dislocation. The epididymes dissected out and spermatozoa expressed out by cutting the distal end of the cauda epididymidal tubule. For *in vitro* fertilization (IVF), superovulation induced by hMG and hCG (10 IU) in normal female and oocytes recovered from ampulla.

Results: Results revelved that fertilization ability and IVF index decreased significantly in treated males ($p<0.05$). However pronucleus formation was not different between groups but results showed that cleavage rate has a reduction in the sperm of mices received chaste tree essential oil.

Conclusion: It can be concluded that chaste tree essential oil can reduce fertilization capacity of sperm.

Keywords: Chaste Tree, Essential Oil, IVF, Sperm

P-110: Effect of Increasing Amount of Oocyte Secreted Factors on Cumulus Expansion of Bovine Cumulus-Oocyte Complexes

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Background: *In vitro* maturation is a good method to

decrease cancer risk of superovulation by gonadotropin hormones. A paracrine effect of oocyte secretions on oocyte developmental competence is under investigation. Apart from oocyte maturation, ovulation *in vivo* requires a precise control of extracellular matrix modification. Cumulus cells secrete hyaluronan to form a muco-elastic extracellular matirx with proteins derived from the serum and the follicle (cumulus expansion). This matrix structure is of importance for oocyte extrusion from the follicle and for pick-up by the fimbria. In addition, a function of selective barrier for sperm has also been reported for this matrix structure. Oocyte secreted factors (OSFs) may be involved in cumulus expansion of oocytes. This study was conducted to identify if the increasing amount of native OSFs improve the rate of cumulus expansion of bovine and ovine oocytes.

Materials and Methods: Collected ovaries from the local abattoir were transported to the laboratory in PBS at 30-35°C. The cumulus - oocyte complexes (COCs) of follicles were recovered by aspiration. Some COCs were denuded using hyaluronidase and vortexing. Then COCs surrounded with at least three layers of cumulus cells were co-cultured with denuded oocytes (Dos) in a 50 µl droplet of oocyte cultue medium (OCM). The selected COCs were randomly co-cultured in four groups (COCs cultured alone, with Dos in 1:1, 1:3 and 1:6 ratios. After an incubation period (24 hours), cumulus expansion was assessad.

Results: In bovine COCs, complete cumulus expansion rate was 80% (control), 75%, 72% and 76%, respectively.

Conclusion: The analysis of data showed that the rate of complete expanded cumulus in treatment groups was not significant when compared with control group. Thus, although these results show that increasing amount of native OSFs does not improve cumulus expansion of bovine COCs, the other aspects of oocyte maturation at molecular level as well as fertilization and preimplantation embryogenes should be more investigated for better understanding the effects of OSFs in cattle

Keywords: Oocyte-Secretsd Factors, Bovine, *In Vitro* Maturation, Cumulus Expansion

P-111: An Attempt to Facilitate the Production of Transgenic Mouse As A Model for Gene Therapy of Gaucher Disease

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Background: Gaucher disease is an autosomal recessive inherited lysosomal storage disorder that affects many of the body's organs and tissues by defective function of the catabolic enzyme β-glucocerebrosidase. Gene therapy is one of the efficient ways for treatment of this disease. Due to the lack of appropriate animal models, in the field of gene therapy little progress has been done.

Materials and Methods: In this study the 845 bp fragment of the GBA gene (mutant glucocerebrosidase gene) was transferred into the male pronucleous of mouse zygote by DNA pronucleous microinjection method

Results: And then it was detected in blastocyste stage by PCR and RT-PCR.

Conclusion: The finding has been reported the detection of transgene in mouse blastocyste for the first time in Iran, which will be instrumental both in developmental studies and in the generation of mouse models of human gene therapy in gaucher disease.

Keywords: Gaucher Disease, Transgenic Mouse, Pronucleous Microinjection, Animal Model

P-112: Potential Applications of Sheep Oocytes As Affected by Vitrification and *In Vitro* Aging

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Background: The present study was carried out to investigate how the interactions between aging, vitrification, and post-warming interval affect the credibility of sheep MII-oocytes for *in vitro* fertilization (IVF), intracytoplasmic injection (ICSI), and parthenogenetic activation (PA).

Materials and Methods: The vitrified-warmed oocytes, either immediately (immediate group: IG) or two hours post-warming (delayed group: DG) along with their corresponding unvitrified controls were used for assessment of (i) survival rate, (ii) meiotic spindle and chromosomal organization, (iii) ultrastructural changes, (iv) gene expression, (v) cortical granule release, and zona hardening and (vi) embryo development using IVF, ICSI, and PA.

Results: According to our results, aged oocytes had significantly higher rates of chromosome and spindle abnormalities compared to young oocytes. However after vitrification-warming, the total rates of these abnormalities were not significantly different between aged and young oocytes. Unvitrified-aged, and vitrified young and aged oocytes had comparable ultrastructural characteristics whereas they were completely dissimilar in compared with unvitrified-young oocytes. Although mRNA abundance was reduced during vitrification-warming in both aged and young oocytes, the post-warming interval could improve the relative mRNA abundance. Aged oocytes had lower capacity for IVF and ICSI in compared with young oocytes, but had similar pattern for PA process.

Conclusion: The vitrification process decreased developmental competence of both aged and young oocytes in compared with young ones, particularly when warmed oocytes were rested for 2 hours before IVF, ICSI and PA. The results of the present study showed that *in vitro* aged oocytes had higher capacity to be used for par-

thenogenetic studies rather than IVF and ICSI. Furthermore, it was shown that vitrified oocytes had a time dependent decline in quality and developmental potential. Notably, the speed of this decline was higher in vitrified-young oocytes, indicating that the vitrified oocytes do not require to be rested post warming. Conclusively, the results of this study can be useful in preserving *in vitro* aged oocytes to provide a valuable and easy access source of oocytes for research purposed studies.

Keywords: Vitrification, Oocyte Aging, ICSI, IVF, PA

P-113: Effect of Bioxell® on Cryopreservation of Goat Epididymal Sperm

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Background: Epididymal sperm is a source of sperm for IVF in patients with ejaculatory insufficiency and also in wild life endangered animals. To use epididymal sperm for IVF or Artificial Insemination, it has to cryopreserved. Bioxell is a sperm freezing extender which does not contain any substance of animal origin. The aim of the present study was to evaluate abnormalities of goat sperm following cryopreservation with Bioxell.

Materials and Methods: Goat testes-epididymes were collected from local slaughterhouse (n=10) and transported to the Lab within an ice container. The caudal epididymis was dissected and cut with a stab incision, then put in sperm collection medium (TALP). After incubation of Epididymis for 15 min in medium prefrezzing sperm parameters were analyzed. Then the sperms were frozen using a commercial known Bioxell extender based on the company's instruction manual. Fourthy eight hours after freezing, straws were thawed and sperm parameters were recorded. In this part of study the focus was on sperm abnormalities that were evaluated with a vital stain (Eosin-Nigrosin B) and traditional Giemsa stain. Sperm abnormalities were evaluated under 40 magnification using Leica light microscopy. Data were analyzed using ttest procedure in SAS.

Results: Sperm viability (%) significantly decreased in frozen (70.46) compare to fresh (94.08) samples ($p<0.0001$). Tail abnormalities were significantly higher in frozen (17%) than fresh (9.04%) samples ($p=0.0003$). Freezing procedure significantly reduced cytoplasmic droplet in frozen (22.2%) compare to fresh (45.6%) samples ($p<0.0001$). sperm head abnormalities including detached heads were similar in both fresh and frozen samples ($p>0.05$).

Conclusion: Sperms parameters following cryopreservation with Bioxell were in acceptable range. Bioxell specially protected the sperm head against freezing stress. Although, increasing tail abnormalities and dead sperms may contribute in redcing sperm fertility. More studies are necessary to find sperm fertility using Bioxell.

Keywords: Epididymal Sperm, Goat, Bioxell

P-114: Effect of In Situ Cool Storage on Goat Epididymal Sperm

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Background: Short time preservation of semen is common in animals (e.g. horse or pig etc…) which their sperm in known as freezing sensitive sperm. It also may use for short transportation of other species to be cryopreserved or use in AI or IVF programs. The aim of the present study was to evaluate effects of duration of cool storage on goat epididymal sperm.

Materials and Methods: Total numbers of 40 testes epididymes of slaughtered bucks were transported in an ice container to the Lab and submitted to 4 time of storage (0, 24, 48 and 72 hours) in 5°C refrigerator. After storage caudal epididymes were dissected and cut with a stab incision and incubated in sperm collection medium (TALP). Sperm parameters were analyzed after time of storage with focus on sperm abnormalities. GLM procedure was used for data analysis in SAS.

Results: The percentages of live sperms were significantly reduced after 48 and 72 hours of cool storage (94.08, 90.08, 76.4 and 79.6 for 0, 24, 48 and 72 hoursrs, respectively $p<.0.01$). An increasing trend of sperm head abnormalities was detected up to 48 hours (1.7%) of cool storage ($p<0.01$). Significant increase in tail abnormalities were recorded after 48 and 72 hours of storage ($p<0.01$). Cytoplasmic droplet significantly decreased after 48 and 72 hours of cool storage ($p<0.001$).

Conclusion: In conclusion, goat epididymal sperm can be preserved in situ in cool enviromnet up to 24 hours. The results showed impact of duration of cool storage on sperm abnormalities of goat epididymal sperm.

Keywords: Epididymal Sperm, Goat, Cool Storage

P-115: The Effect of Intra-Peritoneal Administration of Papaver Bracteatum Lindl. Extract on Development of NMRI Mice Oocytes Treated with Doxorubicin

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Background: In this study the effect of water-alcohol Papaver bracteatum Lindl. Extract on development of mice oocytes treated with doxorubicin (dox) was examined.

Materials and Methods: Mice at 7-8 weeks old were assigned to 4 groups. Control group, mice injected intraperitoneally (IP) with saline. P.bracteatum extract group

alone, mice treated with 200 mg/kg of body weight, IP, twelve consecutive days. Dox group alone, mice were given dox, IP, 10 mg/kg of body weight. Experimental group, mice treated with P.bracteatum extract and doxorubicin together. Effect of the extract on development of mice oocytes treated with dox was evaluated through assisted reproductive technology techniques (ARTs). Ovulated MII oocytes were isolated from the mice oviductal ampullae. The oocytes were transferred into Human Tubal Fluid (HTF) medium supplemented with 4mg/ml BSA and capacitated sperm were added to IVF medium. After 4-6 hours, 2PN were transferred into KSOM medium supplemented with 4 mg/ml BSA. After IVF process, embryos were cultured and their developmental process was monitored for 96 hours.

Results: The results of this study indicated that developmental rate and blastocyst formation improved by using of the extract. We observed a significant increase *in vitro* developmental competence experimental group in comparison with dox group alone ($p<0.05$).

Conclusion: Papaver bracteatum extract, as a medicinal plant with anti-oxidant property, increases oocyte quality. Antioxidative properties of some phytochemicals of the extract are concluded as probable reasons of the extract effects. The purpose of the present study was to assess the doxorubicin toxicity to these follicles and oocytes could be prevented by extract and cause improved embryo development which obtained from mice treated with dox plus papaver compared dox alone. This study was designed to examine the possible ameliorating action of the water-alcohol Papaver bracteatum extract, on doxorubicin-induced detrimental effects in mouse models and imply extract to be a promising adjuvant agent that may attenuate the toxicity of doxorubicin.

Keywords: Antioxidant, Doxorubicin Hydrochloride, *in vitro* Fertilization, Oocyte, Papaver Bracteatum Lindl

P-116: Maturation Genes Expression during *In Vitro* Culture of Vitrified Mouse Preantral Follicles

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Background: Evaluation of maturation genes expression and their pattern during *in vitro* culture of vitrified preantral follicles is an essential and could promote either vitrification procedure or culture condition.

Materials and Methods: Preantral follicles (with 100-130 µm diameter) isolated mechanically from 12-14 days old NMRI mice ovaries and divided into vitrification and control groups. In the vitrification group, follicles were vitrified in a combination of ethylene glycol and dimethylsulfoxide, loaded on cryotop tip and immediately immersed in liquid nitrogen. Vitrified-warmed and fresh control follicles were cultured for 13 days and quantitative expressions of GDF9 and BMP15 as maturation genes and also BMPRII, ALK5, ALK6 as their receptors were assessed by real- time PCR after 3 hours, 4, 8 and 13 days of *in vitro* culture.

Results: The results indicated that the expression of GDF9 and BMP15 in 3 hours and 4 days culture was increased in the follicles of vitrification group compared to the control group and also the expression of BMPRII rose after 4 and 8 days of culture, but with further culture up to 13 days the expression of these three genes were similar to control group. Additionally, the expression of maturation genes receptors such as ALK5 and ALK6 were similar in control and vitrification groups in all of assessed culture days.

Conclusion: Although the expression rate of maturation genes was different in vitrification and control groups' follicles in some of the earliest days of culture, but in general the expression pattern of vitrification group was similar to control group during *in vitro* culture days.

Keywords: Preantral follicle, Vitrification, Cryotop, *In Vitro* Culture, Maturation Gene Expression

P-117: The *In Vitro* Effect of Leptin on Semen Quality of Water Buffalo (Bubal Us Bubalis) Bulls

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Background: Several studies have recently investigated the role of leptin, the polypeptide hormone secreted primarily by white adipose tissue, in the reproduction of rodents, humans, and domestic animals.

Materials and Methods: To investigate the *in vitro* effect of leptin on buffalo semen quality in equilibrated and frozen thawed semen, five healthy buffalo bulls (5 ejaculates from each bull) were used. Each ejaculate was diluted at 37°C with tris-based extender containing 0 (control), 10, 50, 100, and 200 ng/mL leptin. After keeping diluted semen 4 hours in refrigerator to reach to the equilibration time, the semen was packed in 0.5 mL French straws and frozen in liquid nitrogen. The sperm motility, viability, and DNA fragmentation were evaluated at equilibration time and after thawing the frozen semen.

Results: Results showed that in equilibrated semen adding 10 ng/ml leptin on semen extender significantly increased sperm motility and viability compared to control group, but 200 ng/ml leptin decreased semen parameter significantly. In the frozen thawed semen, all leptin concentrations decreased sperm motility and viability whit the significant decrease of 100 and 200 ng/ml. There was no significant difference between leptin and sperm DNA fragmentation in all group in the equilibrated and frozen thawed semen.

Conclusion: This study showed that although *in vitro* addition of 10 ng/ml leptin can increased sperm motility and viability in equilibrated semen, but all leptin concen-

trations decreased these parameters in buffalo bulls frozen thawed semen with the significant decrease of 100 and 200 ng/ml which may be is due to the role of leptin in sperm capacitation.

Keywords: Leptin, Buffalo, Semen Quality

P-118: Effect of Vitrification on Developmental Competence of Parthenogenetic Activation in *In Vitro* Matured Ovine Oocytes

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Background: Cryopreservation of *in vitro* matured oocytes is a useful technique because the oocytes can be used for some assisted reproductive technologies. On the other hand, the cryopreservation of oocytes is an open problem as a result of their structural sensitivity to the freezing process. The purpose of this study was to evaluate the effect of vitrification on *in vitro* development of vitrified *in vitro* matured ovine oocytes after chemical activation.

Materials and Methods: Immature oocytes were collected from abattoir-derived ovaries, matured *in vitro*. Then, *in vitro* matured oocytes divided into two groups: 1. vitrified in cryotop (VTR); 2. without treatment as a control (CTR). 407 matured ovine oocytes were cryopreserved by vitrification. oocytes were exposed to 7.5% EG + 7.5% DMSO for 3 minutes and then 15% EG + 15% DMSO + 0.5 M sucrose for 25 seconds, loaded in cryotops and immersed into liquid nitrogen. After warming, oocytes were cultured *in vitro* for 30 minutes and then parthenogenically activated using ionomycin for 1 minute and subsequently incubated in 6-dimethylaminopurine (6-DMAP) for 2 hours.

Results: When vitrified-warmed oocytes were activated, blastocyst rates in VTR (10.21%) group was significantly lower ($p< 0.05$) than in CTR (39.50%).

Conclusion: Vitrification procedures effect on the structural components and biochemical and molecular events such as spontaneous parthenogenetic activation that could be a reflection of injuries to cytoplasmatic biochemical components leading to abnormalities in the cell cycle control, degeneration and low developmental competence of vitrified MII ovine oocytes.

Keywords: Vitrification, Ovine Oocyte, Chemical Activation, Developmental Competence

P-119: Melatonin Protects Folliculogenesis through Up-Regulation of Estrogen Receptors in Mouse under Treatment with Nicotine

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Background: Melatonin as the major hormone produced by the pineal gland has a significant role in mammalian reproduction. In this study the efficacy of melatonin co-administration with nicotine on mouse follicogenesis was investigated.

Materials and Methods: A total of 32 Female adult NMRI mice were divided into four groups. The control group (n=8) received vehicle, group 2 (n=8) received nicotine (0.4 mg/100 g body weight) for 15 days, group 3 (n=8) was administered melatonin 10 mg/kg for 5 days. Group 4 (n=8) received both nicotine (0.4 mg/100g body weight) and melatonin (10 mg/kg). After autopsy on 16th day, evaluations were made by histopathology, immunohistochemistry methods for evaluation of P53 and estrogen receptor (ER) expression in ovarian follicles and ELISA for serum estradiol level. Statistical analyses were performed by ANOVA.

Results: Nicotine significantly reduced the number and size of follicles and estradiol levels compared to the control ($p<0.01$). While melatonin in group 4 caused a marked normalization in follicogenesis and estradiol levels compared to group 2. There were not significant statistical differences in P53 expression in ovarian follicles in all groups. A significant increase in expression of (ER) were observed in secondary (2/37 ± 1/01 vs. 0.86 ± 0.59) and antral follicles (1.91 ± 1.60 vs. 0.051 ± 0.12) in melatonin treated group compared with controls ($p<0.02$). Co-administration of nicotine-melatonin in last group increased expression of (ER) in compare with group 2.

Conclusion: The results from this study suggest that adverse effects of nicotine on ovary are not dependent to P53 expression. Nicotine reduces serum estradiol level. Administration of melatonin can protect follicogenesis in mouse ovary under treatment with nicotine, partly through up-regulation of estrogen receptors.

Keywords: Nicotine, Melatonin, Estrogen Receptor, P53, Estradiol

P-120: Meiotic Spindle Visualization and Zona Pellucida Birefringence in Relation to Morphology of *In Vivo* and *In Vitro* Matured Human Oocytes

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Background: The meiotic spindle plays an important role in the oocyte during chromosome alignment and separation at meiosis. The zona pellucid (ZP) is a dynamic matrix composed of filaments with the properties that might reflect the history of oocyte cytoplasmic maturation. Since, spindle and ZP in living oocytes are highly birefringent, their structures can be viewed non-invasively by using a Polscope. The aim was to investi-

gate the relationship between the presence of the meiotic spindle and ZP birefringence with morphology of *in vivo* and *in vitro* matured human oocytes.

Materials and Methods: The oocytes were obtained from stimulated ovaries of patients undergoing ICSI. Germinal vesicles (GV; n=13) and metaphase I (MI; n=18) oocytes underwent *in vitro* maturation (IVM) using maturation medium supplemented with FSH + LH. They were checked for maturity 24 hours after culture. With the aid of Polscope, the presence of spindles and ZP birefringence (ZPB) were assessed in *in vivo* (n=21) and *in vitro* (n=21) matured oocytes. In addition, the morphology of each matured oocyte was evaluated using inverted microscope.

Results: The rate of IVM in GV and MI oocytes was 53% and 77%, respectively. Spindle was present in 52.3% and 42.8% of the *in vivo* and *in vitro* matured oocytes, respectively. Spindle detection rates in oocytes derived from GV and MI stage were similar. The percentage of high birefringence oocytes was higher in *in vitro* than in-vivo matured oocytes (76.1 vs. 61.9%). Also, insignificant increases in rates of morphologic abnormalities were seen in *in vitro* in comparison with *in vivo* matured oocytes.

Conclusion: The Findings indicates that IVM is a safe technology for maturation of human immature oocytes. However, application of Polscope is recommended in IVM technology to detect the most suitable oocytes for ICSI.

Keywords: Meiotic Spindle, Zona Pellucida, Morphology, IVM, Human Oocytes

P-121: Assessment of Microtubule and Nuclear Status at Different Intervals of Bovine *In Vitro* Oocyte Maturation

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Background: Mammalian oocyte undergoes a series of structural nuclear modulations during maturation in order to obtain full competence to support fertilization and early embryonic development. Microtubules are major cytoskeleton components and have pivotal modulators for chromosomal movement, and it has been shown that partial or immature spindle organization may compromise correct ploidy of the matured oocytes in mammalian oocytes. Therefore, this study was carried out to investigate the optimum time of *in vitro* maturation in terms of nuclear and spindle organization in bovine oocytes. In this study we investigated the effect of various maturation time point on nuclear status and microtubular organization in order to introduce the best time of maturation for parthenogenetic activation.

Materials and Methods: Oocytes were cultured in maturation medium for 14-32 hours. At 2 hours intervals, cultured oocytes were removed from IVM, fixed and immunostained using a monoclonal anti-β-tubulin antibody to investigate the microtubular organization and counterstained with H33342 to detect the nuclear status.

Results: In this study we observed that: the percentages of MI oocytes were i) increased during 14 to 18 hours after maturation, ii) decreased during 18-32 hr after maturation in bovine oocyte. The percentage of MII oocytes from 14-22 hours (14 hours: 24.1, 16 hours: 28.1, 18 hours: 27.6, 20 hours: 74.3, and 22 hours: 87 %) increased in bovine oocyte. The oocytes were arrested at 22 hours after maturation until 32 hours in MII stage. The best development and organization of microtubules and arrangement of chromosomes on spindle were observed at 22 hours post of maturation.

Conclusion: Our results demonstrated that, the best time for full development of MII spindle and arrangement of MII chromosomes is 22 hours post maturation and increasing time of IVM resulted in increasing degrees of spindle anomalies.

Keywords: Microtubule, Ovine, Oocyte, Nuclear Status, Maturation

P-122: Sperm Intracellular Reactive Oxygen Species Incidence in Ram Fed A Diet with Fish Oil

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Background: Basal level of reactive oxygen species (ROS) is essential for sperm acrosomal reaction, hyperactivation, motility, capacitation and fertilization. Ram spermatozoa contain large quantities of polyunsaturated fatty acids in their plasma membranes so are extremely susceptible to ROS induced damage.

Materials and Methods: Eight rams (36 ± 5 month of age and with a weight of 64 ± 7 kg) were randomly distributed in control (CTR; n=4) and fish oil (FO; 35 g/d/ram; n=4) groups. Rams were fed with fish oil regime in associated with constant levels of vitamin E (45 g/d/ram) for 60 days during the physiological breeding season and after a month of feeding; their semen was collected every week. Computer assisted sperm analyzer system (CASA) with Sperm Class Analyzer software was used to evaluate sperm motility and sperm kinetics (system to setup with analysis values of ovine). Intracellular ROS level was also detected using 2′, 7′- dichlorodihydrofluorescein diacetate (DCFH-DA) for hydrogen peroxide (H_2O_2) and Dihydroethidium (DHE) for superoxide anion (O_2-), by flow cytometry.

Results: As expected, adding FO in diet significantly improved volume and concentration as well as total sperm output (5.40 vs. 3.38×10^9 for FO and CTR, respectively) and improves sperm progressive motility (59.2 vs. and 48.8% for FO and CTR, respectively) as compared to the control group. Totally the rate of O_2 was lower in FO group compared to the control one and this difference was significant ($p>0.05$) between 3rd and 4th week

of sampling. On the other hand, the rate of H_2O_2 didn't indicate significant difference entirely.

Conclusion: In this experiment, using Omega-3 source accompanied by vitamin E could improve the quality and progressive motility of ram spermatozoa, also could decrease the incidence of ROS. As poor diets may impair male fertility potential, probably daily dose of vitamin E and Omega- 3 could compensate dietary deficiencies in some way and thereby optimize male fertility potential.

Keywords: ROS, Sperm Quality, Fish Oil, Ram

P-123: Impact of Oxidative Stress on Differentiation of Testis in Mice

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Background: Antioxidants and reactive oxygen species (ROS) are in balance in the body. Whenever the balance between these molecules is disrupted towards an overabundance of ROS, oxidative stress (OS) occurs. There are evidence that glutathione as an intracellular antioxidant plays an important role against ROS in female reproductive system. Regarding the effect of BSO as a selective inhibitor of glutathione peroxides. On the other hand, primary germ cells after migration to developing ovary differentiate to oogonia and oocytes. Formation and number of primordial follicles in early fetal life is a determining factor in the fertility state of adult life .The aim of the present study is to investigate BSO induced OS on development and differentiation of ovarian follicles in mice.

Materials and Methods: In this study 30 adult female and 15 adult male mice are used. The female mice are divided into two groups of control and experimental .Two female mice at their stereos cycle were put with one male mouse in a cage for mating. Observation of vaginal plaque was considered as the first day of pregnancy and the mice on 13th day of pregnancy received 2 mmol/kg BSO daily until delivery as IP injection. After the pregnancy the 2, 3, days old new born were sacrificed and their testis were fixed and prepared for light and electron microscopic studies. In the sections, the number seminiferous tubules and diameter of them were determined using Motic software and compared with control values using t-test.

Results: Light and electron microscopy and morphometry showed that seminiferous tubules are distinguished on the 2nd and 3rd day's old newborn. Morphometric studies revealed that the number and diameters of seminiferous tubules were significantly ($p<0.001$) reduced in Experimental groups in comparison to control group.

Conclusion: The result indicates that oxidative stress suppresses follicular differentiation at early stages but does not affect the development of already differentiates follicles.

Keywords: Oxidative Stress, BSO, Differentiation, Testis

P-124: Effects of Antioxidants (Vitamins E and C, Astaxanthin) on Sperm Parameters in Rats with High Fat Diet

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Background: Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems such as infertility and sub fertility. Lipid peroxidation contributes to the damage of the sperm plasma membrane and result to infertility. In this study effects of Antioxidants (E, C, Astaxanthin vitamins) on sperm parameters has been investigated in rats with high fat diet.

Materials and Methods: Matured wistar albino male rats weighting approximately 200-250 g were divided to 3 groups; control with access to standard diet, group with high-fat diet and animals received high-fat diet and Antioxidants (vitamins E and C, Astaxanthin). After at three months, the rats were sacrificed and sperm parameters such as sperm count, motility, morphology and viability were analyzed.

Results: The results obtained in the study revealed that sperm number in high-fat diet and Antioxidants group (62.4×10^6 per ml) was higher than control(57×10^6 per ml) and high fat diet group(55.75×10^6 per ml). Progressive motility of spermatozoa in group fed with antioxidants (46.81%) was higher than others. But there was no significant difference between sperm morphology and viability of three groups.

Conclusion: The present results suggest that sperm parameters such as number and motility could affected by high-fat diet, and sperm count and motility reduction due to high fat diet may be compensated and improved by antioxidants such as E, C and Astaxanthin vitamins.

Keywords: High Fat Diet, Sperm Parameters, Vitamins E, Vitamins C, Astaxanthin

P-125: Assessment The Effect of Equisetum Arvense Alcoholic Extract on IVF Rate, Embryo Quality and Development in STZ-Induced Diabetic Female Mice

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Background: Diabetes mellitus is a common metabolic circumstance characterized by high blood glucose level and partial or absolute impairment of insulin secretion. Diabetes in females leads to sexual disorders such as loss of libido, low ovulation rate and disorder in oocyte maturation. Therefore the current study was designed to evaluate the protective effect of Equisetum arvense

alcoholic extract (EE) on IVF rate and embryo development in diabetic mice.

Materials and Methods: To follow-up the present study, 24 mature female mice were divided into four groups as control-sham (n=6), diabetic (n=6) and diabetic + EE (250 and 500 mg/kg, orally, daily gavage). In order to induce diabetes the STZ (50 mg/kg for 5 days) was administered. Following 30 days of STZ administration the animals were humanity sacrificed and the oocytes were collected by dissecting method following superovulation induction (PMSG, 7.5 IU and hCG, 7.5IU after 48 hours). The oocytes were fertilized by fresh mouse sperm in HTF medium containing 4 mg/ml BSA. The rate of fertilization, two cell embryos, arrested embryos and blastocysts rate was examined in period of 120 hours.

Results: The animals in D+EE-administrated groups; especially those in D+EE500, showed remarkably ($p<0.05$) higher fertilizing success rate in comparison to diabetic group. The D+EE-treated groups had significantly ($p<0.05$) higher percentage of fertilized oocytes developed until blastocyst stage and remarkably ($p<0.05$) lower percentage of embryos were blocked in different stages of embryonic development.

Conclusion: The animals in D+EE-administrated groups; especially those in D+EE500, showed remarkably ($p<0.05$) higher fertilizing success rate in comparison to diabetic group. The D+EE-treated groups had significantly ($p<0.05$) higher percentage of fertilized oocytes developed until blastocyst stage and remarkably ($p<0.05$) lower percentage of embryos were blocked in different stages of embryonic development.

Keywords: Diabetes, Equisetum Arvense, Embryo, IVF, Mice

P-126: Stem Cell Factor Increases Blastocyst Formation in Mouse Two-Cell Embryo Culture

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Background: It is demonstrated that c-Kit(receptor of stem cell factor) mRNA is expressed in late 2-cell stage to the expanded and hatched blastocyst and the stem cell factor (SCF) transcript is detected in the oviduct and uterus. The aim of this study was to investigate the effect of different doses of SCF on mouse 2-cell embryo development *in vitro*.

Materials and Methods: 4-6 weeks old female mice were superovulated by i.p. injection of 7.5 IU PMSG and 46-48 hours later 7.5 IU hCG and caged overnight with males. 2-cell stage embryos were collected from oviducts and cultured in T6 medium supplemented with 0, 1, 20 and 50 ng/ml SCF to blastocyst stage then the number of blastocysts, hatching/ hatched and survived blastocysts was assessed.

Results: The proportion of 2-cell embryos which developed to the blastocyst stage in group of 50 ng/ml SCF were significantly higher than control group ($p<0.05$). There was no difference in blastocyst formation between 1 and 20 ng/ml SCF and control groups ($p>0.05$). Hatching rate and blastocyst survival among control and treatment groups was not significant ($p>0.05$).

Conclusion: Culture of mouse 2-cell embryos in the presence of SCF has beneficial effects on the rate of blastocyst formation.

Keywords: Stem Cell Factor, 2-Cell Embryo, Blastocyst Formation, Hatching Rate

P-127: *In Vitro* Assessment of Trehalose for Cryopreservation of Ram Sperm in Extender Containing Soybean Lecithin

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Background: Trehalose, a non-permeable disaccharide, has a protective role related both to osmotic effect and specific interactions with membrane phospholipids, rendering hypertonic media, causing cellular osmotic dehydration before freezing, and then decreasing the amount of cell injury by ice crystallization. Within this study, an attempt was made to evaluation of two levels of 50 and 100 mM trehalose in Tris based extender containing soybean lecithin on post-thaw quality of ram sperm.

Materials and Methods: A Tris based extender containing 1% soybean lecithin and 7% glycerol was prepared and then divided to 3 aliquots. Then, different concentrations of trehalose (0, 50 and 100 mM) were added to extenders. Semen samples were collected by artificial vagina from four mature zandi native breed rams (3 and 5 yr of age) during the breeding season and after primary evaluation, samples were pooled in order to eliminate individual effect. The sperm were evaluated and accepted for evaluation if the following criteria were met: volume varying between 1 and 2 mL, sperm concentration of 3×10^9 sperm/mL, motile sperm percentage higher than 70%, and less than 10% abnormal sperm. The diluted semen was gradually cooled to for 2 hours. The cooled semen was loaded into 0.25-mL French straws (Biovet, L'Agile France). For assessment of sperm after freezing, the sperm were thawed and then prepared for evaluation. Motion parameters were assessed by CASA. Viability and membrane integrity and abnormality were evaluated by eosin-nigrosin, Host and Hanckok solution, respectively.

Results: The extender supplemented with 50 mM trehalose exhibited the greatest percentages of sperm motility (58.43 ± 1.86), membrane integrity (52.34 ± 1.46), viability (63.71 ± 1.52), in compare to 0 (48.71 ± 1.86, 45.46 ± 1.46, 56.71 ± 1.52, respectively) and 100 (44.57 ± 1.86, 41.01 ± 1.46, 55.29 ± 1.52 respectively) mM trehalose ($p<0.05$). Also, 50 mM trehalose significantly reduced percentages of post-thaw total abnormalities (14.94 ±

1.16) compare to control (19.25 ± 1.16) and 100 mM trehalose (20.13 ± 1.16).
Conclusion: The findings of this experiment show that when the trehalose in the diluents containing lecithin 1% and glycerol 7% a concentration of 50 mM had better performance than to 100 mM, and significant improve sperm mobility parameters.
Keywords: Trehalose, Soybean Lecithin, Ram Semen, Freezing-Thawing

P-128: Fibroblast Growth Factor Improves Oocyte Maturation in Vitrified-Thawed Mouse Follicles

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Background: The fibroblast growth factors (FGF-4) are a group of heparin-binding single chain polypeptides that play a pivotal role in development, cell growth, tissue repairing and transformation. The aim of this study is to improve development and maturation outcome of vitrified mouse pre-antral follicle by adding of fibroblast growth factor (FGF) into medium culture.
Materials and Methods: Pre-antral follicle with diameter of 150-180 µm were mechanically isolated from 18-21 day old NMRI mouse ovaries follicles were vitrified and thawed and then cultured in medium supplemented with 0 , 20 ng/ml FGF. Comparison between FGF group and control was performed.
Results: The results showed that MII oocyte rate of vitrified- thawed follicles increased significantly in the culture medium supplemented with growth factor as compared to the control group ($p<0.05$). There was not any significant difference between the degeneration rate of vitrified follicles, in the control and treatment group ($p>0.05$).
Conclusion: Addition of growth factor in the culture has favorable effects on *in vitro* maturation of virified pre-antral follicle.
Keywords: Fibroblast Growth Factor, Vitrification, Oocyte Maturation, Pre-Antral Follicle

P-129: Study of GnRH Potential in Inhibition of Side Effect of Drugs Used in Chemotherapy on Spermatogenesis

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Background: Male factors, mainly spermatogenesis disorder, are responsible for 20-30% of infertility occurs in different societies. One of the known causes of spermatogenesis disorder is chemotherapy in patients with cancer. The side effect of chemotherapic agents may last from 10 years up to the end of the life. Since dividing cells are mainly affected by anticancer drugs, the aim of the present study is to investigate the preventive effect of GnRH antagonist as a suppressor of spermatogonial proliferation, on spermatogenic defect produced by anticancer drug (vincristine).
Materials and Methods: In the present study 30 adult male mice aging 6-8 weeks were used. The mice were divided into 3 equal groups as: control, exp I group and exp II group. In exp I group. A single dose of vincristine was injected as ip, at 1.5 mg/kg. In expII group, cetrorelix injection was started one week before vincristine treatment and continued for 3 more weeks. The mice in all groups were sacrificed 3 weeks after the last dose of Cetrorelix injection. Testicular specimens were prepared for LM studies.
Results: LM study showed that rate of spermatogonia in control group was 47.42 ± 1.96 µm, in exp I group was16.53 ± 2.37 µm, and in exp II group was 36.28 ± 7.98 µm. The rate of spermatogenesis Index (SI) in control group was 3.03 ± 0.21 µm, and in exp I group was 1.04 ± 0.48 µm, and in exp II group 1.90 ± 0.40 µm. Statistical analysis of data showed significant differences in rate of spermatogonia and SI between control and exp I group ($p<0.05$).and the rate of spermatogonia in exp II group was similar to control group, but there were significant differences in rate SI between exp II group and control group ($p<0.05$).
Conclusion: According to the result it is concluded that GnRH antagonist administration before cancer treatment could partially prevent the side effect of anticancer drugs.
Keywords: Vincristin, Spermatogenesis, GnRH Antagonist

P-130: Designing and Construction of An Appropriate Eukaryotic Expression Vector to Generate Soluble Form of Human Hyaluronidase Type PH20 in Cell Culture Feasible for Application in IVF and ICSI

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Background: The hyaluronidases are the enzymes hydrolyze β-1, 4 glycosidic linkage of hyaluronan. Hyaluronan is a polymer consisting of a repeating disaccharide unit found in cumulus ovuforus complex, semen liquid and other tissue. Addition to hydrolyzing the hyaluronan, hyaluronidase can penetrate through the cumulus cells layer that surrounds the oocyte, thus it terms spreading factor. Moreover, it is used to increase the absorption and dispersion of injected drugs. This enzyme used to denude oocytes from surrounding cumulus cells in IVF and ICSI.

Materials and Methods: At first step, total mRNA was extracted from testis tissue and cDNA was synthesized. Ph20 coding sequence deleted GPI anchor was amplified by means of specific primers designed based on ph20 special CDS and also contained additional regions encoding His tag and distinctive sequence recognized by entrokinase enzyme. Then, an amplified fragment was inserted into pTZ57R and treated by appropriate restriction enzyme to sub clone into pBudCE4.1. In this recombinant expression vector, attB region was added for insertion of this construct into genome by phiC31 integrase produced by another vector termed as pCMV-Int.
Results: The constructed expression vector was confirmed successfully as verified by sequence analysis. After transfection, culture media was extracted and tested on Granulosa cells. The cell mass was separated effectively that indicate this protein is active.
Conclusion: In this study, we produced an appropriate vehicle encoding recombinant hyaluronidase for therapeutic approach and recombinant protein for MS and infertility therapy.
Keywords: Hyaluronidase, PH20, CHO, IVF, ICSI

P-131: Autologous Transplantation Encapsulated Preantral Follicles into Fibrin Hydrogel Supplemented with Platelet Lysate

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Background: Recent improvement in treatments of cancer has significantly increased survival rates of cancer patients. Unfortunately these treatments such as chemotherapy and radiotherapy can sever damage to the ovaries. Ovarian cryopreservation prior to chemo/radiotherapy and auto-transplantation post-treatment can restore fertility to cancer patients. Hypoxia in the transplant from delayed revascularization is one of the concerns about this strategy. Thus, we assumed that isolated follicles transplantation with fibrin hydrogel supplemented platelet lysate (PL) lessen this concern.
Materials and Methods: In this study NMRI mice (4 weeks) heterotopic grafted with own follicles. Follicles were mechanically isolated using insulin-gauge needles and encapsulated into fibrin hydrogel droplets supplemented with PL 15%. Follicles with centrally located oocytes and two layers of granulosa cells (100-130 µm in diameter) were selected. In this study a total of 844 follicles were divided into two groups: A. 389 follicles encapsulated into fibrin hydrogel B. 455 follicles encapsulated into fibrin hydrogel supplemented PL 15%, Then implanted heterotopic under the neck skin (hypoderm) for a period of 14 days. Finally, survival, growth and number of grafted follicles were being assessment with Hematoxylin and Eosin staining and histological sections were analyzed to determine follicle number and stage.
Results: We observed in group A (encapsulated follicles into fibrin without PL supplement) from 389 transplanted follicles 115 follicles survived (survival rate 29.56%) that 78 follicles were in pre-antral stage (more than 2-3 layers of granulosa cells, pre-antral rate 67.82%) and 37 follicles were in antral stage (antral rate 32.17%). In group B (encapsulated follicles into fibrin supplemented PL 15%) from 455 transplanted follicles 178 follicles survived (survival rate 39.12%) that 122 follicles were in pre-antral stage (more than 2-3 layers of granulosa cells, pre-antral rate 68.53%) and 56 follicles were in antral stage (antral rate 31.81%).
Conclusion: Our results demonstrate the autologous transplantation encapsulated pre-antral follicles into fibrin hydrogel supplemented platelet lysate improves survival rate and folliculogenesis. Also fibrin hydrogel matrix as biodegradable scaffold physically bridges the transplant and host tissue, and platelet lysate as a growth and angiogenic rich product would promote revascularization and follicle survival.
Keywords: Autologous Transplantation, Preantral Follicle, Fibrin Hydrogel, Platelet Lysate

P-132: The Effect of Vitrification on Mouse Oocytes Survivale Rate and Apoptosis Using Cryotop

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Background: Vitrification of oocyte is one of the most important topics in the field of assisted reproductive techniques (ART). Considering the importance of oocyte vitrification in clinics, in the present study the effect of vitrification on mouse oocytes survivale rate and apoptosis by cryotop were investigated.
Materials and Methods: 200 GV oocytes and 200 MII oocytes were obtained respectively from NMRI mouse ovaries and oviducts. GV and MII oocytes group, each again divided into two control and freezing groups. The oocytes in freezing group were vitrified after dehydration in eqilibration and vitrification media (Origio) using cryotop. Mouse oocytes survivale rate and apoptosis after staining with TUNEL technique were compared in control and freezing groups using Fisher's exact test. Differences less than 0.05 were considered to be significant.
Results: The survivale rates of GV and MII oocytes after vitrification and thawing was significantly different compared to control group ($p<0.01$ and $p<0.01$). The apoptosis rate of GV oocytes after vitrification and thawing did not show a significant difference compared to control group while the apoptosis rate of MII oocytes after vitrification and thawing was indicative of a significant difference compared to control group ($p<0.001$). Meanwhile, there was a significant difference in apoptosis rate between GV and MII oocytes after vitrification and thawing ($p<0.03$).
Conclusion: The present study indicated that vitrifica-

tion result in detrimental effect by reducing oocyte survivale rate and increasing of oocyte apoptosis.

Keywords: Vitrification, Mouse Oocytes, Survivale Rate, Apoptosis, Cryotop

P-133: Effect of Thawing Rate on Kinetic Parameters of Frozen - Thawed Buffalo Spermatozoa Extended in Tris - Egg Yolk

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Background: Optimal thawing rate is critical for quality and fertilizability of frozen -thawed spermatozoa. Kinetic parameters are the most important factors which are affected by thawing rate so, that have close relation between kinetic parameters and fertility of the spermatozoa. Tris - egg yolk have been widely used for cryopreservation of the buffalo and other species semen. According to our findings no studies have examined the influence of different thawing rate on kinetic parameters of buffalo spermatozoa. Therefore, the objective of the current study is to evaluate the effect of different thawing rate on post thaw kinetic parameters of buffalo spermatozoa extended in Tris - egg yolk.

Materials and Methods: Fifteen ejaculations of five mature buffalo semen were diluted with tris -citrate-egg yolk extender and frozen .Three straws of each bull thawed at 37°C for 30 seconds, 50°C for 15 seconds and 70°C for 7 seconds then placed in makler chamber and analyzed with computer assisted sperm analyzer system (CASA) immediately and after 2 hours incubation.

Results: Semen thawing in 50°C significantly increased the progressive motility (PM) and curvilinear velocity (VCL) (29.8 ± 4.00% and 59.40 ± 7.30 µm/s respectively) at 0 hour when it compared to 37°C (19.08 ± 4.63% and 47.29 ± 3.0 µm/s). There was no significant difference in PM between 50°C and 70°C (26.92 ± 9.22%) while VCL was significantly improved in 50°C in comparison with 70°C (46.99 ± 7.41 µm/s) at 0 hour. After 2 hour incubation there were no significant differences in PM between all groups, while VCL in 50°C was significantly higher than 37°C and 70°C (40.88 ± 3.04 vs. 31.63 ± 2.80 and 38.20 ± 11.03 µm/s respectively). Average path velocity (VAP) in 50°C was significantly higher than other groups, 0 and 2 hours after thawing ($p<0.05$).

Conclusion: It seems that thawing the buffalo semen in 50°C for 15 seconds could increase sperm kinetic parameters and it can improve subsequent fertility of buffalo spermatozoa.

Keywords: Buffalo, Kinetics Parameters, Sperm, Thawing Rate

P-134: *In Vitro* Assessment of Cysteine Effect on DNA Integrity of Frozen-Thawed Buffalo Spermatozoa

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Background: Cysteine has been used as additive in cryopreservation extenders. It contains of thiol group which plays an antioxidant role to eliminate reactive oxygen species (ROS) during freeze -thaw procedure. Excessive ROS production cause DNA damage and subsequently reduce sperm fertility. On the other hand, protamine protein have high levels of cysteine which is critical in packaging and compaction of sperm chromatin. To date there is no report regarding the cysteine effect on DNA integrity of buffalo spermatozoa following cryopreservation.

Materials and Methods: Sixteen ejaculations of four mature buffalo bulls were spilt into four experimental groups and diluted with Tris - citrate -egg yolk extender which contains 0, 5, 7.5, 10 mM of cysteine. Frozen sperm were thawed and diluted with Tris -Null -EDTA buffer at the final sperm concentration of 5×10^6 cell/ml. 100 µl aliquots of this suspension was mixed with 200 µl detergent/acid solution. After 30 seconds acridine orange (AO) solution was added to the samples and cells were subjected to flowcytometry. The green fluorescence (intact DNA) detected by the FL1 detector was compared with red fluorescence (single strand DNA) detected by FL-3 detector ten thousand sperm cell were analyzed by cyflogic software.

Results: Mean percentage of sperm with damaged DNA in cysteine 5, 7.5, 10 mM were 4.08 ± 0.69, 4.05 ± 0.85, 4.22 ± 0.18 respectively were reduced significantly in comparison with control group 6.2 ± 0.53 ($p<0.05$).

Conclusion: It seems that 7.5 mM concentration of cysteine could improve DNA damage of frozen - thawed buffalo spermatozoa.

Keywords: Buffalo, Cysteine, DNA Integrity, Spermatozoa

P-135: Effect of Oxidative Stress on Follicular Growth Using Morphological Criteria

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Background: Infertility problem affect more than 15% of young couples in different societies. One of the known causes of ovulation disorder is chemotherapy in patients

with cancer. This side effect may last from ten years up to the end of the life. Antioxidents and reactive oxygen species (ROS) are in balance in the body. Oxidative stress is mainly insert their effect through decreasing of cell proliferation and viability. The aim of the present study is to investigate, adverse effect of oxidative stress on folliculogenesis using electron and light microscopy.

Materials and Methods: For this purpose, 30 adult female and 5 adult male bulb/c mice were kept in standard condition and divided into 3 groups of experimental, sham and control. In the experimental group, oxidative stress was induced by injection of 2 mmol/kg BSO daily for 2 weeks as IP injection. The sham group was received the solvent of BSO and the control group did not received any. The mice were killed by cervical dislocation. The mice were sacrificed and ovarian specimens were removed, fixed in formaldehyde fixative and 2.5% Glutaraldehide then prepared for light and electronmicroscopic study.

Results: light microscopy showed that the ovarian antral follicles was decreased in experimental group and many follicles have abnormal structure with decreased granulosa cellular layers, and electron microscopy revealed that in experimental group several vacuolated or ruptured mitochondria, nuclear chromatin was marginated and condensed with apoptotic cell features.

Conclusion: The results indicate that oxidative stress affects ovulation by both cytotoxic effect and decreasing of cell proliferation and differentiation.

Keywords: Oxidative Stress, BSO, Morphology, Ovary

P-136: *In Vitro* Development of Ovine Oocytes Matured in A Chemically Defined Medium

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Background: Despite great advances achieved in the field of *in vitro* oocyte maturation (IVM), almost all IVM media are supplemented with undefined component which not only pose the risk of pathogen transmission but also hinders our knowledge of molecular mechanisms of maturation. One of these components is serum that containing various concentration of unknown molecules affecting cellular processes, therefore the use of serum needs to be studied. We aimed at comparing developmental competency of ovine oocyte grown with and without serum.

Materials and Methods: Abattoir-derived ovaries were transported to the laboratory in saline. After aspiration of follicles, good quality oocytes were divided into two experiments. In the first experiment oocytes were cultured in defined maturation medium. The second experiment was carried out in undefined maturation medium. Then cleavage rate, blastocyst yield and morphological Cumulus cell expansion were evaluated between two experimental groups.

Results: In general, the use of chemically defined maturation media leads to significantly lower cleavage rate and blastocyst yields (91.24 ± 3.06 vs. 78.77 ± 3.66 and 42.65 ± 1.66 vs. 22.02 ± 8.44 respectively). The cumulus expansion index (CEI) of defined maturation group significantly lower than other treatment group.

Conclusion: Thus to improve the developmental competence of mammalian oocyte, several common growth factors, hormones and antioxidants have been added to defined maturation media. This purpose needs to more investigation about different defined maturation media.

Keywords: Ovine, Oocyte, *In Vitro* Maturation, Defined Maturation Media

P-137: Improvement in Post-Thawed Ram Sperm Quality by Adding Cysteine in Soybean Lecithin Based Extender

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Background: Ram sperm have a higher polyunsaturated fatty acids and a lower cholesterol/phospholipid ratio than other species which renders the sperm much more vulnerable to oxidative damage caused by reactive oxygen species (ROS). Oxidative stress can affects the integrity of cells at both the structural and sub structural levels. Therefore, a suitable antioxidant may prevent cryo-injuries and preserve sperm fertilizing ability after freezing-Thawing. So far, assessment of cysteine in ram cryopreservation medium based on soybean lecithin has not been studied. The aim of the current study was to evaluate the effects of cysteine (0, 5 and 10 mM) on the post-thawed sperm parameters, and acrosome status.

Materials and Methods: Semen samples were diluted with extenders containing 5 or 10 mM cysteine which were based on Tris and soybean lecithin and then frozen. The sperm parameters were assessed after thawing for motility, viability and acrosome status. All experiments were repeated for seven times. Means of treatments were compared using Duncan's multiple range tests by significant level of 0.05.

Results: Results show that the addition of 10mM cysteine led to higher motility and viability compare to extender without cysteine (55.9 ± 1.56 and 60.5 ± 1.34 vs. 47.1 ± 1.56 and 54.1 ± 1.34). Unlike motility and viability, Cysteine had not significant effect on the membrane integrity, acrosome status and other CASA parameters like progressive motility, VCL, VSL, ALH, BCF, STR and LIN. When sperm parameters were compared between 5 and 10 mM cysteine, no significant were observed for all of sperm parameters.

Conclusion: We conclude that addition of 10 mM cysteine to the ram sperm cryopreservation medium based on soybean lecithin led to production of higher motile and viable sperm so that produce sperm with higher fertilizing ability.

Keywords: Reactive Oxygen Species, Ram Sperm,

Cysteine, Lipid Peroxidation, Motility

P-138: The Effects of Omega-3, 6, 9 Fatty Acids on The Quality of Bovine Chilled Semen

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Background: Many studies are being conducted to improve the quality of bull sperm in storage conditions. It seems that increasing sperm membrane unsaturated fatty acids can enhance sperm quality. This study was aimed to investigate the effects of combination of omega- 3, 6, 9 fatty acids on characteristics of bovine chilled semen.
Materials and Methods: Different levels of oil containing omega- 3,6,9 fatty acids was added to semen extender. Five proven bulls were randomly selected and their ejaculates were collected by artificial vagina. Fresh semen was analyzed in terms of several parameters including volume, concentration, motility, viability and morphology. To emulsify the oil in semen extender, polyethylene glycol (PEG) was added as a more suitable solvent and solution was finally sonicated. Experimental groups included: control and treatments 1, 2, 3 and 4 as sham, levels 1, 2.5 and 5% of omega-3, 6, 9 fatty acids, respectively. In treatment 1, PEG was added alone to the diluents and in treatments 2, 3 and 4 different concentration of omega-3,6,9 fatty acids in combination with PEG were added to extender based Tris- citrate buffer, egg yolk and glycerol. After dilution, semen samples were packed into 0.5 ml straws and then cooling process was performed. Samples were evaluated in terms of motility, viability and morphology after 24 and 48 hours of storage in refrigerator (5°C). Motility and other dynamic parameters were analyzed by computer aided sperm analyzer (CASA) and viability and morphology were measured using phase contrast microscope after staining. The results were evaluated by repeated measure ANOVA using SPSS and $p<0.05$ was considered significant.
Results: Immobility increased and all other parameters (including motility, progressive sperm, viability, morphology…) significantly decreased in all groups including control compared with fresh samples. Moreover, some parameters including motility and progressive sperm were significant different between groups ($p<0.05$). Our result showed that PEG has significant detrimental effects on motility ($p<0.05$) while its effects was not significant on the viability and morphology ($p> 0.05$). Combination of omega-3, 6, 9 fatty acids could not attenuate harmful effects of PEG significantly. Moreover, they could not improve sperm motility, viability and morphology of bovine sperm in chilled storage condition.
Conclusion: Our result showed that this fatty acid combination does not appear to be a proper candidate for improving bovine sperm quality in cold conditions.

Keywords: Bovine, Sperm Quality, Cooling, Omega- 3,6,9 Fatty Acids

P-139: Compaison of Swim Up and Histoprep® Continuous Gradient for Isolation of Motile Epididymal Sperm in Ram

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Background: Isolation of motile sperm is essential for routine IVF programs. One of most important source of sperm for IVF is isolation of epididymal sperm (ESP). The problem regarding use of ESP is somatic cells which are recovered through sperm collection. The swim up and the swim down procedures are use to isolate motile sperm. The aim of the present study was to compare swim up and Histoprep® continuous gradient (HP) for isolation of ram ESP.
Materials and Methods: Ram testes epididymes were collected from local slaughterhouse and transported to the Lab. Basic medium for separation of sperms from epididymis was caffeine treated BO solution. Histoprep was provided from the company. After incubating of cauda epididymis in the basic medium for 15 minutes, primary sperm parameters including sperm motility and concentration were recorded. Two equal volumes of sperm solution were use in swim up solution and on Histoprep gradient. We followed the routine swim up procedure by incubation of 800 µl of BO-caff in CO_2 incubator for 30-45 minutes. For Histoprep, we followed the company protocol for isolation of human lymphocytes. After sperm recovery in two procedures, the secondary sperm parameters were recorded. To find the effects of the procedure on sperm parameters, proportion of secondary to primary sperm parameters, for each method, was calculated.
Results: The results showed that proportion of motile sperms were 1.094 ± 0.04 and 1.19 ± 0.06 in Histoprep and swim up respectively ($p>0.05$). The proportion of secondary to primary sperm count for two procedure was similar (0.27 ± 0.05 and 0.25 ± 0.07 for Histoprep and swim up, respectively).
Conclusion: The results showed that Histoprep and swim up have similar effects on motile sperm isolation for ram ESP.

Keywords: Epididymal Sperm, Swim Up, Histoprep, Ram

P-140: Effects of The Method of Motile Epididymal Sperm Isolation on Embryo Production in Ewe

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Background: Motile sperm is essential for successful embryo production. The aim of the present study was to compare the *in vitro* embryo development following

isolation of Ram epididymal sperm isolation by swim up (SP) or Histoprep® continuous gradient (HP).

Materials and Methods: Ram testes-epididymis were provided from the local slaughterhouse and transported to the lab. Sperms were recovered from cauda epididymis and submitted to swim up or HP procedure for motile sperm isolation. Ewe ovaries were collected from sloutherhouse and their oocytes were picked up and transferred to TCM199 supplemneted with 10% FCS, 10% BFF and 3 IU hCG and 1 IU Merional (LH/FSH). After 22 hrs of incubation in 5% CO_2 and 95% RH, submitted to IVF with sperms of both groups (total cultured oocyte numbers for HP and SP were 166 and 148) which were capacitized in BO medium. Embryo development was followed by incubation in TCM199 supplemented with 5 and 10 % of FCS for cleavage and blastocyst formation and embryo development was evaluated at das 3 and 6 post insemination.

Results: The results showed similar efficacy of both procedures for Ewe IVF in cleavage rate (54.6 and 55.6 % for HP and SP respectively). Blastocyst rate also was similar between groups (5.6 and 3 % for HP and SP, respectively). The low rate of blastocyst in this project may return to our embryo culture medium.

Conclusion: HP may have similar efficacy to SP for producing *in vitro* Ewe embryo.

Keywords: Swim up, Histoperp, Epididymal Sperm, Embryoo Development, Ewe

P-141: Comparison Study The Effect of *In Vitro* Maturation and IVF Fertilization of Cryopreserved Oocyte with Fresh Oocyte in Mice

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Background: Cryobiology and *in vitro* maturation of oocytes are very important tools of reproductive technology. Oocyte plays an important role in fertility. The objective of this research is comparison study the effect of invitro maturation and IVF fertilization of cryopreserved oocyte with fresh oocyte in mice.

Materials and Methods: For this purpose, after superovulation was performed oocytes were isolated mechanically from the ovary and evaluated in three groups as follows: group A: fresh oocytes, group B: frozen oocytes. group A, B oocytes were transferred to TCM-199 tissue culture medium for *in vitro* maturation. Then isolated oocytes vitrified with rapid freeze-rapid thaw method. Fresh and freeze-thawed oocytes moved into culture medium and some factors were evaluated such as growth rate, destraction, cumulus expansion, for evalution of fertility, collected oocytes in each group were transferred to culture medium and sperms were added. 48 hours post insemination the number of fertilized oocytes was determined.

Results: For this reason, oocytes were put into the CO_2 incubator for 48 hours. The results demonstrated that growth rate of fresh group was better than frozen groups and oocytes with intact cumulus cells had better growth rate to metaphase II. In this research, oocytes with intact cumulus from high to low number were in groups A, B. Also growth rate was quite consistent with it and showed a significant difference between these groups.

Conclusion: Quality of oocyte isolated from by using this method of freeze-thaw cause low growth rate than fresh of oocytes.

Keywords: Oocyte, Maturation, Vitrification, Mouse, IVF

P-142: Effects of A Synthetic Antioxidant (4-Hydroxy Tempo) Additive to The Semen Extender on the Ejaculated Spermatozoa Characteristics before and after Freezing in Water Buffaloes (Bubalus Bubalis)

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Background: Semen cryopreservation is the most important section of artificial insemination programs; it allows preservation of semen fertility for a long time.

Materials and Methods: The aim of the present study was to detect the effect of *in vitro* supplementation of 4-hydroxy Tempo on fresh and frozen spermatozoa quality of buffalo bulls. Five healthy buffalo bulls (5 ejaculates from each bull) were used. Each ejaculate was diluted at 37°C with tris-based extender containing 0 (control), 0.5, 1, 4, 8 and 12 mM 4-hydroxy Tempo and the sperm motility and viability were evaluated at 0(T0) (immediately after dilution), 60 (T1) and 120 (T2) minutes after diluting semen. In the second step, semen samples were diluted with tris-egg yolk-glycerol extender containing the same amounts of 4-hydroxy Tempo, cooled to 4°C, equilibrated and semen parameters (motility, viability, membrane integrity and DNA fragmentation) were estimated. Then, the semen was packed in 0.5 mL French straws and frozen in liquid nitrogen. Later, the semen was thawed and analyzed for the same parameters, as well as total antioxidant capacity.

Results: Results showed that addition of 0.5 and 1mM 4-hydroxy Tempo to the semen extender significantly increased the sperm motility of fresh and equilibrated semen compared to the control without affecting other parameters. However, in frozen-thawed semen, extenders containing 0.5 and 1mM 4-hydroxy Tempo significantly improved sperm motility, viability, membrane integrity and semen total antioxidant capacity and also resulted in lower DNA fragmented sperms. In this study 4-hydroxy Tempo supplementation of semen extender of 8 and 12 mM had deleterious effects on sperm parameters as early as the samples were prepared for freezing.

Conclusion: This study indicates that Tempo supplementation may help to ameliorate negative effects of semen storage on characteristics of sperm quality; improved sperm motility, viability, plasma membrane integrity and DNA fragmentation, with higher values obtained after adding 0.5 mM 4-hydroxy Tempo to the extender.

Keywords: Buffalo, Semen, Antioxidant, 4-Hydroxy Tempo

P-143: Effects of *In Vitro* Copper Supplementation on The Ejaculated Semen Parameters in Water Buffaloes (Bubalus Bubalis)

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Background: A number of studies have demonstrated membrane lipid peroxidation (LPO) as one of the causes of defective sperm function in liquid semen preserved at 4◦C and cryopreserved semen. Some attempt has been made to preserve sperm parameters, particularly sperm motility, by adding some elements and Materials to the semen before freezing.
Materials and Methods: To investigate effects of copper (Cu) additive to semen extenders on sperm parameters (motility, viability, membrane integrity and DNA fragmentation) after semen dilution and cryopreservation, semen samples of 5 buffalo bulls of 2-4 years old was collected at 5 different occasions during the autumn 2011. A number of 25 samples were used in each examination. Sperm motility and viability were measured before and at 0 (T0), 60 (T1) and 120 (T2) minutes after diluting semen in tris extender containing 0 (control), 0.004, 0.008, 0.016, 0.032 and 0.064 mg/L copper sulphate. A tris-egg yolk-glycerol extender containing the same amounts of copper sulphate was prepared, semen diluted, cooled to 4°C and kept refrigerated for 4 hours to equilibrate, sperm motility, viability, membrane integrity and DNA fragmentation were estimated, then, semen was packed in 0.5 ml French straws and frozen in liquid nitrogen. Later, the frozen semen was thawed in 37 °C water bath for 30 seconds, and the same parameters as well as total anteioxidant capacity (TAC) of the frozen-thawed semen were estimated.
Results: The results showed that copper additive at the rate of 0.032 mg/L gives a better protection of sperms through the process of dilution, equilibration and freeze-thawing than that in control and other Cu concentrations.
Conclusion: Our findings show that copper supplementation of semen extenders seems to be a promising procedure for preserving spermatozoa quality, motility in particular, during cryopreservation processes.
Keywords: Buffalo, Semen Extender, Copper

P-144: Glucose-6-Phosphate Dehydrogenase Activity in Ovine Oocytes in Association with Developmental Characteristics and Expression of Bax Gene

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Background: Recent studies have revealed that oocyte competence can be assessed through the presence of the glucose-6-phosphate dehydrogenase (G6PDH) enzyme, as indicated by brilliant cresyl blue (BCB), a dye that can be degraded by G6PDH. In the present study, we aimed to examine the validity of BCB test to select developmentally competent oocyte in ovine and its association with stage-specific expression profile of an apoptosis regulatory gene (Bax).
Materials and Methods: Ovine cumulus-oocyte complexes (COCs) were exposed to 26 µM BCB diluted in mDPBS for 90 min. After BCB exposure, COCs were divided into 2 groups: BCB+ (blue cytoplasm, low G6PD-activity) and BCB- (colourless cytoplasm, high G6PD-activity). Oocytes in control group were incubated without exposure to BCB dye. After IVM, oocytes were subjected to IVF followed by embryo culture for 9 days. Concerning the transcript level, real-time PCR was used to quantify the expression of Bax mRNAs between Control, BCB+ and BCB- oocytes at different maturational stages: Germinal vesicle (GV) and Metaphase II (MII).
Results: There were significantly more MII oocytes in BCB+ (78.9%) and control (73.8%) groups than in BCB- group (52.4%, $p<0.05$). Both, BCB+ (71.7%) and Control (67.3%) groups showed significantly higher cleavage rates compared to BCB- group (50.5%, $p<0.05$), whereas no difference was found between Control and BCB+ group. Blastocyst rate was also significantly higher for BCB+ (34.5%) group compared to control (20.8%) and BCB- (4.4%, $p<0.05$) groups, with control group being significantly higher than BCB- group. Moreover, the expression of Bax gene at mRNA level showed no significant differences among the groups, neither at GV nor at MII stage.
Conclusion: Using BCB test, we successfully incorporated the G6PDH-activity into selecting a subset of oocytes with superior developmental capacity with no correlation with transcription level of the Bax gene.
Keywords: Ovine, Oocyte, G6PDH-Activity, BCB, Bax Gene

P-145: Maturation Capacity and Viability Assessment of Human Immature Oocytes After Vitrification and *In Vitro* Maturation (IVM)

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Background: 15% of oocytes collected in ART cycles are immature. These oocytes may be matured following IVM program. It is possible to cryopreserve the immature oocytes for further use in ART after application of IVM. The aim was to determine the maturation rate, morphology and viability of human immature oocytes after fresh

IVM and vitrified-IVM program.
Materials and Methods: 63 women who underwent controlled ovarian stimulation for ART were included. 103 immature oocytes were retrieved from these infertile women aged between 18-43 years old. 53 immature oocytes were used for fresh group and 50 immature oocytes for vitrification group. The maturation medium was Ham's F10 supplemented with 0.75 IU FSH, 0.75 IU LH and 40% human follicular fluid (HFF). After 48h, maturation and morphology were assessed in fresh-IVM and vitrified-IVM oocytes. Also, viability was assessed using PI/Hoechst staining.
Results: Oocytes Maturation rate were reduced in vitrification group (56.0%), in comparison with fresh group (88.7%, p<0.001). Oocyte viability rate after staining were reduced in vitrification group (56.0%), in comparison with fresh group (86.8%, p<0.007).
Conclusion: Vitrification reduces both the maturation capacity and viability of human immature oocytes. It is recommended to apply IVM on fresh immature oocytes, instead.
Keywords: IVM, Oocyte, Vitrification, Maturation, Viability

Ethics and Reproductive Health

P-146: The Holy Quran, Genetic Gender Determination and Recognition, and Divine Knowledge and Creativeness

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Background: Determining the genetic gender has been a human's inaccessible will during the history. Today science resolves the problem; pre implantation diagnosis (PGD) is used as a method of genetic disease diagnosis and gender determination. The holy Quran in many verses ascribes genetic gender determination and recognition to Allah, the Almighty. Hence, according to some, genetic gender determination is a divine action and human interfering with this process is in direct contradiction to the presumption of divine creativeness and is interference with divine wisdom. In other hand, some believe that though it originally is due to Allah, its substances and Materials causes have been bestowed to people by Allah. The present survey studies the theoretical problems of this modern technique and its contact with divine knowledge and creativeness. We aim to study and criticize the proposed contradictions between this technology and divine presumptions and affairs.
Materials and Methods: Basic, Desk, Analytical.
Results: 1. Allah's will and decree is prior to all affairs and so all natural and Materials causes solely by the will of Allah could be effective. 2. Knowing divine presumptions of the universe order, the modern technology of genetic gender determination and recognition not only is not interfering with Allah's actions and contrary with divine presumptions but also is a sign of being human the successor of Allah and a sign of being heavens and the earth at his control and disposal, so long as there is neither interfering with divine affairs nor change of divine legal rules. 3. The holy Quranic verses (related to divine creativeness) aim to show the power of Allah the Almighty, not to negate the effective causes of determining and recognizing the genetic gender. 4. Whatever found in traditions encouraging couples to eat some special foods, because of the gender and other characters of the embryo, could connote the human's power in such cases.
Conclusion: 1. Allah's will and decree is prior to all affairs and so all natural and Materials causes solely by the will of Allah could be effective. 2. Knowing divine presumptions of the universe order, the modern technology of genetic gender determination and recognition not only is not interfering with Allah's actions and contrary with divine presumptions but also is a sign of being human the successor of Allah and a sign of being heavens and the earth at his control and disposal, so long as there is neither interfering with divine affairs nor change of divine legal rules. 3. The holy Quranic verses (related to divine creativeness) aim to show the power of Allah the Almighty, not to negate the effective causes of determining and recognizing the genetic gender. 4. Whatever found in traditions encouraging couples to eat some special foods, because of the gender and other characters of the embryo, could connote the human's power in such cases.
Keywords: Genetic Gender Determination, Divine Knowledge, Divine Presumptions, Divine Creativeness

P-147: An Epidemiologic Study of Infertility Factors in Patients Referring to Infertility Centers in Iran

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Background: Infertility is one of the most common health disorders among young people. Several epidemiological reports have been published in different countries to evaluate the status of the infertile population. This study examined the causes of infertility in four main infertility Center of Iran to Identified reasons for Infertility and its distribution in Iran.
Materials and Methods: Data is collected from 4 Infertility Center in Iran over the 5 years. In all cases, infertility has been confirmed definitively by two specialists.

Data were analyzed by SPSS™ 17 software.
Results: Of 2870 couples that referred to these centers during this period, 1851 couples (64%) were diagnosed as a definitive infertile. In this study mean age was 39.55 ± 2.18 and 28.11 ± 3.5 years in males and females respectively. The mean duration of infertility was 2.14 ± 0.85 years. Masculinity reasons were found in 944 cases (51%). primary infertility was seen in 1110 cases (60%). Following the masculinity reasons, Oocyte disorders in 444 cases (24%) and tubular obstruction in 203 (11%) comprised the next reasons. Infertility factors were diagnosed only in 38% of female and 30% of males. In 13% cases combination factors was observed and in 14% infertility reason was unknown.
Conclusion: Since the factors related to males almost makes up more than half of the reasons, sperm analysis seems to be mandatory before any specific treatment. Factors related to the males are more important and common of other factors.
Keywords: Sperm Analysis, Non-Reproductive Epidemiology, Infertility

P-148: The Process of Couples' Decision Making Towards Using Family Planning Methods

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Background: More than 30% of pregnancies in Iran are unintended and most of them happen among the women who use various contraceptive methods. This study was conducted to explore the process of making decision towards using family planning methods in women of reproductive age.
Materials and Methods: In this grounded theory study semi-structured interviews were conducted with 24 participants living in urban society of Mashhad, Iran, 2011 and continued until data saturation. Data were analyzed adopting Strauss and Corbin's mode of analysis through constant comparative method applying levels of open, axial and selective coding using MAXqda software. Study rigor was verified via prolonged engagement, member validation of codes and thick description of the data.
Results: The core category that describes the process of couples' decision making towards using family planning methods was "caring the comprehensive health of my family". The categories were presented into a paradigm model consisted of: 1. forming fertility thoughts, 2. seeking information about the methods, 3. assessing available choices based on appraising self-condition, 4. managing the course of receiving and using methods and 5. realizing the fertility intentions.
Conclusion: It is important that family planning providers to comprehend the motivations, perceptions and knowledge of women about contraceptive methods in their contextual situation, which illustrate their mode of interaction in the arenas of family planning decision making.
Keywords: Decision Making, Family Planning, Couple

P-149: Relying on A Higher Being: A Grounded Theory of Experiencing Infertility in A Religious and Spiritual Context

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Background: Infertility is an acute life crisis which may last for an indeterminate length of time. In a holistic approach to infertile women's care, which is believed to assist patients cope better with their experiences, all aspects of holistic care including religious and spiritual needs of individuals should be considered. A literature review revealed that religious and spiritual dimensions of infertility have received very little attention. This study examined how women experienced infertility in its religious and spiritual context and how this perspective affected the strategies infertile women adopted to handle infertility.
Materials and Methods: Using a grounded theory approach 30 infertile women affiliated to different denominations of Christianity (Protestantism, Catholicism, Orthodoxy) and Islam (Shiite and Sunni) were interviewed. Seven infertile women had no formal religion. Data were collected through semi structured in-depth interviews in one Iranian and two UK fertility clinics and analyzed using Strauss and Corbin's mode of grounded theory analysis.
Results: The substantive theory of "relying on a higher being" was generated from data which encompassed four sequential stages: encountering the problem, challenging acceptance, struggling for a resolution and coming to terms. The majority of participants experienced a feeling of spiritual strengthening or spiritual awakening as they came to terms with their fertility problem in their journey. Although infertile women mostly worked through this process in a coordinated fashion, variations in the process happened because of different orientations towards religion.
Conclusion: We argue that such a theory contributes to a greater understanding of the experience of infertility in a religious and spiritual context. It represents an initial attempt to conceptualize this experience and further studies are needed to validate this initial substantive theory, both in order to better understand religious and spiritual dimensions of infertility and to formalize this theory by testing it in other relevant substantive areas.
Keywords: Infertility, Religion, Spirituality, Grounded Theory

P-150: Ethicals Challenges in Infertile Patients

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Background: Medical ethics is an interdisciplinary sci-

ence that must be resolved the ethical issues with modern developments.
Materials and Methods: A total of 980 infertile women that select with simple and available method from doctor Rasekh clinic and about 51 (5.2%) of them have ethical problems regarding infertility. We review 5 Cases in this study.
Case 1: The patient is a 18 year old housewife with 3 years primary infertility but man avoids testing spermogram and saying to her wife that I am healthy, And you are sick and infertile.
Case 2: A 36 year old woman with 20 year primary infertility and her husband have history of pertosis in child hood .and now he is azospermic. After testicular biopsy he tears up all the document.
Case 3: 30 year old woman and a 38 year old man with infertility but the man is addict with decreased sexual desire and libido, and her mother in law would rebuke.
Case 4: 20 year old female who is married two months ago, and her Mother-in-law said that if you do not be pregnant till 2 month later, you should be divorce.
Case 5: A 29 year old housewife who had an abortion 3 years ago. Her husband is a farmer and drug addicted 42 years old man .and he said that the cause of infertility is related to her wife and should take divorce. Her wife has suicidal attempt for several time.
Results: In this cases what is the doctor's moral duty? 2-Gynecologist should talk to these couples and their family with which of aspects (social or medical)?
Conclusion: All of the ethical issues that been created by this treatment must be resolved by the medical ethics. Cultural promotion in infertile couple and their families Through TV and radio in our country.
Keywords: Ethic, Challenges, Infertility

P-151: Violence Against Women and Children: Causes, Challenges and Prevention

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Background: Violence against women is a problem of public health on a global scale. Two decades of international research organizations including the World Health Organization show the problem of violence against women is more serious and more common than was previously considered. These studies aimed to investigate, the most common causes of violence against women and prevention strategy from it.
Materials and Methods: This article is an overview of the use of reference books and more than 18 articles through the review of computer: Yahoo: Iran medex: magiran; Google; Pub Med; Sid databases using key words; Violence, women, children: causes, challenges and prevention has been set.
Results: Cultural and ethnic factors, economic and cultural poverty, unemployment or addiction in parents, remarried parents, including the causes of violence against children. Women may be several causes of violence which varies according to culture. The most important of them are talking to a man who has no relation with him, Consensual sexual intercourse with any marriage, victim of sexual assault, refusing to marry the man of the desired family, disrespect to the husband, or divorce.
Conclusion: Given the extent of violence against children and women and the role of multiple factors and multiple causality, it is recommended established intersectional collaboration for the promotion of education and public information in the field of reproductive health issues to reduce violence against children and women.
Keywords: Violence, Women, Children, Causes, Challenges and Prevention Has Been Set

P-152: Satisfaction and Preferences of Cesarean Delivery in Nulliparous Women in Babol 2010: A Postpartum Survey

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Background: Despite international policy documents calling for reduction in medical interventions in birth. Cesarean Section rates throughout the world, so as in Iran, have continuously rising. Many publications have examined the reasons behind the worldwide rising cesarean delivery rate. The purpose of this study was to investigate pregnant women's intentions for opting for CS and postpartum satisfaction.
Materials and Methods: This cross-sectional study was conducted at six medical centers (4 public-2 private) in Babol over a 1 year period including rural, semi-rural and urban women after the childbirth. Randomly 180 women were eligible to include this survey. Semi structured questionnaires were completed just a day after giving birth by CS. The questionnaire was developed on the basis of a review of published reports, a review of existing questionnaires, and consulting with obstetricians, midwife and epidemiologist. Data gathered and analyzed by SPSS18 software and Chi-square test.
Results: The mean age of the individuals was 25 ± 4.92. 59.7% were occupied in urban area. 53 (29%) subjects had chosen CS by their own demand and the others (71%) which most of them preferred vaginal delivery had to be performed CS because of specific medical indications. In the first group, the main reasons mentioned by mothers for choosing CS were respectively: fear of pain (62%), fear of fetal damage during vaginal delivery (37%) and relatives' suggestions (35%). On the other hand, in obligative group, the main indications by the physician's diagnosis were prolonged labor (27%), fetal endangered (24%) and cephalopelvic disproportion (16%). In selective group 92.5% were satisfied after labor which in the other group 62% of mothers express some degrees of satisfaction ($p<0.001$, OR=9.30).
Conclusion: As many studies revealed, if a woman chooses cesarean delivery in her first delivery, she is more likely to have subsequent deliveries by cesarean. Then specific and scientific based supportive care during first labor and delivery, and preparation or education on issues surrounding cesarean section, could play a pivotal role in making the true decision and improves women's satisfaction with birth.
Keywords: Cesarean Section, Satisfaction, Nulliparous, Babol

Female Infertility

P-153: Effect of Consulting on The Sexual Satisfaction of The Interfile Females

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Background: Infertility problem has become to a social concern and many couples experience it during their reproductive years. This problem has substantial negative impacts on their life and especially on their sexual relationships. The purpose of this study was to determine the impact of counseling on sexual satisfaction of infertile women referred to infertility clinics in Tabriz.
Materials and Methods: This study was an empirical one that was performed by using of a control group. 120 females who had the inclusion criteria were selected by convenience sampling and then put in two consulting and control groups by random allocation. The study was conducted in two stages of pre-test and four months follow-up. In the counseling group, intervention was held for each individual separately during the two 45 minutes session with two weeks interval. Data collecting tool include a personal profile questionnaire and sexual satisfaction questionnaire. The collected data was analyzed by using the SPSS-VER15 software and descriptive and deductive statistical methods.
Results: The study results showed that in the pre-intervention stage there wasn't any significant statistical difference in the rate of sexual satisfaction of consulting and control groups (p=0.2), while after the four month of intervention there was a significant difference in the sexual satisfaction rate of consultation and control group (p<0.001). Similarly, there was a significant difference in the sexual satisfaction of consulting and control groups before and 4 months after the intervention (p=0.001).
Conclusion: Consulting has a significant effect on the sexual satisfaction rate of infertile females.
Keywords: Infertility, Consulting, Sexual Satisfaction

P-154: Comparison of Pregnancy Outcome between Low Dose HCG in Late Follicular Phase in GnRH Long Protocol and Standard Long Protocol in ART Cycles

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Background: A safe, simple and cost-effective protocol is important goal in ART cycles. The aim of this prospective study was whether administration of low dose HCG in late follicular phase can be used clinically to replace gonadotropins administration in GnRH long protocol.
Materials and Methods: 69 patients who were candidate for assisted reproductive technology (ART) enrolled the study and randomly divided into two groups. The control group (n=38) received standard long protocol and gonadotropins administration were continued until the day of HCG injection (10000 IU) for final follicular maturation. The study group (n=31) received GnRH long protocol and when at least ≥ 6 follicles with mean diameter ≥ 13 mm were observed in both ovaries, gonadotropins administration were discontinued and low dose HCG 200 IU daily was started and was continued until the day of HCG injection (10000 IU) for final follicular maturation.
Results: There were no significant differences in age, basal FSH, infertility duration and infertility etiology in two groups. There were no statistical differences between two groups regarding to chemical pregnancy, clinical pregnancy, ongoing pregnancy, abortion, and multiple pregnancy rates per transfer between two groups (48%, 36%, 36%, 8%, and 4% in study group vs. 54.3%, 25.7%, 25.7%, 17.1% and 5.7% in control group, respectively). Mean doses of used gonadotropins were significantly higher in control group than that in study group and were 2524 ± 893 IU in control group and 1439 ± 433 IU in study group (p=0.000).
Conclusion: According to our data, we recommended use of low dose HCG in GnRH long protocol because of lower doses of used gonadotropins and its more cost-effectiveness.
Keywords: Pregnancy Outcome, Low Dose HCG, Late Follicular Phase, GnRH Long Protocol, ART Cycles

P-155: Outcome of Assisted Reproductive Technology in Women Aged 40 Years and Older

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Background: Human fertility has been declined all over the world. Advanced women's age is one of the most important factors in determining the success of reproduction and ageing has negative impact on ART outcome and advanced female age decreases the chance of live birth rates achieved using ART, especially after 40 years of age. The purpose of this study was to evaluate ART outcomes regarding to pregnancy, abortion, cycle cancellation and live birth rates in women 40 years and older.
Materials and Methods: A retrospective study was performed on three hundred-thirteen women undergoing ART cycles in the Madar Hospital in Yazd. Women with age ≥ 40 years who indicated for ART enrolled the study regardless of the infertility type or etiology. In this study, we used data from IVF or ICSI cycles using fresh embryo transfer. Follow up was performed in regard to pregnancy, abortion, cycle cancellation and live birth rates.
Results: The mean age of women was 41.87 ± 1.97

years. Chemical pregnancy rate was 8.6% (27/313) per cycle. Clinical pregnancy rate was 3.8% (12/313) per cycle. Spontaneous abortion was observed in 63% (17/27) of patients with positive pregnancy test. The overall cancellation rate was 23.3% per oocytes retrieval. The overall live birth rate per cycle for all women who initiated an ART cycle at age ≥40 years was 3.2% (10/313) that eight of those women were under 42 years old.

Conclusion: Based on our results, we suggest that women with age 42 years and above should be advised to use other options, including oocyte donation or adoption.

Keywords: Advanced Age, Assisted Reproductive Technology, Pregnancy Rate, Live Birth

P-156: Adolescent Pregnancy

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Background: It is estimated that 15 million births occur every year to adolescents. This represents about 11%of all births each year. The global average rate of births per 1000 females aged 15-19 years is 65. A range of social, cultural, biological and service delivery factors contribute to the high levels of adolescent pregnancy and childbirth. This study was conducted to determine the causes of adolescent pregnancy.

Materials and Methods: By searching more than 35 related articles in WHO, Google, Science direct and Pubmed, reference books and by using key words of pregnancy, Adolescens, factors contributing this study had been done.

Results: The study showed that pregnant teenagers are involved in causes that include: 1. Declining age of menarche, 2. longer periods of education and delayed marriage for some course and delayed marriage adolescents, 3. forced early marriage and pregnancy, 4. initiation of sexual activity during Adolescence, 5. sexual coercion and rape, 6. education level, 7. socio-economic factors, 8. other risk behaviors, 9. lack of knowledge and 10. lack of access to service centers are.

Conclusion: Considering that approximately 21% of Iran's population (15 million) are young and High risk behavior such as early and unprotected sexual activity, alcohol use, smoking and drug addiction among teenagers is rising, a quick Planning and precise and serious policy in this area seems necessary.

Keywords: Causes, Rrelated Factors, Pregnancy, Adolescent

P-157: TLR5 Gene Expression in Endometrium of Women with Unexplained Recurrent Spontaneous Abortion

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Background: Recurrent spontaneous abortion (RSA) is usually defined as three or more consecutive pregnancy losses before 20th week of gestation. Although different factors are considered as etiology of RSA but in some cases, despite of extensive work up, the cause of RSA remains unknown which called unexplained RSA. Immunological factors are suggested as etiological factors of unexplained RSA. On the other hand, pattern recognition receptors (PRRs) are one of the most imperative components of innate immunity which can recognize ligands derived from various pathogens. Toll like receptors (TLRs) as a main group of PRRs consist of at least 10 functional proteins in human. After ligand recognition, TLRs activate intracellular signaling cascade which induce inflammatory and / or anti-viral responses. TLR5 is one of the cell surface TLRs which recognizes bacterial flagellin. In present study, the expression of TLR5 gene was tested in endometrium of women suffering from unexplained RSA.

Materials and Methods: Endometrial samples were obtained between day 19th and 24th of menstrual cycle (window of implantation) from 10 women with unexplained RSA and 6 fertile women who had at least one successful pregnancy (control group). TLR5 gene expression was studied by RT-PCR and then quantified by real time PCR. Beta actin was used as housekeeping gene.

Results: TLR5 gene expression was detected in endometrium of patients with unexplained RSA and normal women. The mean relative expression of TLR5 gene was higher in endometrium of women with unexplained RSA in compare to normal ones at significant level.

Conclusion: This finding suggests that TLR5 might play important role in the pathogenesis of unexplained RSA since TLR5 signaling could result in inflammatory cytokine production. It has been also proposed that increased inflammatory microenvironment of endometrium may lead to implantation failure.

Keywords: Gene Expression, Innate Immunity, Recurrent Spontaneous Abortion, Toll Like Receptors

P-158: Metformin versus Chromium Picolinate in Clomiphene Citrate-Resistant Patients with Polycystic Ovary

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Background: To compare the chromium picolinate with metformin in Clomiphene citrate resistant PCOS patients.

Materials and Methods: This was a randomized clinical trial being performed at university affiliated clinics including 92 women with Clomiphene citrate-resistant PCOS who were randomly assigned to receive chromium pi-

colinate (200 µg daily) or metformin (1500 mg daily) for 3 months. Anthropometric and hormonal profile were measured and compared before and after treatment. We also measured ovulation and pregnancy rate between two study groups.

Results: Chromium picolinate decreased FBS significantly after 3 months of treatment (p=0.042). In the same way, the serum levels of fasting insulin decreased significantly leading to increased insulin sensitivity as measured by QUICKI index (p=0.014). Patients who received metformin had significantly lower levels of testosterone (p<0.001) and free testosterone (p<0.001) after 3 months of treatment compared to those who received chromium picolinate. Overall patients who received metformin experienced more side effects compared to chromium picolinate (23.9% vs. 56.2%; p<0.001). There wasn't any significant difference between two study groups regarding the ovulation (p=0.417) and pregnancy rates (p=0.500).

Conclusion: Chromium picolinate decreases the FBS and insulin levels and thus increasing insulin sensitivity in Clomiphene citrate-resistance PCOS women. These effects were comparable with metformin however metformin treatment was associated with decreased hyperandrogenism. Chromium picolinate is better tolerated than metformin. However ovulation and pregnancy rate didn't differ significantly between groups.

Keywords: Metformin, Chromium Picolinate, Polycystic Ovary

P-159: Immunological Aspect of Ectopic Pregnancy with Emphasis on TLR2, 3, 4 and 5 Genes

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Background: Toll-like receptors (TLRs) play a crucial role in early host defense against invading pathogens. Recognition of some bacterial pathogen associated molecular patterns (PAMPs) is mediated by TLR2, 4 and 5 while TLR3 distinguishes double stranded RNA. Interactions between the immune system and female reproductive system have important consequences for fertility and reproductive health. Infection of the fallopian tube (FT) can result in adverse reproductive outcomes including ectopic oregnancy (EP). EP is an abnormal pregnancy that occurs outside the uterus, most often in the FT. Little has been done regarding TLRs expression in abnormal pregnancy. Therefore our objective was to clarify TLRs expression in FTs of women carrying EP.

Materials and Methods: Our samples were divided into two groups: Case and control groups. Case group was women underwent salpingectomy for EP. Control group was women with healthy tube underwent hysterectomy. For control group, women were injected with human chorionic gonadotropin (hCG) in 14 days leading up to hysterectomy to produce state of pseudo-pregnancy. Then biopsies from Infundibulum, Ampulla and Isthmus of FT were obtained from both groups. In this investigation TLR2, 3, 4, 5 genes expression was survey with RT-PCR. Also Q-PCR was used to compare quantitative expression of TLR2, 3, 4, 5 genes between two groups.

Results: TLR2, 3, 4, 5 genes expression have been demonstrated in all regions of the FTs in both groups. Lower expressions of TLR2, 3, 4, 5 genes were detected in all parts of FTs of case in compare to control groups.

Conclusion: Reduction in TLR2, 3, 4, 5 genes expression in fallopian tube could result in increasing of infection which predispose to EP.

Keywords: Ectopic Pregnancy, Fallopian Tube, Innate Immunity, TLR2, 3, 4, 5

P-160: A Comparative Study of Luteal Estradiol Pre-Treatment in GnRH Antagonist Protocols AndIn Micro Dose Flare Protocols for Poor Responding Patients

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Background: This study aims to verify if luteal estradiol pre-treatment improves IVF/ICSI outcomes in a GnRH antagonist protocol as compared to a micro dose GnRH agonist protocol in poor-responding patients.

Materials and Methods: A total of 116 IVF/ICSI cycles were included in this prospective randomized clinical trial. The selected women were randomly assigned to receive an estradiol pre-treatment in a GnRH antagonist protocol (daily oral Estradiol Valerate 4 mg preceding the IVF cycle from the 21st day until the first day of the next cycle) or in a micro dose GnRH agonist protocol.

Results: The patients in the luteal estradiol protocol required more days of stimulation (10.9 ± 1.6 vs. 10.2 ± 1.8) and a greater gonadotropin requirement (3247.8 ± 634.6 vs. 2994.8 ± 611 IU) ,yet similar numbers of oocytes were retrieved and fertilized. There was no significant difference between the two groups in terms of the implantation rates (9.8% vs. 7.9%), and the clinical pregnancy rates per transfer (16.3% vs. 15.6%).

Conclusion: This study demonstrates that the use of estradiol during a preceding luteal phase in a GnRH antagonist protocol can provide similar IVF outcomes when compared to a micro dose GnRH agonist protocol.

Keywords: Poor Responders, IVF Outcome, Luteal Phase, Estradiol, Micro Dose Protocol, GnRH Antagonist

P-161: Independent to Glucose and E2 the PCOS Women Show Significantly Higher Hemocysteine Level versus non PCOS

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Background: Hyperhomocysteinemia is a recognized risk factor for atherosclerosis. Elevated homocysteine (Hcy) results in severe oxidative stress, platelets hyper activity, and impairs arterial circulation and smooth muscle proliferation in vascular wall. This study was carried out to evaluate follicular fluid (FF) Hcy, glucose and estradiol levels in IVF candidate PCOS women.
Materials and Methods: 52 infertile patients from Dr-Tizro Day Care and IVF Center divided in to two groups including; 26 PCOS and 26 non PCOS women. Long protocol performed for all patients. FF Hcy, glucose and estradiol (E2) levels were analyzed at the time of oocyte retrieval. Automated Chemiluminescence technique (ELECYS2010HITACHI, Roche Diag. Germany, Diasorin kit), enzyme conversion immunoassay kit (Axis-Shield, Dundee, UK) and enzymatic reaction techniques (Pars Azmoon kit) were used in order to evaluate E2, Hcy and glucose respectively.
Results: In PCOS patients, the FF Hcy level was manifested significantly higher in comparison to controls ($p<0.001$). Observations demonstrated that in PCOS group the Hcy level increased independent to E2, Glucose levels, BMI and age, while PCOS group showed significantly higher BMI in comparison to non-PCOS patients ($p<0.03$). The pregnancy rate in non PCOS women (50%) remarkably was higher versus PCOS group (33%). No significant differences were revealed between two groups for FF glucose and estradiol levels.
Conclusion: Present data showed that although FF glucose, E2 levels were constant in PCOS and non PCOS patients, the FF Hcy levels in PCOS significantly increased.
Keywords: Homocysteine, Estradiol, PCOS, Follicular Fluid, Glucose

P-162: Body Mass Index and Polycystic Ovary Syndrome Women

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Background: Polycystic ovarian syndrome (PCOS) is one of the most common endocrinology problems of infertile women. Since lifestyle modification and weight loss is the most effective treatment and associated with failure in many cases, this study evaluated the factors impact on weight loss of PCOS women.
Materials and Methods: This cross- sectional study was conducted on 150 infertile women of the PCOS clinic of Fatemeh-Zahra infertility research center in 2010- 2011. The questionnaire contains demographic information, clinical and laboratory findings and also ultrasonography of all patients, and then anthropometric measurements were taken. After completing the data, diet for weight loss and exercise was recommended and all patients received metformin. The samples were examined for 4 months monthly.
Results: 150 women in age18-35 entered the study. 91.5% of women were overweight and obese80.22 ± 13.37 Kg. The majority of them were housewives 90.2andhad under high school education 83.5% and 54.9% of them lived in urban areas. The mean weight loss was 5.32 ± 4.89 up to maximum 17 kg, which related directly within creasing women age. Mean weight loss in people over 25 years was7.36 kg between fewer than 25, 4.5 kg is. Also in those households with higher education level, the mean weight has increased, so women with post high school education showed mean weight loss 7.7 kg and who were under high school had weight loss 5.4 kg. Finding showed that no significant relationship between the duration of infertility and place of life.
Conclusion: The results revealed weight loss in women with PCOS has a direct relationship with level of education and women age, while place of life and duration of infertility is not correlated in the process of weight loss in these patients.
Keywords: PCOS, Weight Loss, Metformin, Education Level, Age

P-163: Effects of Bilateral Tubal Ligation on Serum Oxidative Stress and Ovarian Function in Female Rats

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Background: Tubal ligation is one of the most common contraceptive methods in the world. Some researchers believe that tubal ligation has complications. It is assumed oxidative stress can produce these complications. The aim of this study was to evaluate oxidative stress and ovarian function after tubal ligation.
Materials and Methods: In this study, female rats (weighting 200-250 g) were divided into 2 groups (tubal ligation and Sham). Serum (Prooxidant Antioxidant Balance), MDA (Malondialdehyde), estradiol and progesterone were measured on days 15 and 45 and 3 months and 6 months after the intervention in diestrous phase.
Results: After tubal ligation, serum MDA increased however serum PAB increased after 15 days, 45 days, 3 months and then decreased after 6 months ($p<0.05$). Serum concentration of estradiol decreased remarkably,

after 15 and 45 days ($p<0.05$). Serum concentration of progesterone decreased remarkably, after 15 days, 45 days, 3 months and 6 months in tubal ligation group compared to the sham group ($p<0.05$).

Conclusion: Tubal ligation increased oxidative stress level and decreased estradiol and Progesterone of serum. Complications of tubal ligation may be due to increased oxidants and decreased estrogen and progesterone. As for the tubal ligation probably reduced serum estrogen levels, as a potent antioxidant, is involved in imbalance. By prescribing antioxidant, effects of tubal ligation can be reduced.

Keywords: Tubal ligation, Ovarian Function, Prooxidant-Antioxidant Balance, Malondialdhyde, Rat

P-164: Human Leukocyte Antigen Class Ib and Pregnancy Success

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Background: During pregnancy, the maternal immune system is in close contact with cells and tissue from the semiallogenic fetus .The Human Leucocyte Antigen (HLA) class Ib molecules, HLA-E, -F and -G, are expressed at the materno-fetal interface. Because of the apparent immunoregulatory functions of these proteins, they may be involved in successful acceptance of the semi-allogenic fetus during pregnancy.In this presentation we have reviewed studies regarding role of hla class Ib in pregnancy success and gathered their results in order to gain a better understanding of the relationship of these molecule and pregnancy success.

Materials and Methods: We have conducted a thorough literature search using search engines in the medical databases and collected the data and research results regarding these molecules and pregnancy.We focused on polymorphisms of the three genes, expression patterns of the proteins, and interactions with immune cells and the have been evaluated to elucidate whether HLA-E, -F and -G are involved in the pathogenesis recurrent miscarriages.

Results: Non-classical antigens such as Human leukocyte Antigen class Ib may interact with receptors of NK cells ,Macrophage, CD8+ T cell, CD4+ T cell and monocyte such as Killer Inhibitory Receptor,Leukocyte Ig Like Receptor which results into limit activity of these immune cells and downregulation of immune response and helps in the maintenance of pregnancy and any changes in pattern of expression of these HLA molecules because of polymorphisms or other reasons may cause misscarriage.

Conclusion: The HLA class Ib molecules seem to induce suppression of the maternal immune system. Evidences show changes in expression pattern and function of these molecules are associated with miscarriage . To clarify the functions of HLA-E, -F and -G, future studies need to link investigations of the polymorphisms in these genes to measurements of protein levels, and examine the role of these proteins in the complex interplay of immune cells and cytokines at the materno-fetal interface.

Keywords: HLA classIb, Polymorphisms, Miscarriage, NK Cells

P-165: Comparison of Urinary and Recombinant Human Chorionic Gonadotropin during Ovulation Induction in Intrauterine Insemination Cycles

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Background: Urinary human chorionic gonadotropin is the most widely used medicine for ovulation in ovarian stimulation cycles. Recently however, recombinant HCG has also become commercially available. This study presents compare the use of recombinant hCG with urinary hCG during controlled ovarian hyperstimulation and intra uterine insemination (COH-IUI) cycles

Materials and Methods: This is study is a randomly-controlled clinical trial that includes women aged between 20 and 44 years who were admitted to the infertility ward of Zanan Hospital, Tehran. The subjects were undergoing COH-IUI during the period of years 2010 to 2011. Ovulation induction was started on patients who had criteria for COH-IUI ,when there was at least one dominant follicle (≥ 15), single IUI was performed after 36 hours of hCG injection (either recombinant or urinary). Rate of ovulation and pregnancy were compared in two groups.

Results: In 537 cycles of COH-IUI that were analyzed, pregnancy occurred in 147 cases (27%). In urinary hCG group, 92 out of 333 cases became pregnant (27%) while in the recombinant hCG group 56 out of 204 cases became pregnant (27.5%)

Conclusion: Findings of this study indicate that the pregnancy and ovulation rate in two groups are not different.

Keywords: IO-IUI, rhCG, uhCG

P-166: Assisted Reproductive Techniques Outcomes in Hypogonadotropic Hypogonadism Women

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Background: To evaluate the outcomes of using *in vitro* fertilization (IVF)/ intracytoplasmic sperm injection (ICSI cycle) techniques in hypogonadotropic hypogonadism women and comparing them to women with tubal factor infertility.

Materials and Methods: Data from 81 hypogonado-

tropic hypogonadism (HH) patients treated with IVF/ICSI in the period from early 2009 until the end of 2010 were analyzed and compared with treatment results from 89 patients with Tubal factor infertility. Moreover, data from hypogonadotropic hypogonadism patients were analyzed with respect to the age factor. P<0.05 was considered statistically significant.

Results: Despite a higher fertilization rate and higher number of grade A/B embryos transferred in the Tubal factor group, the implantation, pregnancy and live birth rates were found to be similar between the two groups (p=0.3, p=0.1, p=0.6, respectively). When HH patients were evaluated according to the age factor, no significant difference was found regarding outcome parameters (p=0.2).

Conclusion: HH women that were treated with IVF/ICSI cycles were found to have a good chance for pregnancy, even in coexistence with age factor.

Keywords: Hypogonadotropic Hypogonadism, Assisted Reproductive Techniques Outcome, Advanced Age

P-167: Association between Pregnancy Dietary Iron Intake and Gestational Diabetes Mellitus

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Background: Gestational diabetes mellitus (GDM) is one of the most common pregnancy complications affecting approximately 7% of all pregnancies and up to 14% of pregnancies in high-risk populations. Although, dietary iron intake is one of preventive factors for Iron deficiency which leads to less preterm delivery, prematurity, and SGA but iron is a strong pro-oxidant and high body iron levels can damage pancreatic β-cell function and impair glucose metabolism. The aim of this study is to investigate the association between Pregnancy Dietary Iron Intake and Gestational Diabetes Mellitus.

Materials and Methods: To gain insight into this potential association, we performed a review of various literatures which focused on this association.

Results: a study among13, 475 pregnant women Dietary iron intake was positivelyand significantly associated with GDM risk. After adjusting for age, BMI and other risk factors, RRs (95% CIs) across increasing quintiles of iron were 1.0 (reference), 1.11 (0.87-1.43), 1.31 (1.03-1.68), 1.51 (1.17-1.93), and 1.58 (1.21-2.08), respectively (Pfor linear trend 0.0001). Consistent findings were reported in a study which showed iron intake was positivelyand significantly associated with GDM risk (Ptrend=0.04)After adjusting for confounders, women reporting the highest iron intake levels (1.52 vs. <0.48 mg per day) experienced a 3.31 - fold-increased GDM risk (95% CI 1.02-10.72). Also, a prospective study of 1,456 healthy pregnant women reported that maternal elevated serum iron concentrations were associated with a twofold increased GDM risk. However, some previous studies focusing on supplementary iron intakes during pregnancy and GDM risk have produced no significant association between iron supplementation and GDM risk. Similar findings were reported in a study done on 1164 pregnant women which demonstrated no significant difference in the incidence of GDM in the iron supplement and placebo groups at 28 weeks (OR:1.04,95% confidence interval (CI): 0.7-1.53 at 90% power) or 36 weeks.

Conclusion: According to adverse effects of GDM on mothers and infants health, it seems that prevention programs targeting pregnant women with GDM and further investigations on the effect of iron intake could be effective.

Keywords: Pregnancy, Iron Intake, Gestational Diabetes Mellitus

P-168: Evaluating The Raelationship between Infertility and Nutrition

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Background: Infertility is an emotional crisis with physical economic and psychosocial challenge because it interferes with all personal life aspects. Infertility can be due to many reasons Many hypotheses have been proposed to explain this including issues of genetics, physiology, nutrition and management, Consuming vegetable protein (soy) instead of carbohydrates or animal protein is associated wit substantially lower risk of ovulatory infertility.

Materials and Methods: These paper reviews Evaluating the raelationship between infertility and nutrition , using 19 articles published from 2005 t0 2011 in PubMed. gov, SID, Science Direct, Medline, Proquest.

Results: Fertility can be affected by many variables such as hormonal levels, body fat,activity, and stress.. Nutrition can make a critical difference whether you are overweight or under weight, or at normal weight in balancing the endocrine system There was an inverse association between frequency of multivitamin and folic acid use and ovulatory infertility. Selenium antioxidant mineral is vital for healthy sperm formation, particularly motility. Vitamin C is a most important role in male fertility is the prevention of agglutination. Zinc deficiency decreases both testosterone and sperm counts. Alcohol consumption can affect male semen quality, and higher consumption may lead to serious problem with sperm morphology, leading to an increase in malformed sperm. Caffeine may be associated with chromosome damage and the effect on sperm appears.

Conclusion: Nutrition can help Regulate menstrual cycle Decrease stress and anxiety associated with infertility Normalize hormone and endocrine systems. Improve blood flow in the uterus, Decrease chance of miscarriage, Increase the chance of pregnancy for women undergoing *in vitro* fertilization (IVF).

Keywords: Infertility, Nutrition

P-169: Melatonin and Fertility

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Background: Melatonin is a hormone secreted by the pineal gland in the brain. It helps regulate other hormones and maintains the body's daily rhythm, telling us when to fall asleep and when to wake up. Ever since we developed electricity and the lightbulb, we have been decreasing our body's secretions of melatonin by being exposed to bright light after it is dark. Jet lag, shift work and poor vision also affect regular melatonin levels. In Chinese Medicine, melatonin could be likened to the functions of healthy Liver energy. Melatonin also helps control the timing and release of female reproductive hormones. It helps determine when a woman starts to menstruate, the frequency and duration of menstrual cycles, and when a woman stops menstruating (menopause).
Materials and Methods: This is review article.
Results: Melatonin intake also improved pregnancy rates, which were 19.6 percent among women taking supplements compared with 10.2% for the women not taking any. Melatonin has strong antioxidant effects. Preliminary evidence suggests that it may help strengthen the immune system. This could have an important effect on male fertility. In an *in vitro* study involving semen samples taken from 52 men receiving counseling for infertility, semen samples were incubated for 30 minutes with or without 1mm melatonin. Positive correlations were found between melatonin concentrations and sperm concentration, motility and morphology. Additionally, samples incubated with melatonin showed improved percentage of motile and progressively motile cells, and decreased static cells.
Conclusion: High endogenous melatonin concentrations enhance sperm quality and short-term in vitro exposure to melatonin improves aspects of sperm motility.
Keywords: Menlation, Antioxidant, Fertility

P-170: Zinc and Copper Serum Level Alterations after An Intra Uterine Device Insertion

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Background: Intra uterine devices (IUDs) are among the most commonly used contraception methods around the world. The most common form of IUDs used in Iran, is IUD Copper T-380A. As recently there is great emphasis on the role of blood zinc levels on general health conditions, this study was performed to assess the effects of IUD T-380-A insertion on zinc and also copper serum levels.
Materials and Methods: This was a prospective cohort study performed on 66 women clients attending Health Centers of Tehran. After giving adequate explanations about the study and obtaining the clients' written consent, a blood sample was obtained before and 3 months after the insertion of a Copper T-380-A IUD, and serum levels of copper and zinc were measured by an auto analyzer Instrument in a single laboratory. None of the participants were anemic, and so none received ferrous-sulfate complements during the study. The statistical software and methods used were: SPSS version 16 and paired sample t tests. $P<0.05$ were considered significant.
Results: There were significant elevations in both copper (166.70 ± 32.31 vs. 153.74 ± 39.29 µg/dl) and zinc (105.47 ± 16.13 vs. 90.76 ± 16.22 µg/dl) mean serum levels, 3 months after IUD insertion.
Conclusion: In this study, there was a slight increase in copper serum levels- but not reaching toxic levels- after IUD insertion, which was expectable, as the IUDs contained copper. As zinc has an inverse relationship with copper in the body, the excess of copper can be a concern. In this study this concern was removed, due to not the decrease, but the remarkable increase of serum zinc levels after IUD insertion. This finding needs further investigations in larger studies with different study settings, to evaluate the exact health risks or benefits of IUDs.
Keywords: T-380-A IUD, Copper, Zinc

P-171: Pregnancy Rate after Laparoscopy in Infertile Women

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Background: Infertility not only as a disease but also as a serious problem affects the family and harms its health. Approximately 50 to 80 million people in the world suffer from infertility. Laparoscopy is a golden standard which helps diagnose peritoneal and the fallopian tubal problems and treat them.
Materials and Methods: In this cross-sectional study, 162 infertile women referring to the Honari Clinic of Jahrom, Iran from 2008 to 2010 were enrolled. Laparoscopy was applied for these women due to other unsuccessful treatments. Information was collected from medical documents and analyzed by SPSS 11.5.
Results: With the mean age of 27.2 ± 5.31years, 95.7% of the cases had their first marriage, 80.2% had primary infertility, and 93.8% were housewives. The result of hysterosalpingography revealed normal fallopian tubes in 126 cases (77.8%). Of all the cases 18.5% had had unsuccessful intra-uterine injection of sperm. According to the laparoscopic findings and evaluation of ovarian problems, the causes of infertility included 66% ovarian cysts, 4.3% adhesions, 9.9% endometrioses. Also, the evaluation of the fallopian tubes showed 77.8% open tubes and 22.2% closed ones. The rate of pregnancy after laparoscopic surgery was 54.9%, most of which (84.6%) had been after cauterization of polycystic ovaries. All of these pregnancies had been spontaneous, of which 83.15% had led to termination and 16.85% had ended in abortion. None of the cases had had ectopic pregnancy or any other problem.
Conclusion: The most common cause of women's infertility in Jahrom is polycystic ovaries, which in most cases is fortunately treated after laparoscopy. Hence cauterization of ovaries by laparoscopy can be an effec-

tive treatment for infertility.

Keywords: Infertility, Polycystic Ovary, Laparoscopy, Pregnancy

P-172: Intracellular Toll Like Receptors Expression in Endometriosis

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Background: Endometriosis is a complex disease that profoundly affects the quality of life in many women. This disease affects roughly one in ten women of reproductive age. Endometriosis induces a variable amount of inflammatory reaction in pelvic environment. An active immune system needs to recognize these inflammatory agents. Rapid innate immune system defenses against infections involve the recognition of invading viral and bacterial pathogens, by the family of Toll like receptors (TLRs). Among TLRs family only TLR3, 7, 8 and 9 that expressed in the intracellular endosomal compartments, which can detect viral infections.TLR3 distinguishes double strand RNA viral motifs. TLR 7/8 are specific for single strand RNA. While TLR9 recognizes unmethylated CPG DNA of viruses. The objective of this study is to clarify the expression of intracellular TLRs in the woman with endometriosis.
Materials and Methods: In this study three groups were examined. Ectopic biopsies were obtained with laparoscopic procedure from patient with endometriosis. Eutopic and control biopsies were gained with piplle from endometrium of women with and without endometriosis. Reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative-PCR (Q-PCR) for 5 samples of each groups used to show the existence of TLR3, 7, 8 and 9 genes.
Results: TLR3, 7, 8 and 9 mRNA were expressed in the each groups but in ectopic and eutopic samples we showed variable expression.
Conclusion: Our finding suggested that TLRs is involved in endometriosis pathophysiology. It is shown that some products of TLRs signaling such as TNf-α and IL-1 were increased in endometriosis.

Keywords: Innate Immune System, Toll Like Receptors, Endometriosis

P-173: Conservative Treatment in Young Patients with Cervical Cancer: A Review

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Background: Fertility preservation in young patients with cervical cancer is a novel point which considers these days and in Iran we have a new experiment that was successful.
Materials and Methods: Evaluation of literature review and our experiences in conservative management in patients with early stage cervical cancer. The key words in this research were conservative management, young women and cervical cancer.
Results: For cervical cancer cases with a low risk of relapse who wish to maintain their fertility, radical trachelectomy is an alternative to radical hysterectomy. Pelvic magnetic resonance imaging is recommended before surgery, with laparoscopic assisted lymphatic dissection required for assessment of lymphatic metastasis. If there is a visible lesion in the cervix, the specimen taken during trachelectomy should be sent for frozen section. The complications of radical trachelectomy are chronic vaginal discharge, irregular vaginal bleeding, dysmenorrhea, ulceration, amenorrhea and cervical stenosis. The probability of cervical cancer recurrence with a lesion of similar size is comparable with radical trechelectomy and radical hysterectomy. Two thirds of pregnancies after trachelectomy lead to live births of which approximately 40% of them are healthy. However, the probability of second trimester abortion and pre-term labor is greater than in the general population. Because of the possibility of uterine arterial injury in short cervix, vaginal delivery should be avoided and a cesarean operation in 37-38th week is recommended. Adjuvant treatment with chemotherapy followed by radical trachelectomy is a suitable option for larger lesions. On the other hand, conization or simple trachelectomy are more proper approaches for very small lesions.
Conclusion: Conservative management in early stage cervical cancer is an optional procedure and before doing the radical surgery, we should consider and ask the couples who want to preserve their fertility.

Keywords: Conservative Management, Young Women, Cervical Cancer

P-174: Good Pregnancy Outcome after Prenatal Exposure to Bleomycin, Etoposide and Cisplatin for Ovarian Immature Teratoma: A Case Report and Literature Review

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Background: The administration of bleomycin plus etoposide and cisplatin during pregnancy is rare.
Materials and Methods: We describe a case with good pregnancy outcome after exposure to these chemotherapeutic agents at the third trimester of pregnancy.
Results: Pregnant woman with stage IIIc immature teratoma underwent surgical staging, and received two cycles of bleomycin, etoposide and cisplatin from the 29th week of pregnancy until delivery. The patient did not have any evidence of recurrence of ovarian cancer for 1.5 years. Her infant did not have any evidence of minor or major malformations, and showed normal neurological development during 1.5 years of follow-up.
Conclusion: Germ cell malignant tumors in pregnant women are present at young age. Diagnosis is usually established at approximately 20 weeks of gestational age or after, and pregnancy outcome seems to be favorable after combination therapy with bleomycin, etoposide and cisplatin. However, a definitive conclusion cannot be derived yet because of the limited number of cases currently available in the literature. Further research is needed to acquire more information on the long-term impact of treatment modalities like chemotherapy on the offspring.
Keywords: Combined Antineoplastic Agents, Germ Cell Tumor, Ovary, Pregnancy Outcome, Teratogens

P-175: Relationship between Dietary Fat Intake and Its Major Food Sources and Assisted Reproduction Parameters

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Background: To examine the relative contributions of dietary fat intake level and its food sources and assisted reproduction parameters.
Materials and Methods: A prospective study was conducted on 240 infertile women. In assisted reproduction treatment cycle, body mass index (BMI), physical activity (PA), calorie intake, fat consumption and major food sources were assessed. The number of retrieved oocytes, fertilization rate and embryo quality were also determined.
Results: Total fat intake adjusted for age, BMI, PA and etiology of infertility was positively associated with the number of retrieved oocytes and inversely associated with the high embryo quality rate. An inverse association was observed between sausage and ham intake and the number of retrieved oocytes. Vegetable oil intake level and good cleavage rate had an inverse association. Nuts intake was positively related to the number of retrieved oocytes and number of high embryo quality.
Conclusion: The results revealed that higher fat consumption level tends to raise the number of retrieved oocytes, and adversely relates to embryonic development. Of the food sources of fat, high intake of vegetable oil, sausage and ham may adversely affect assisted reproduction parameters. Also, nuts intake may have positive effect on assisted reproduction treatment.
Keywords: Dietary Fats, Assisted Reproduction, Embryo Quality

P-176: Infertility and iIts Association with Helicobacter Pylori Infection

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Background: Infertile couples in the reproductive failure, after a year of sex without contraception is described and in 10 to 15% of couples can be seen, that the 1/3 because of the male, 1/3 female and the rest are a common cause. Factors involved in human infertility, the expression of microbial infection (25%), biological factors, environmental and genetic (50%) and unknown causes (25%) are. Studies from 1975 until now prove that genital infections can help women suffering from infertility and in the male reproductive system, will be leading to microbial colonization of semen. The infectious agents involved in causing infertility, bacteria such as Neisseria gonorrhoea, Chlamydia trachomatis, enterobacteria, gram-positive cocci, Gardnella and Helicobacter pylori. This study investigated the relationship between Helicobacter pylori and infertility has been prepared.
Materials and Methods: The present study was retrospective and by using library and Internet resources are provided.
Results: Helicobacter pylori may be direct or indirect factor in infertility in both sexes. The bacteria of different ways such as oral-genital, anal-genital or oral-oral are transmitted to sexual partners or other people and often its source, the mouth, dental plaque and saliva are listed. Vaginal environment and ecological conditions of the stomach and pH are very similar and these bacteria in these conditions can be colonized. A theory that in cases of infertility by this bacterium is it have been strong antigens, which can trigger phagocytosis and Phagocyte is attracting in the region which results in severe inflammation in the uterus or fallopian tubes and followed by closure of the tube occurs or infertility may be due to production safety conditions, Ag-Ab complexes or linear homology between the bacterial protein (as Ag) with tubular human proteins. In most studies in this field, this antibody has bacteria in infertile women than fertile men.
Conclusion: Due to the high H. pylori antibodies in infertile women, this bacteria can be considered as a cause of infertility in infertile men and recommended that screening tests be done on these individuals
Keywords: Blood Plexus, Embryo Development, Morphine, Rat

P-177: The Relationship between Perimenstrual

Nause, Vomiting and Menstrual Characteristics

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Background: The premenstrual symptoms may be a sensitive marker of psycho-physiological problems that applied as a diagnostic and a reproductive health education tool and can be influenced different factors such as menstrual characteristics. So, the aim of this study was to evaluate the relationship between premenstrual nausea, vomiting and menstrual characteristics.

Materials and Methods: This study was a cross-sectional study that carried on 407 high-school female in Mashhad city during in the year 2011. The sampling was random clustering method with exclusion criteria including: use combinations of hormones; suffering from medical illnesses; being a professional athlete and etc. Then participants completed written consent form. Tools study were including of: demographic/menstrual questionnaire, Higham Chart for bleeding amount and MDQ-C form (4-point Likert Scale) that recorded nausea, vomiting symptom in the last 6 months and in during of three phases of the menstrual cycle (a week before the menstrual period, menstrual bleeding and a weeks after menstruation) at menstrual cycles. Data was analyzed using SPSS version 14 software and statistical test such as Pearson correlation test and regression linear.

Results: Results showed that mean ± SD age, BMI, age of menarche was 16.24 ± 1.0 (y), 21.16 ± 1.02 kg/m^2, 12.78 ± 1.11 (y). 83% were passive smoker and 97% single. 5.7% had premenstrual nausea and vomiting, 20.1% menstrual nausea and vomiting and 3.9% postmenstrual nausea and vomiting. Also, the mean ± SD duration of menstrual cycle 6.8 ±1.6, length of menstrual cycle 28.06 ± 8.5, amount of menstrual bleeding 131.3 (± 1.1). 93% had menstrual duration of 3-10 (day), 46.2% amount of more than 100cc, 78.6% length of 21-42 (day), 36.4% irregular. There was a significant negative correlation between premenstrual nausea and vomiting and duration of menstrual cycle (p=0.018, r = -0.52).

Conclusion: This study shows a significant association between some menstrual characteristics and nausea, and vomiting related to menstrual cycle, so it is necessary provide appropriate solutions to improving menstrual problem for students as feature mothers.

Keywords: Menstrual Characteristics, Premenstrual, Nausea, Vomiting

P-178: Is There Any Role for Acupuncture in Infertility Treatment?

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Background: Acupuncture is one of the most popular forms of alternative medicine used in the treatment of infertility. But what is acupuncture? Can it really help to get pregnant? Acupuncture involves placing hair-thin needles into particular points in the body. According to traditional Chinese medicine, these points run along "energy lines" called meridians. By needling specific points, acupuncture is said to regulate the body's hormones and organs. Whether or not acupuncture can improve pregnancy rates is controversial. Some research studies have shown promise, while others have found no link between acupuncture treatment and improved pregnancy rates. The only thing just about everyone agrees on is that acupuncture can increase relaxation, help with pain, and lower stress levels. Given the stress of infertility, acupuncture may be worth a shot. No one is certain how acupuncture works, but there are theories.

Materials and Methods: In this review article it was decided to search in internet about the acupuncture and its role in infertility and this review summarizes the recent studies which investigated the role of acupuncture in infertility the search was performed with key word of acupuncture and infertility and there were about 68 papers in regard to the acupuncture and infertility.

Results: About the mechanism of acupuncture there is one theory says that by needling points on the body, chemicals and hormones are triggered and released. These chemicals either change the experience of pain, or they trigger a cascade of chemicals and hormones which influence the body's own internal regulating system. The improved energy flow and biochemical balance produced by acupuncture stimulates the body's natural healing abilities, and enhances physical and emotional well-being. Research has shown specifically that acupuncture increases the amount of beta-endorphins flowing through the body. Beta-endorphins Are feel-good hormones that help lessen pain. Exercise is also known to boost the amount of beta-endorphins in the body. The research on acupuncture and infertility is ongoing, and the topic is controversial. Some studies have been too small to prove a definitive connection to improved pregnancy rates, and some studies contradict each other in their results. With that said, here are a few of possible benefits of acupuncture, according to the preliminary research: Improved pregnancy rates during IVF treatment cycles, when acupuncture takes place on the day of embryo transfer Increased blood flow to the uterus, leading to an improved endometrial lining Reduced stress and anxiety levels Possible improvement in ovulation for women with PCOS Possible improvement in sperm count and quality in men with infertility. Possible regulation of gonadotropin-releasing hormone, which in turn could help regulate ovulation.

Conclusion: Even though a positive effect of acupuncture in infertility has been found, well-designed multicenter, prospective randomized controlled studies are still needed to provide more reliable and valid scientific evidence. Furthermore, it is urgent and necessary to clarify the mechanism of acupuncture for infertility.

Keywords: Acupuncture, Infertility

P-179: Spinal Anesthesia in Laparoscopic Surgery of Infertile Patients

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Background: Laparoscopy is one of the most common surgical procedures. General anesthesia as the only suitable technique for laparoscopic procedures is a concept of the past. A problem with modern general anesthetics is that even though patients can be awake and oriented shortly after cessation of the anesthetic. There is growing evidence suggesting that regional anesthesia has an important role to play in the care of patients undergoing laparoscopy. The key benefits of regional anesthesia include less emesis, less postoperative pain, shorter postoperative stay, improved patient satisfaction, and overall safety. The outcome of regional anesthesia depends on the creativeness of surgeons and anesthesiologists, and patient acceptance. In this study it was decided to compare spinal anesthesia with the gold standard general anesthesia for elective laparoscopic procedure in patients with infertility.
Materials and Methods: A prospective descriptive study was performed on 10 cases with infertility that was candid to do Laparoscopy as a work up of infertility. The study group had no contraindication for laparoscopic Surgery, spinal anesthesia and were underwent spinal anesthesia and diagnostic or Therapeutic laparoscopy consists of ovarian drilling or adhesionolysis if it was necessary. Conversion of anesthesia to general, insufflations Pain during surgery, duration of surgery and postoperative variable consists of duration of Hospital stay, presence of nausea or vomiting, use of analgesic or antiemetic and headache were Recorded.
Results: The mean age of the study group was 5/7 ±7/2 and there was no conversion to general anesthesia. Insufflations pain was present in 3 of 10 cases (30%) that were treated with use of propofol and the mean duration of surgery was 31miutes.in regard to postoperative variable all 10 patients were discharged at same day of surgery .Two cases (20%) received one dose of analgesic after surgery and none of them developed nausea, vomiting and headache .so, there was no need to use anti-emetic.
Conclusion: It seems that use of spinal anesthesia in work up of infertility cases that are underwent laparoscopy is safe and has minimal complication such as insufflations pain ,but it need more study with more cases for the confirmation of this results.
Keywords: Laparoscopy, Anesthesia, Spinal, General

P-180: The Fate of Motherhood, Fetuses and Neonates in Drug Addicted Pregnant Women

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Background: Pregnancy in opioid-dependent women is a major public health issue. According to a national household survey in 2001, 3.7% of pregnant women in the United States used illicit drugs. In 2009 this number increased to 4.5% . Women who are afflicted by opioid addiction form a highly vulnerable group of patients frequently facing unplanned pregnancy with adverse outcomes including perinatal complications. The purpose of this study was to assess the effect of addiction on the fetus, and neonatal life in pregnant women.
Materials and Methods: For this review, computerized literature searches of Pubmed , Scopus, Science Direct and SID were conducted. The search included 20 articles in English from January 2006 to December 2011.
Results: According to the search, due to inappropriate effects of opioids on female reproductive system, addicted women frequently develop (face) unplanned pregnancies, leading to irregular menstruation or amenorrhea. Additionally, chaotic lifestyles associated with drug abuse often foster insufficient birth control measures and consequently unexpected pregnancies. In fact, the rate of unintended pregnancies has been found to range between 80% and 90% among opioid-dependent women. The addicted mothers manifested significantly greater levels of complications such as placental abruption, preterm labor, preeclampsia, hypertension, PROM, cesarean, hepatitis B, meconium in the amniotic fluid, intrauterine fetal growth retardation, anomalies in infant, low Apgar score in the first and fifth minutes, fetal death, hypoglycemia, neonatal convulsions, breathing problems, RDS, need to neonatal resuscitation, admission in NICU, more neonatal deaths, weight loss, and low circumference neonatal head size.
Conclusion: Opioid-dependent pregnant women form a highly vulnerable group of patients with many unplanned pregnancy. The fetus and newborn in such women develop serious complications.
Keywords: Pregnancy, Addiction, Neonate and Fetus

P-181: The Effect of Estradiol Valerate and rFSH Combination As Ovarian Stimulator on *In Vitro* Fertilization and Embryo Developmental Rate of Ovulated Oocytes in Mice

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Background: Sex steriods play important roles in the growth and differentiation of reproduction tissues and in maintatinance of pregnancy. 17-β oestradiol (E2) is the most important one of them that has a positive effect an the granulosa cells proliferation and ovarian follicle maturation. Then the aim of this study was determine of the effectes of rFSH+ E2 combination as an ovarian stimulator on the quality of MII oocyte and post -IVF embryos.
Materials and Methods: 6-8 weeks NMRI female mice were injected intraperitonealy 10 IU rFSH and 26 µg Estradiol valerate per mouse . After 48 hours, they received 10 IU hCG as same manner and then ovulated oocytes collected from dilated part of uterine tube 18 hours after latter injection for *in vitro* fertilization of in T6+ 15 mg/ml BSA. Post 6-hour fertilizated ocytes (2PNS) transfer to droplts containing T6+ 5 mg/ml BSA for 120 hours. The data were analyzed by the independent sample t-

test that was two-tailed, with a confidence level of 95% ($p<0.05$).
Results: The mean number of MII oocyte were 19.11 ± 3.79 and 14.47 ± 4.7 in control (rFSH) and experimental groups (rFSH + E2) respectively with significant difference ($p=0.001$) but mean of fertilization rate had no statistically difference. The mean of 2-cell embryo is better in experimental group than control. However , there were no statistically singnificant differences between the two groups in the 2PN , 4 cell, and 8-cell,embryo rates but the embryos of control group could reach to blastocycte stage.
Conclusion: In this study ,we concluded that rFSH itself can cause to produce more in MII oocyte than rFSH+ E2 combination as ovarian stimulator and also Supports embryo development to blastocycte stage.
Keywords: Estradiol, Ovarian Stimulator, IVF, Blastocyst, Mice

P-182: The Role of Vascular Endothelial Growth Factor Gene Expression in Patients with the History of Endometriosis

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Background: Endometriosis is the presence of endometrium-like tissue in sites outside the uterine cavity, primarily on the pelvic peritoneum and ovaries. Ectopic endometrium for replacement and growth require to blood supply. Vascular endothelial growth factor (VEGF) is one of the most important intermediate of locality angiogenesis that product by monocytes and macrophages. This study evaluates gene expression of VEGF in patients with a history of endometriosis.
Materials and Methods: This study contains 3 groups (n=5). In patient with endometriosis, ectopic biopsies were obtained by laparoscopic procedure and eutopic biopsies were obtain by pipelle. In women with no sign of endometriosis, control biopsies gained with piplle. Gene expression of VEGF was determined by RT-PCR and the quantitative level of gene expression was tested by real time PCR.
Results: VEGF gene was expressed in all groups. However, Q-PCR analysis showed that the expression of VEGF in ectopic group was higher in compare to control and eutopic groups.
Conclusion: VEGF is a potent angiogenic factor involved in physiology and pathology of angiogenesis. Elevated levels of VEGF are found in peritoneal fluid of patients with endometriosis. Peritoneal fluid macrophages elevate levels of VEGF by expressed both VEGF receptors.
Keywords: Endometriosis, Angiogenesis, VEGF

P-183: A Low Risk Approach for Treatment of Infertile PCOS Patients

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Background: Ovarian hyperstimulation syndrome is one of the most common complications of infertility treatment. As the fluid-filled egg follicles begin to grow within the ovary, the ovary enlarges. Sometimes, that fluid can cause fluid elsewhere in the body to shift into the abdominal cavity or the lungs. Women with PCOS are at a greater risk for developing OHSS due to the already large number of follicles on the ovary and the tendency for women with PCOS to over-respond to the hormones. The purpose of this study was decreased OHSS due to induction of ovulation with clomiphene citrate and then letrozole.
Materials and Methods: This is prospective clinical research from Dr.Rasekh clinic. 36 infertile polycystic ovarian syndrome women were selected with 47 months infertility. The average age of them is 27.3 years (STD=5). The 36patients (40%) with drug regimen; initially tab clomiphene citrare (from day 3 of menstrual cycle).Then the second drug Letrozole was started from day 8 to 11 menstrual cycle. OHSS was 0%. Pregnancy rate was 8 (22%). The patients were monitored for ovulation by translational ultrasonography, with measurement of number and size of the follicles, as well as endometrial thickness. Human chorionic gonadotrophin (HCG) was injected intramuscularly when at least one mature follicle >18 mm diameter was detected. Data was analyzed with SPSS software.
Results: The rate of OHSS is at minimum in this method ($p<0.05$). This means that can be completely prevented the creation of OHSS. Pregnancy rate is almost considered desirable ($p<0.05$).
Conclusion: We suggest that this method will be employed for the prevention of OHSS that is a serious complication in treatment of infertile women .Whilst this method has been favorite fertility rate. Another feature of this method is its low cost.
Keywords: OHSS, PCOS, Clomiphene, Letrozole

P-184: The Role of Cell Surface Toll Like Receptors in Endometriosis

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Background: Toll like receptors (TLRs) are a major family of innate immune systems which recognize specific pathogen associated molecular patterns (PAMPS)

in bacterial, fungi, virus and parasites. Human TLRs comprise a large family of 10 different type proteins that are expressed on various immune cells. Among these receptors, TLR1, 2, 4, 5, 6 and 10 were expressed on the cell surface. TLR2 forms heterodimers with TLR1 or TLR6 which recognize bacterial lipoproteins and lipopeptides, TLR4 that recognizes lipopolysaccharides (LPS) and TLR5 that recognizes flagellin. TLR10 is highly homologous to TLR2, but its function is still unknown and no specific ligand has been yet identified. Endometriosis is a benign gynecologic disorder characterized by the ectopic growth of misplaced endometrial cells. Endometriosis is involved mainly innate immune System. Whereas TLRs are expressed in the endometrial cells as a result their expression and their regulation might be vital for the pathogenesis of endometrial diseases especially endometriosis.

Materials and Methods: This study contains three groups (n=5). Ectopic biopsies were obtained with laparoscopic procedure from patient with endometriosis. Eutopic and control biopsies were gained with piplle from endometrium of women with and without endometriosis. The existence of TLR1,2,4,5,6 and 10 genes were tested with RT-PCR and Q-PCR has shown different levels of genes expression in this test.

Results: TLR1, 2, 4, 5, 6 and10 mRNA were expressed in the all groups. However, Q-PCR analysis showed that the TLRs expression in ectopic group was higher than other groups.

Conclusion: Our finding suggested that TLRs is involved in endometriosis pathophysiology. It is shown that TNF-α and IL-1 were increased in endometriosis which these are products in TLR signaling.

Keywords: Endometriosis, Innate Immune System, TLR

P-185: Expression and Function of Vascular Endothelial Growth Factor in Fallopian Tube Carrying Ectopic Pregnancy

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Background: Vascular endothelial growth factor (VEGF) is an angiogenic growth factor that is produced by different tissues, including the endometrium and ovary. VEGF is involved in many processes related to reproductive physiology, including angiogenesis, which is essential for implantation and placentation. It has been suggested that VEGF may be the angiogenic factor responsible for the implantation and placentation of ectopic pregnancy (EP). EP is a complication of pregnancy in which the embryo implants outside the uterine cavity, usually in fallopian tube (FT). Thus we investigate the role of VEGF in tubal EP.

Materials and Methods: Biopsies from Isthmus, Ampulla and Infundibulum of FT were obtained from case and control groups. Case group was women who underwent salpingectomy for EP. Control group was women with healthy tube who underwent hysterectomy for benign gynaecological conditions. For control group, Human chorionic gonadotropin (hCG) was injected in 14 days leading up to hysterectomy to produce a state of pseudo-pregnancy. In this study, VEGF expression was investigate with RT-PCR in Infundibulum, Ampulla and Isthmus. Pseudo-pregnant and EP groups were compared with Q- PCR for VEGF gene.

Results: RT-PCR has shown VEGF gene expression in Isthmus, Ampulla and Infundibulum of FT in both case and control groups. Variable expression of this gene was observed in all regions of FT carrying EP compared with control group by Q-PCR.

Conclusion: Expression of VEGF is stimulated by tissue hypoxia. It is likely that implantation in the unfavourable FT would be associated with increased tissue hypoxia which results in variation of the expression of VEGF in tubal EP.

Keywords: Ectopic Pregnancy, Fallopian Tube, VEGF

P-186: Evaluating The Role of Nutrition on The Infertility

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Background: According to WHO report, there are around 80 million people in the world that suffer from infertility and about 12-15% of the people in Iran are infertile. The nutrition has an important rule on infertility treatment. So we should improve the couple's information and knowledge about the effective nutrition on the infertility to increase the chance of fertility and decrease negative emotional and mental effects of infertility on the couple.

Materials and Methods: Information of this survey with numerous articles from 1999 to 2012 in internet different sites and books collected and evaluated.

Results: The nutritionists say that mineral, vitamin low deficiency and chemical poisons can intervene on ovum and sperm production and failure it. Zink has been the widest nutritious element that has been studied on couple's fertility improvement. Zink deficiency can cause some changes in the couple's chromosome that decrease the fertility and increase the rate of abortion. Also use of iron supplement (after loss other disorders) cause a decrease in the rate of ovarial infertility dangerous meaningfully. Change or replace animal resources protein with herbal resources, may cause a decrease in ovarial infertility. The food that is full of vitamin E like sunflower oil, fish liver oil and gourd seeds may cause an improvement on infertility. Essential fatty acids that exist in black raisin seeds oil, primrose oil and Lenin seeds oil for gonadal normal function in both sex is necessity. Therefore nutritional supports can help the fertility and improve fertility's methods.

Conclusion: Proper and enough nutrition must be the base of every disease like infertility. Infertility is an important physiological and physical problem in the couple that requires a systematic assessment and continuous follow up. If health is defined punctuality physical, physiological and social status so can't dissemble social and

physiological backwash of infertility.
Keywords: Infertility, Nutrition

P-187: Relaxation Technique Effect on Stress Score and Pregnancy Test of Infertile Women in Isfahan Fertility and Infertility Center (2010)

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Background: Infertility effects on psychological and emotional tension in couples and make severe stress in infertile men and women. Relaxation technique is one of the methods which reduce stress and balance human emotions. The purpose of this study was determination of relaxation effect on stress score in the infertile women and pregnancy test as outcome of their treatment.
Materials and Methods: This study was a semi experimental and clinical trial study. Participants were studied by randomize sampling in two groups. At first, the stress score was determined in both groups, by Newton's infertility stress questionnaire. Stress score was evaluated in participants and then relaxation technique was administered in the intervention group. This technique performed in 12 sessions. All questionnaires were completed under supervision of the researcher after embryo transfer to the uterus (after 2 weeks) and before pregnancy test administering.
Results: Independent t-test showed total stress score does not have significant difference in groups before intervention ($p>0.05$). Whereas, independent t test indicated significant difference in stress scores between the two groups after intervention ($p<0.05$). Stress score was higher in the control group than the intervention group. Chi-square test also showed that positive pregnancy test was significantly higher in the intervention group ($p<0.05$).
Conclusion: Relaxation technique as a complementary and alternative medicine (CAM) method can reduce stress score in infertile women and effect on treatment outcome.
Keywords: Relaxation, Stress, Infertile Women

P-188: Mammary Gland Changes during Chlorpromazine Treatment in Female Rats

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Background: Antipsychotic drugs are medications used to treat schizophrenia and other psychotic disorders. Antipsychotic drugs can cause unwanted side effects. Hyperprolactinemia is an important but neglected adverse effect. The aim of the this study was to consider the effects of Chlropromazine -induced hyperprolactinaemia on mammary glands of female rats .
Materials and Methods: In this study 32 adult female wistar rats (160 ± 5 g) were randomly divided into four groups (n=8 each) with one group serving as control sham((5 mL/kg of 0.5% methylcellulose solution.), in the drug treated groups female rats were orally administered CPZ (3, 10, 30 mg/kg) once daily for 28 days .The test compound was suspended in 0.5% methylcellulose solution . On day 29th all rats were sacrificed by CO_2 inhalation and blood samples were collected for biochemicals analyses. The mammary glands were removed surgically and prepared for histological study.
Results: This study showed that treated by CPZ had significant increase in prolactin level in plasma . In the mammary gland, acinous hyperplasia with an increased secretion was observed in females treated with CPZ.
Conclusion: These results showed that CPZ-induced hyperprolactinaemia negatively affects on mammary glands and there were mammary gland changes and unexpected breast development.
Keywords: Chlropromazine, Prolactin, Hyperprolactinaemia, Mammary Glands, Rat

P-189: Investigation of Vascular Endothelial Growth Factor Receptors Expression in Ectopic Pregnancy

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Background: Ectopic pregnancy (EP) is a complication of conception in which the embryo implants outside of uterine cavity. The increasing incidence of serious maternal morbidity resulting from EP has prompted the search for biomarkers to aid in early diagnosis and take advantage of conservative treatments. One of the effective biomarkers in EP is vascular endothelial growth factor (VEGF). VEGF is an angiogenic growth factor that has an important role in regulating trophoblast functions in the process of placentation and implantation. This molecule acts on endothelial cells through their receptors flt-1 (VEGFR1) and fkl-1/KDR (VEGFR2) to exert their effects. VEGFR-2 mediates almost all of the known cellular responses to VEGF. VEGFR-1 modulates VEGFR-2 signaling. The purpose of our study is to determine levels of VEGFR1 and VEGFR2 in fallopian tubes (FTs) carrying EP.
Materials and Methods: In this study, biopsies from Infundibulum, Ampulla and Isthmus of FTs were obtained from case and control groups. Case and control groups were women who underwent salpingectomy for EP and hysterectomy respectively. Human chorionic gonadotropin (hCG) was injected in 14 days leading up to hysterectomy to produce a state of pseudo-pregnancy. In this investigation expression of VEGFR1-2 were tested with RT-PCR. Also Q-PCR was used to compare quantitative expression of these receptors between two groups.

Results: RT-PCR has shown expression of VEGFR1-2 in infundibulum, Ampulla and Isthmus from case and control groups. Q-PCR has shown, variable expression of VEGFR1-2 in all parts of FTs carrying EP compared with control group.
Conclusion: Hypoxia plays important role in the regulation of VEGFR1-2 expression. It is possible that implantation in the tubal ectopic environment associated with increasing of hypoxia. This may lead to variation of VEGFR1-2 expression in FTs carrying EP compared with control group.
Keywords: Ectopic Pregnancy, Fallopian Tubes, VEGF, VEGF Receptors

P-190: Pregnancy Outcomes in Women with Polycystic Ovary Syndrome Compared with Normal Women

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Background: Polycystic ovary syndrome (PCOS) is a common reproductive disorder associated with many characteristic features, including hyperandrogenaemia, insulin resistance and obesity which may have significant implications for pregnancy outcomes and long-term health of the woman. It has an incidence ranging from 5 to 10%.
Materials and Methods: This study is conducted to evaluate the pregnancy and perinatal outcome in women with PCOS. Pregnancy outcomes in 208 women with PCOS and were compared with 240 normal women.
Results: Women with PCOS demonstrated a significantly higher risk of developing gestational diabetes (133 in PCOS groups vs. 98 in normal group, p=0.00), pregnancy-induced hypertension and preeclampsia (67 in PCOS groups vs. 21 in normal group, p=0.01) and preterm labor (22 in PCOS groups vs. 14 in normal group p=0.01). Furthermore, women with PCOS seem to experience increased risk of cesarean delivery than normal group. There was no increased risk of congenital anomalies.
Conclusion: Women with PCOS are at increased risk of pregnancy complications. Pre-pregnancy, antenatal and intrapartum care should be aimed at reducing these risks.
Keywords: PCOS, Pregnancy Outcome, Normal Women

Genetics

P-191: Cloning and Expression of Recombinant Ovine FSH Hormone in Pichia Pastoris

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Background: Follicle stimulating hormone is a heterodimeric protein composed of two subunits, α and ß, which are linked noncovalently. The hypophysial gonadotropin FSH plays an important role in the regulation of oocyte maturation, and a key component for growth of ovulator follicles in ewes.
Materials and Methods: This study seeks to clone and express the ovine follicle stimulating hormone subunits in Pichia pastoris, which has numerous advantages over other systems for expression of eukaryotic proteins. The ORF region of alpha and beta fragments were amplified by PCR and inserted into PTZ57R/T vector and the follow subcloned in pPIC9 expression vector with α-factor signal peptide under the control of AOX1 promoter. The recombined plasmids were cotransformed into the P. pastoris GS115 strain by electroporation. Incubation was continued for 102 hours at 29.5 °C and 0.5% methanol was added every 24 hours during the period of incubation as an inducer. Finally, collected supernatant in during incubation were investigated by SDS-PAGE and Western blotting.
Results: Over results confirmed integration of linearized expression cassettes into the P. pastoris genome. Also expression of the recombinant protein was confirmed by SDS-PAGE and Western blotting.
Conclusion: Production of recombinant protein in eukaryotic expression systems such as P. pastoris is a reliable method for producing of therapeutic ovine FSH. Whereas the secretion of the recombinant protein in supernatant could be cause the α-factor sequence as a signal peptide.
Keywords: Recombinant FSH, Cloning, Ovine, Pichia Pastoris

P-192: The Study of Cystic Fibrosis Transmembrane Conductance Regulator Gene Mutations and Polymorphisms in Iranian Patients with Mayer Rokitansky Kuster Hauser Syndrome

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Background: Mayer - Rokitansky - Kuster - Hauser (MRKH) syndrome is characterized by congenital aplasia of the uterus and the upper part of the vagina in women showing normal development of secondary sexual characteristics and a normal 46, XX karyotype. Congenital anomaly of the female genital tract, estimated to occur in approximately 1 in every 5,000 females. It is caused by a failure of development of the caudal portion of the embryonic paramesonephric (Mullerian). Because the embryologic development of the Müllerian ducts directly depends on the prior normal development

of the Wolffian ducts, the same gene products may be necessary for normal embryologic development of both ductal systems. CFTR mutations have previously been associated with congenital bilateral absence of the vas deferens (CBAVD). CBAVD is caused by a disruption in the vas deferens, a Wolffian duct derivative. This study evaluated the role of CFTR mutations in patients with MRKH syndrome.

Materials and Methods: DNA samples of 15 females with MRKH syndrome and 15 health females (control group) were tested for common mutations of CFTR gene including (DF508 ,G542X ,N1303K ,W1282X,G551D)by ARMS-PCR and IVS8-Tn polymorphism by using RFLP-PCR were analyzed.

Results: No mutation and polymorphism was found in patient's and normal samples.

Conclusion: We only detected most common (DF508, G542X, N1303K, G551D, W1282X) of this gene in patients, could not find An association between CFTR gene mutations and MRKH syndrome, Therefore we should all 27 CFTR exons were analyzed to suggest that mutations of this gene could be associated or not.

Keywords: MRKH Syndrome, CFTR Gene, ARMS-PCR

P-193: The Association of Apolipoprotien E Polymorphisms with Recurrent Pregnancy Loss

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Background: The role of apolipoprotien E polymorphisms do not diagnostic correctly in recurrent pregnancy loss etiology but Apo E has been shown to play an important role in lipid metabolism in pregnancy. We evaluated these polymorphisms in Iranian women with unexplained recurrent pregnancy loss.

Materials and Methods: 5 ml Blood were sampling from 81 women with a history of two or more consecutive spontaneous abortions and 81 women with at least two live births and not miscarriage. DNA was extracted from the leukocytes and PCR amplification of Apo E was performed. For determining Apo E genotypes used restriction fragment length polymorphism analysis.

Results: Women with consecutive spontaneous abortions had greater significantly frequency from Apo E E3/E4 and E4/E4 genotype (19.75%) comparing with control group (2.46%) (p=0.032).

Conclusion: Polymorphism Apo E4 may help to thrombophilic risk factors in loss of fetus during pungency. Our data supported association polymorphisms apolipoprotein E with recurrent pregnancy loss.

Keywords: Apolipoprotein E, Recurrent Pregnancy Loss, Apo E4 Polymorphism

P-194: SEPT12-G800A Polymorphism in Azoospermic Infertile Men Referred to Royan Institute

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Background: SEPTINS belong to a family of polymerizing GTP-binding proteins that are required for many cellular functions, such as vesicular trafficking, mitosis, membrane compartmentalization and cytoskeletal remodeling. Among all SEPTIN genes, SEPT12 is dominantly expressed in testis tissue of adults, known as an essential annulus component of mature sperm. Therefore, it is hypothesized that genetic alterations of SEPT12 may be associated with male infertility. In the current work we tried to find gene polymorphisms of SEPT12 in infertile men with azoospermia.

Materials and Methods: Genomic DNA was extracted from periphery blood samples of 30 infertile men and 30 normal controls referred to the Royan Institute, and DNA fragments were amplified by PCR and analyzed by sequencing.

Results: Genotype analyses indicated that G800A polymorphic SEPT12 alleles were distributed in three peaks of frequency in both control and disease groups. Categorization of the alleles into (GG), (GA), (AA) types revealed a significant difference between azoospermia and control samples (p less than 0.05 by chi-squre test).

Conclusion: These results suggest that G800A polymorphism in SEPT12 gene may have a determinative role in the pathogenesis of male infertility.

Keywords: SEPT12, Polymorphism, Male Infertility

P-195: Analysis of Expression Level of Tex11 Gene in Obstructive and Non-Obstructive Azoospermic Men Referred to Royan Institute

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Background: About 15% of couples worldwide suffer from infertility problem that half of these cases are related to male infertility. Spermatogenesis is a cumulative process and thousands of genes are involved in it. Change in one of these genes or their products can

cause male infertility. Tex11 is a germ cell specific gene that is located on the X chromosome (Xq13.1 region). This gene was identified at 2001 for the first time as a germ cell-specific gene with unknown function. Tex11 gene in mice and human is only expressed in testis. TEX11 promote formation of meiotic cross overs and it is essential for meiosis and male fertility and is involved in the initiation and maintenance of chromosome synapses in males.

Materials and Methods: The samples retrieved from patients who underwent diagnostic testicular biopsy in Royan institute. 5 patients with obstructive azoospermia and normal spermatogenesis and 5 patients with non-obstructive azoospermia were recruited. Total RNA was extracted with Trizol reagent and cDNA was synthesized. Quantitative real-time RT-PCR for Tex11 was performed using Power SYBR Green kit.

Results: After normalizing the relative amount of Tex11 transcript by the amount of GAPDH transcript in the same cDNA, it was shown that tissue samples from non-obstructive azoospermic patients had lower level of tex11 gene expression than obstructive azoospermic samples.

Conclusion: As expression level of Tex11 gene is reduced in non-obstructive azoospermic men, we can conclude that Tex11 expression levels is essential for normal spermatogenesis and deficiency in this gene can cause spermatogenic failure and infertility in men.

Keywords: Male Infertility, Azoospermia, Tex11, Spermatogenesis

P-196: Association rs3819392 Single Nucleotide Polymorphism within The KIT Gene with Azoospermic Male Infertility

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Background: Recent studies have shown that KIT is expressed in the cytoplasm of the spermatogonia, acrosomal granules and leydig cells. Reduction in KIT expression in oligozoospermia with an increase in the germ cell apoptosis process. Three single-nucleotide polymorphisms (SNPs) have been identified and these have been studied to discover KIT role in the male infertility. The aim of this study was evaluation single-nucleotide polymorphism SNP rs3819392 located within the genomic region of the KIT.

Materials and Methods: We have studied one single-nucleotide polymorphism SNP rs3819392 located within the genomic region of the KIT. We carried out a genetic association study to provide the role of the KIT gene in male infertility. These have been studied in 100 normal male cases and 100 azoospermia male. PCR and RFLP techniques were used for this study of the polymorphism.

Results: Our results showed a positive correlation between SNP rs3819392 of KIT 1101 G>A and male infertility (p= 0.032).

Conclusion: Our data revealed the KIT may play an important role in male infertility and thus KIT 1101 G>A can be one of the genetical risk factor for the male azoospermic infertility.

Keywords: Single-Nucleotide Polymorphism, KIT, Azoospermic, Male Infertility

P-197: Expression of Bovine Follicle Stimulating Hormone Subunits in Pichia Pastoris

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Background: Bovine follicle stimulating hormone (bFSH) belongs to the family of glycoprotein hormones that are released from the pituitary gland or the placenta. This hormone is a heterodimer consisting of common α- and specific β-subunit. Bovine FSH is used for reproductive technology such as superovulation in farm animals and polycystic ovary syndrome treatment. Also this hormone is produced for clinical, veterinary purposes and infertility treatment. Recombinant DNA technology provides a useful tool for production of FSH free from hormonal, viral or prions contaminants. The objective of our study was the production of bFSH hormone in Pichia pastoris expression system.

Materials and Methods: For this purpose, two ORF strands containing α and β subunits were separately cloned into pTZ57R/T vector and then were subcloned into pPIC9 expression vector and confirmed by PCR and sequencing. The two recombinant plasmids were linearized using the SalI and SacI restriction enzymes in order to generation of His+ Mut+ GS115. The products were co-transfected into the competent yeast cells by electroporation and examined by PCR.

Results: Expression of recombinant bovine FSH in both yeast cells supernatant and pellet was confirmed by SDS-PAGE and Western blotting techniques.

Conclusion: Production of recombinant protein in eukaryotic expression systems such as pichia pastoris is a reliable method for producing of therapeutic bovine FSH. Also the detection of the recombinant produced protein by specific antibody confirmed a correct post translated modified structure.

Keywords: Bovine Follicle Stimulating Hormone, Expression, Pichia Pastoris

P-198: Utilization of Pichia Pastoris Secretion System for Expression of Equine Follicle Stimulating Hormone

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Background: Equine follicle stimulating hormone (eFSH) is a pituitary heterodimeric glycoprotein consists of noncovalently linked of generic alpha subunit and a hormone specific beta subunit. The molecular weights of the subunits are similar and about 16 KD. In general, FSH plays a key role in controlling vertebrate gonadal functions. In female mammals, ovarian maturation and follicular growth is critically dependent on FSH stimulation.
Materials and Methods: The purpose of this study was to clone and express the Equine follicular stimulating hormone in Pichia Pastoris. Extracted total mRNA from Iranian Thoroughbred horse's anterior pituitary gland was used to synthesize the appropriate cDNA. The two genes encoding the alpha and beta subunits were cloned after amplification using the two specific primers. The genes were separately cloned into the pTZ57R/T vector before transforming into the DH5α strains of E.coli. Cloning was then confirmed by PCR and sequencing methods following by subcloning into the yeast pic-9 expression vector. The recombinant vectors were linearized by sall and sacl restriction enzymes and transformation into the Pichia Pastoris using the electroporation method. Positive cell lines were grown on Buffered Complex Methanol Medium (BMMY) containing methanol as the sole carbon source.
Results: Finally, the expression of recombinant eFSH protein was confirmed by SDS-PAGE and Western blotting methods.
Conclusion: Production of recombinant protein in eukaryotic expression systems such as pichia pastoris is a reliable method for producing of therapeutic equine FSH. Also the detection of the recombinant produced protein by specific antibody confirmed a correct post translated modified structure.
Keywords: FSH, Pichia Pastoris, Equine, Cloning

P-199: Effect of Oxidative Stress on Sperm Quality and Mmu-miR-34a Expression in Testis of Mature Mouse

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Background: Male infertility is responsible for approximately 50% of infertility in the world. Reactive oxygen species (ROS) is one of the causative agents of infertility in males which effects on sperm quality and function. In this study, the effects of oxidative stress induced by tertiary-butyl hydroperoxide (TBHP) were investigated on sperm quality, testis tissue and miRNAs expression.
Materials and Methods: Adult male mice (9-10 weeks) strain Balb/c was randomly selected from mouse colony. After a primary study to determine LD50, TBHP was injected at the concentration of 1:10 LD50 for 2 weeks. The mice were sacrificed and their testis tissues were used for cell viability, ROS assay and miRNAs expression. Epididymis was also surveyed for sperm analysis by CASA system.
Results: The sperm motility, count and viability were decreased in the TBHP treated mice in comparison of the control mice. The flowcytometry analysis showed a significant increase in H_2O_2 and O_2•- levels in both testis and sperm 2 weeks after intra-peritoneal (ip) injection. Body weights revealed no treatment-related effects but atrophy of testis and decrease of testis cells viability was observed. Also, the expression of mmu-miR-34a in the experimental group decreased.
Conclusion: TBHP-induced oxidative stress caused to decrease in sperm vitality and motility and testis cells viability. Results indicated that oxidative stress induction in testis reduced its normal function. That is due to an increased level of H_2O_2 and O_2•- in testis and their deleterious effects on genomic levels.
Keywords: Male infertility, Oxidative Stress, Reactive Oxygen Species (ROS), Tertiary-Butyl Hydroperoxide (TBHP), MiRNA

P-200: Frequency of Heterochromatin Polymorphisms in Couples with Recurrent Abortions in Patients Refer to IVF Clinic of Yazd

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Background: Recurrent pregnancy loss (RPL) is a multifactorial problem associated with genetic abnormalities

reflected by inherited disorders. The aim of the present study was to investigate the contribution of heterochromatin polymorphism in couples with recurrent miscarriages compared with couples without miscarriages.

Materials and Methods: Over a 3 year period, we made a study of the diagnostic significance of heterochromatic variants in 467couples with a history of recurrent abortions and a control group of 189 couples who referred for investigation rather than abortion or diseases.

Results: The results indicated significant increases of heterochromatin polymorphic variants in couples with RPL compared with couples without RPL (12.2% vs. 7.3%) p=0.0.

Conclusion: Most often, chromosomes vary in size and position of heterochromatin in 1qh, 9qh, and 16qh regions. Although inherited variants have been reported not to be associated with any risk for phenotypic abnormalities, chromosomal heteromorphisms have been found to have a higher frequency relative to the normal population and have been regarded as abnormalities in some studies. Potential epigenetic, genetic, and chromosomal modifications could be associated with certain complex disorders such as infertility and bad obstetric history.

Keywords: Heterochromatin, Polymorphism, Infertility, Karyotype, Recurrent Abortion

P-201: Prevalence of 4977bp Deletion in Mitochondrial DNA in IVF Failure Women

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Background: Successful IVF process is limited by factors such as oocyte quality. Oocyte quality can be defined as its abilities to be fertilized, mature and give rise to normal offspring and it is dependent on nuclear maturation and cytoplasm maturation. Damage to mitochondrial DNA (mtDNA) has been described in oocytes in IVF failure women that decrease cytoplasmic quality because Mitochondria are essential for respiration and oxidative phosphorylation of the cell. The presence of mtDNA 4977bp deletion in many different tissues can serve as a marker of this damage. However, no attempt has been made to detect the presence of mtDNA 4977bp in blood cells of these women. This is the first report concerning the 4977bp deletion in IVF failure women.

Materials and Methods: Polymerase chain reaction techniques (PCR) were used to detect mtDNA 4977bp deletion in blood samples of 100 IVF failure women and compared with 100 women who had success IVF procedure.

Results: The prevalence of 4977bp deletion in mtDNA was 88% in IVF failure women and 45% in control samples.

Conclusion: The higher prevalence of mtDNA 4977bp deletion in patients in this study indicates that damage to mtDNA in blood cells can serve as a marker that demonstrate reduced quality of oocyte.

Keywords: Mitichondrial DNA, IVF Failure

P-202: StuI Polymorphism on the Androgen Receptor Gene in Women with Endometriosis

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Background: Androgens have an anti-proliferative effect on endometrial cells. Human androgen receptor (AR) gene contains two polymorphic short tandem repeats of GGC and CAG, and a single-nucleotide polymorphism on exon 1 that is recognized by the restriction enzyme, StuI. Prior studies have shown that the lengths of the CAG and GGC repeats are inversely and linearly related to AR activity and associated with endometriosis and endometrial cancer. However, little is known about the single nucleotide polymorphism of the AR gene. Thus, we investigated whether these AR polymorphisms are risk factors for endometriosis.

Materials and Methods: The genetic distributions of these polymorphisms were investigated in blood samples from 100 endometriosis patients and healthy controls. The allelic profiles were analyzed by polymerase chain reaction (PCR), PCR-restriction fragment length polymorphism (PCR-RFLP) and analyzed statistically.

Results: The observed frequencies of GG, GA and AA genotypes of the G1733A polymorphism were 0.14, 0.68 and 0.18, respectively, for the patient group and 0.68, 0.3, and 0.02, respectively, for the control group. Allele frequencies were 0.48 and 0.83, respectively, for the patient and control groups for the G allele (wild type) and 0.52 and 0.17, respectively, for the patient and control groups for the A allele (mutant). Statistical analysis of these results indicated significant differences between the two groups (p=0.00014).

Conclusion: These results indicated for the first time that the androgen receptor G1733A polymorphism is strongly associated with increased risk for endometriosis.

Keywords: Endometriosis, Androgen Receptor

P-203: Investigating Association of HLA-G Gene Polymorphisms and Failed Implantation in Human Embryos

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Background: HLA-G is a non-classical human leukocyte antigen expressed primarily in fetal tissues at the maternal-fetal interface. The unique structure of HLA-G molecule permits a restricted peptide presentation and allows the modulation of the immune system. There is now general agreement that HLA-G is an important immunotolerant molecule with the capability of inhibiting immune cell functions, such as those of natural killer (NK) cells, T lymphocytes and dendritic cell. Recently, 44 HLA-G alleles have been described which encode 14 distinct functional proteins.
Materials and Methods: To investigate whether HLA-G gene polymorphism is associated with failed implantation, we used polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) for exon 4 of HLA-G gene in 100 couples with failed assisted reproductive technology (ART), such as IVF/ICSI, in their history and 100 couples with normal fertility in their history from Roy an institute DNA bank. PCR products of exon 4 were digested with three restriction endonucleases (MspI, TspRI and NspI) that have restriction site for HLA-G 010404, 0108 and 0106 alleles. Therefore digested fragments of the PCR products were separated by 2% agarose gel and detected by ethidium bromide staining.
Results: The obtained results indicate that HLA-G 0106 alleles in failed ART group were significantly higher than control group ($p<0.05$). In the other hand, the data have shown numbers of cases with HLA-G 010404 polymorphism in the two groups had no significant difference. Also HLA-G 0108 polymorphism was not seen in the groups.
Conclusion: The significant genotype-specific risk in failed ART group suggested that allelic variation in α3 domain of HLA-G have associated to failed implantation.
Keywords: HLA-G Polymorphism, Implantation, Assisted Reproductive Technology

P-204: Evaluation of DPY19L2 Gene Deletion As A Major Cause of Globozoospermia, in Iranian Globozoospermic Infertile Men

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Background: Male infertility is a Multifactorial syndrome encompassing a wide variety of disorders. In more than half of infertile men, the cause of their infertility is unknown (idiopathic) and could be congenital or acquired. Globozoospermia, also called round-headed spermatozoa, is a rare disease with incidence< 0.1% among male infertile patients. The most prominent feature of globozoospermia is the malformation or totally absence of the acrosome, caused by spermatogenesis defects. Through the study of family cases of globozoospermia, researchers recently identified a large deletion about 200kb encompassing the whole DPY19L2 gene. This gene is located on 12q14.2, has 22 coding exons and is flanked by two low copy repeat (LCR) regions sharing 96.5% of homology. The mechanism underlying the deletion is most probably due to non-allelic homologous recombination (NAHR) between the LCR. Due to the lack of any report study regarding to the prevalence of globozoospermia in Iranian infertile men, the aim of this study is detection of DPY19L2 gene deletion in Iranian globozoospermic infertile men.
Materials and Methods: Blood samples were taken from total of 25 globozoospermic patient after filling a consent form. Genome samples were extracted and were subjected to PCR for detection of exon 1 in DPY19L2 gene.
Results: Data were evident that there was a deletion in DPY19L2 gene in 20 out of 25 patients.
Conclusion: This deletion in DPY19L2 gene could be considered as a pathogenic factor for onset of globozoospermia.
Keywords: Globozoospermia, DPLY19L2

P-205: Analysis of Mlh3 C2531T Polymorphism in Iranian Women with Unexplained Infertility

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Background: Infertility is increasingly recognized as a major health problem. Meiotic genes are very important candidates for genes contributing to female and male infertility. Mammalian MutL homologues have dual roles in DNA mismatch repair (MMR) after replication errors and meiotic reciprocal recombination. There are four MutL homologues in eukaryotes that mutations of three of them (Mlh1, Mlh3, and Pms2) result in meiotic defects. Previous studies demonstrated that male and female Mlh3-deficient mice mated normally but were infertile. In female mice Mlh3-/- oocytes showed abnormalities in extrusion of the first and second polar bodies after *in vitro* fertilization with normal sperms of wild type mice, .The MutL homologs, Mlh1 and Mlh3, are crucial for meiotic reciprocal recombination. In our current study, we show that common SNP C2531T in the Mlh3 gene is associated with unexplained infertility in Iranian women.
Materials and Methods: We investigated Iranian patients, including 105 women with unexplained infertility and 100 women with at least one child and no history of infertility or abortion as controls.
Results: Genotypes CC, CT and TT of the Mlh3gene

presented frequencies of 4.76%,77.15% and 18.1%, respectively, in the women with unexplained infertility and 25%,73% and 2% in the controls (p<0.001).
Conclusion: The data suggest that the Mlh3 C2531T polymorphism can be associated with risk of female infertility and presence of the polymorphic allele T leads to an increased risk of 2.09 times (OR =2.09, 95% CI =1.38-3.16; p<0.001) to develop infertility in relation to the control group.
Keywords: Infertility, Meiotic Genes

P-206: Mov10l1 Gene Polymorphisms Are Probably Associated to Male Infertility in Azoospermic Men with Complete Maturation Arrest

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Background: Thousands of genes are involved in spermatogenesis. Alterations in any of these genes could lead to male infertility. Moloney leukemia virus 10-Like 1 (Mov10l1) gene is one of the genes that are expressed specifically in germ cells. Genetic disruption of this gene in mouse stops spermatogenesis during Meiosis I and causes azoospermia.
Materials and Methods: In this study, the genetic changes of mov10L1 gene, analyzed in a population of 30 infertile patients with a complete maturation arrest in spermatocyte level as the patient group and 70 fertile men who had at least one child as the control group. After DNA extraction from blood samples of selected individuals, PCR-SSCP method was done to verify presence of any polymorphisms/mutations. Ultimately sequencing was used to confirm genetic changes of the mentioned gene.
Results: As a result, eight single nucleotide transitions were identified, including; a missense (rs2272837) and four nonsense polymorphisms (rs2272836, rs11704548, rs2272838, rs138271) in the exonic sequences and 3 polymorphisms (rs12170772, rs2272840, rs17248147) in the intronic regions. All these have been registered as single nucleotide polymorphisms in NCBI-SNP database. Except rs2272838, a statistically significant association was shown in all polymorphisms between two groups (p<0.05).
Conclusion: Based on the results, it is expected that mutations and polymorphisms in mov10L1 gene could be a genetic factor in the incidence of infertility in men which requires further studies.
Keywords: Azoospermia, Polymorphism, Mov10l1

P-207: Study of Relationship among Six SNPs in PRM1, PRM2 and TNP2 Genes and Idiopathic Azoospermia in Iranian Infertile Men

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Background: Histones are replaced by protamines to condensate and package DNA into the sperm head during mammalian spermatogenesis. Protamine genes defects have been reported to cause sperm DNA damage and male infertility. In this study relationship among some protamines genes family SNPs include PRM1 (C321A), PRM2 (C248T) and TNP2 (T1019C), (G1272C), (G del in 1036 and 1046 bp) were studied in 96 idiopathic infertile men with azospermia or oligospermia and 100 normal control men.
Materials and Methods: Analysis of SNPs was performed using restriction fragment length polymorphism (PCR-RFLP), single strand conformational polymorphism (PCR-SSCP) and PCR sequencing.
Results: No polymorphisms were found for tested SNPs except for PRM1 (C321A) and TNP2 (G1272C) in which frequency of altered AA and GG genotypes were slightly higher in infertile case group. Statistical analysis showed no significant association related to PRM1 (C321A) p=0.8 and TNP2 (G1272C) loci p=0.6.
Conclusion: These results are consistent with previous studies and indicating that all tested SNPs were not associated with oligospermia and azospermia and idiopatic male infertility in Iranian population.
Keywords: Male Infertility, SNP, PRM1Gene, PRM2 Gene, TNP2 Gene

Reproductive Imaging

P-209: The Value of Hysterosalpingography in the Diagnosis of Tubal Pathology Among in Infertile Patients

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Background: Infertility, defined by the failure to achieve a clinical pregnancy after twelve months or more of regular unprotected sexual intercourse. infertility is an important health problem in the society .tubal pathology is one of the main causes of infertility.it is estimated to account for 12-33%.hysterosalpingography is a method used for screening purposes in the routine infertility evaluation.
Materials and Methods: These paper reviews the value

of hysterosalpingography in the diagnosis of tubal pathology among in infertile patients, using 20 articles published from 2004 t0 2011 in PubMed.gov, SID, science direct, Medline, Proquest.

Results: The diagnostic accuracy of hysterosalpingography in the diagnosis of tubal pathology depends on selected target condition. Diagnostic accuracy of hysterosalpingography is lacking in the diagnosis of general tubal pathology, peritubal adhesions, and tubal occlusion when target condition is defined as any form of tubal occlusion. Diagnostic accuracy of hysterosalpingography is good in the diagnosis of tubal occlusion when target condition is defined as two-sided tubal occlusion. Hysterosalpingography is useless test in ruling in and ruling out the diagnosis of general tubal pathology and peritubal adhesions. Hysterosalpingography is a fair clinical test in ruling in and ruling out the diagnosis of tubal occlusion when pathology is defined as any form of tubal occlusion. Hysterosalpingography is a good clinical test in ruling in and ruling out the diagnosis of tubal occlusion when pathology is defined as two-sided tubal occlusion.

Conclusion: hysterosalpingography is more accurate in the diagnosis of tubal occlusion .HSG is helpful in the investigation of infertile women.

Keywords: Hysterosalpingography, Infertility, Tubal Pathology

P-210: The Antifertility Effects of Cornus Mas in Adult Male Rats

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Background: Fertility control is an issue of global and national public health concern. Though considerable progress has been made in the development of methods of contraception among females, progress and possibilities are still slow and limited. Therefore, there is a need to develop new contraceptive modalities for males. In this study the antifertility activities of Cornus mas were evaluated in male rats.

Materials and Methods: In 40 male adult rats, weighing 180-210 g, the methanolic extract of Cornus mas was administered at four doses 400,200,100 and 50 mg/kg for 21 days. The control group received normal saline for the same duration. Animals were sacrificed and the testes were collected for histopathological studies.

Results: Cornus mas treatment for 21 days caused appreciable alterations in histological appearance of the testes. There were various degrees of damage and lytic necrosis to the seminiferous tubules. Severe interstitial edema, hyperemia and dissociation of germinal epithelium were prominent. Intertubular multinucleated giant cells, also, were found. Histological changes were severe in the groups which received higher doses of Cornus mas extract.

Conclusion: The results of this study showed that methanolic extract of Cornus mas in higher doses has antifertility properties.

Keywords: Cornus Mas, Male Rats, Fertility, Testes

P-211: The Effects of Vasectomy on Histological Structure of Epididymis and Testis in Rat Model

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Background: Vasectomy is a simple and highly effective contraceptive method with a low morbidity rate and an extremely low mortality rate. during the past decade millions of men have undergone vasectomy, but there have been surprisingly few studies of morphological effects of genital organs after vasectomy. The most important issues about vasectomy are degenerative changes in testicular tissue, epididymitis, and the occurrence of infectious mass. Also the reversibility of pregnancy should be mentioned. Then the aim of the present study was to evaluate effect of vasectomy on the testicular functions of rat.

Materials and Methods: In this study 20 adult male wistar rats (220 ± 20 g) were randomly divided into two groups with one group was sham operated model and vasectomized group . All rats were sacrificed by CO_2 inhalation 120 days after operation and the testis was removed and fixed in 10% formalin fluid and processed for paraffin embedding. Five µm thick sections were stained with HandE.Light microscopical analysis was made to evaluate the structural changes induced in the testis ($p<0.05$).

Results: The histological and histomorphometrical study of testis confirmed that there was an obvious structural changes such as apearance of vacuolated edema in the interstitial connective tissue between the seminiferous tubules with increased hypertrophicf mass of leidig cells and decrease in seminiferous tubules diameter (STD), Tubule Differentiation Index (TDI) and spermatogenesis in the semniferous tubules ($p<0.05$).

Conclusion: In this investigate we observed degenerative changes of testes tissue by pressure of accumulated sperms and semen fluid in epididymis on epithelial tissue of seminifrous tubules.

Keywords: Vasectomy, Histological, Testis, Seminiferous Tubule, Rat

P-212: The Effect of Ginger Hydro-Cohol Essence on Cyclophosphamide Side Effects in Spermatogenesis Processes

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Background: Cyclophosphamide as an alkalizing and cytotoxic agent contributes in treatment of different kinds of cancer. It can pass the blood-brain barrier. Linking two strains of DNA in cell nucleus and breaking the inner and outer strain linkages and inhibiting RNA synthesis and as a consequence, inhibiting protein synthesis, cyclophosphamide demonstrates severe pharmacological side effects on the body. The most important cyclophosphamide side effects consist of azoospermia, reduction of gonadal hormones in hypothalamo-hypophyseal axis, reduction of sperm, infertility, cardio-vascular disorder, nephrotoxicity, bladder fibrosis and hemorrhagics cyct.

Materials and Methods: In this study, forty adult male Balb/c mice (about 20 g) were examined in four groups of ten mice. A group was received neither solotion nor the drug, control group received only the vehicle and experimental groups received cyclophosphamide (5 mg/Kg/BW) Intraperitoneally (IP). Then hydroalcoholic ginger extract was administered (1g/Kg/BW) orally along with cyclophosphamide in 14-day period.

Results: In this study, Kruskal–Wallis test was used to analyse and calculate plasma concentrations of hypophyseal gonadotropic hormones (FSH, LH), testosterone and also spermatogenesis. Our result demonstrated that the experimental groups which received hydroalcoholic ginger extract simultaneously with the drug cyclophosphamide, significantly ($p<0.05$) had eleveted plasma concentrations of gonadotropic hormones and spermatogenesis related factors during receive of hydroalcoholic ginger extract.

Conclusion: This work has shown that hydroalcoholic ginger extract (1g/Kg/BW) is able to reduce pharmacological side effects of cyclophosphamide ($p<0.05$).

Keywords: Cyclophosphamide, Ginger Hydro-Cohol Essence, Spermatogenesis

P-213: Comparison of Controlled Ovarian Stimulation with Clomiphen Citrate + HMG or Clomiphen Citrate + rFSH in IUI Cycles

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Background: Different protocols are used for controlled ovarian hyper stimulation (COH), but the optimal method has not yet been determined. So we decided to compare the outcome of controlled ovarian stimulation using clomiphen citrate +HMG versus clomiphen citrate +rFSH in IUI cycles.

Materials and Methods: 144 women with unexplained or male factor infertility undergoing IUI cycles (72 patients in CC+ rFSH group and 72 patients in CC+ HMG group) were randomized and included in this single blind study. The study was performed in Amir-Al-Momenin university hospital from October 2006 to June 2010.The primary outcomes were clinical and ongoing pregnancy rates. The number of dominant follicles, mean of follicular size, endometrial thickness on the day of HCG administration, total dose of gonadotropins and duration of stimulation with gonadotropins were secondary outcomes.

Results: Clinical and ongoing pregnancy rates were not significantly different from each other in the two groups (11.1%, 9.7% in CC + HMG group and 12.5%, 12.5% in CC + rFSH group, respectively; $p>0.05$).There were no statistically significant differences in secondary outcome measures between the two groups.

Conclusion: Considering the similar pregnancy rate, the similar total dose and duration of stimulation with gonadotropins in both groups and the higher cost per ampoule in CC + rFSH group versus CC + HMG group, it seems that CC + HMG is a more suitable and cost-effective regimen than CC + rFSH in IUI cycles in patients with unexplained or male factor infertility.0ur sample size was small.So further larger studies are necessary to confirm our results.

Keywords: Clomiphen Citrate, Controlled Ovarian Stimulation, HMG,IUI,r-hFSH

P-214: The Rate of *In Vitro* Oocyte Maturation, Fertilization and Embryo Development in Presence of Melatonin in Mice

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Background: It is important to protect oocytes and embryos from oxidative stress in the culture medium. Melatonin has been shown to be a direct free radical scavenger. Therefore, effect of melatonin during *in vitro* oocyte maturation (IVM), fertilization and embryo development of mouse oocytes was evaluated.

Materials and Methods: Oocytes from supper-ovulated mouse were divided to two groups: cumulus–oocyte complexes (COCs, group i) and denuded COC (d-COC, group ii). The oocytes were cultured in maturation medium with different dosages of melatonin (1 – 110 – 105 nM). The cumulus expansion and nuclear status were evaluated after 24 hours of *in vitro* maturation. The oocytes were used for *in vitro* fertilization (IVF). The fertilized oocytes were cultured in medium supplemented with different doses of melatonin.

Results: The expansion (86.79%) and maturation (80.55%) rate of COCs increased in supplemented medium with 10 nM of melatonin Vs control group (73.33%, $p<0.01$), but oocytes without cumulus cells indicated higher maturation rate at higher melatonin doses (10 and 100 μM, 84.34 and 79.5% vs. 69.33% in control group ($p<0.01$). Fertlization rate was higher in treated medium with 1 μM of melatonin (93.75 %, $p<0.01$). The

rate of cleavage and blastocyst formation was promoted in medium supplemented with 10 and 100 nM of melatonin (92.37 and 89.36% vs. 81.25% in control group ($p<0.01$). We observed a dose dependent response to melatonin treatment in this experiment.

Conclusion: Exogenous melatonin can promote cumulus cell expansion, IVM, and embryo development. However we investigated a dose-dependent response in different stages of maturation and development. It may reflect sensitive rate of oocytes and embryos to culture conditions.

Keywords: IVM, Development, IVF, Melatonin, Cumulus–Oocyte Complex

P-215: Study of Some Related Factors with Fetal Macrosomia and Low Birth Weight

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Background: Birth weight are the major factors determining the physical and mental development of infants and credible signs of intrauterine growth. The purpose of this study was the Study of birth weight on maternal and neonatal factors in the women referred to delivery room of Specialized Hospital in 2010.

Materials and Methods: In this cross sectional study, 3076 women with the normal vaginal delivery (NVD) entered into the study population. The Statistical Package for the Social Sciences, SPSS 17 for Windows, was used for the analysis. Data analysis was performed by using descriptive and analytical statistics (mean ± SD), Chi-square test, independent sample t test, ANOVA, post hoc scheffe and liner regression). The significant level was set at less than 0.05 ($p<0.05$).

Results: The prevalence rate of low birth weight was 6.7% (205) and macrosomia 3.2% (97). Average neonatal birth weight was 3248.18 ± 458 g and maternal age was 25.33 ± 5.7 years. On the basis of linear regression, types and number of delivery, neonatal sex, preeclampia and age of mother were respectively the most factors of low birth weight. None of them relate to macrosomia.

Conclusion: Our findings showed by detection and control of many factors which relate to birth weight, we can decrease prevalence of low birth weight and macrosomia and result in normal birth weight.

Keywords: Low Birth Weight, Fetal Macrosomia, Newborn

P-216: The Protective Effect of Saturejakhuzistanicaagainst Female Mice Follicle Defects Induced by Busulfan

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Background: In the past 2 decades, the survival rates for many of the malignancies that affectyoung women have significantly improved. For many of these malignancies, survival rates exceed 80-90%. Therefore, the remote effects of cancer treatment have recently gained worldwide interest, andthe protection against iatrogenic infertility caused by chemotherapy assumes high priority. Busulfan is a bifunctional alkylating agent.This produces reactive carbonium ions that can alkylate DNA and effects on ovary.. In this study we use extract of saturejakhuzistanicaas protective agent for restoration follicles againstthe side effects ofbusulfan on ovarian follicles.

Materials and Methods: 48 adult female mice were randomly divided into 6 groups (n=8): control group received only vehicle orally once a day,two chemotrapy groups were gavaged and injected (ip) with Busulfan,a group were gavaged with saturejakhuzistanica,and another two groups were gavaged and injected (ip) with Busulfan andweregavaged with saturejakhuzistanica.after 35 days we prepare cross section of ovarian tissues. the tissue section were stained with HandE and then consider underlight microscope observation.the number of different stage of growing and corpus loteumswere counted and then in this experiment the rate of healthy and atretic follicles were evaluated.

Results: The results obtained from this study showed thattreated mouse with busulfan can reduce thenumber of follicles and increased percent of atreticfollicles,Administration of saturejakhuzistanica in group 5 and 6 significantly restored the primordial follicles the deterioration of follicles by busulfan and improved quality and number of follicles in different stage of growth ($p<0.05$).

Conclusion: the present study indicates that administration of busulfan decrease number and quality of follicles and saturejakhuzistanica may have protective effect against busulfan defect.

Keywords: Busulfan, Saturejakhuzistanica, Ovary, Follicle, Aatresia, Mouse

P-217: The Effect of Progesterone on The *In Vitro* Maturation of Mouse Germinal Vesicle Oocytes Derived from Polycystic Ovary

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Background: Polycystic ovary syndrome (PCOS) is characterized by chronic anovulation and hyperandro-

genism and affects approximately 5-10% of women of reproductive age. *In vitro* maturation (IVM) of mammalian oocytes has become an efficient method to produce mature oocytes in order to use in assisted reproductive techniques such as *in vitro* fertilization (IVF), intra cytoplasmic sperm injection (ICSI), and cloning. In some mammals, oocytes maturation and its progression to metaphase II are steroid–dependent. Progesterone exists in the follicular fluid that contributes to normal mammalian ovarian function. It has been shown that, *in vivo* levels of progesterone in follicular fluid are closely associated with developmental competence of an oocyte. In PCOS, progesterone secretion and levels in ovary and follicular fluid is low. In this study, we examined the effects of progesterone on *in vitro* maturation of oocytes derived frompolycystic ovary in mouse.

Materials and Methods: To induce polycystic ovary phenotype, female NMRI mice 6-8 weeks-old were injected daily with testosterone enantate (TE)1 mg/100 g body weight dissolved in sesame oil (experiment group), while control group were injected only with vehicle for two weeks.Collected immature oocytes (germinal vesicle oocytes) of both groups were matured in modified medium α-MEM containing combinations of 5% FBS, 100 mIU/ml rFSH (Gonal-f), 10 ng/ml rEGF, 7.5 IU/ml HCG with progesterone (3 mg/ml), (treatment I) and without progesterone (treatment II) for 24 hours. The number of oocytes at the germinal vesicle (GV), germinal vesicle breakdown (GVBD) and metaphase II (MII) stages were counted.

Results: After 24 hours of transfer to maturation media, the percentage of MII oocytes in experiment group,treatments I and II, was 49.65% and 19.40% and in control group, treatments I and II was 31.51% and 36.21%. After 24 hours, exposure of progesterone, the percentage of MII oocytes were significantly increased in experiment group compared to control group (ANOVA, $p<0.05$).

Conclusion: Adding progesterone to the medium could improve maturation of mouse germinal vesicle oocytes. It be concluded that progesterone can be to improve oocyte *in vitro* maturation in polycystic ovaries.

Keywords: Polycystic Ovary Syndrome, Progesterone, Germinal Vesicle Oocytes, Mouse

P-218: Demographic Characteristics and Factors Affecting Infertility in Men

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Background: Infertility about half of the men. Some factors affecting infertility is related to genetic and environmental factors. This study examined demographic characteristics of men with infertility were referred to the Shariati Hospital Infertility Clinic.

Materials and Methods: This is a cross-sectional study. 200 eligible patients were enrolled with written consent Face-to-face interviews were based on a questionnaire that included variables on socio-demographic characteristics and Sperm analysis test performed by all individuals. Their height and weight measurements were recorded.

Results: The mean age was 5.7 ± 34.1. The average years after marriage number was 1.3 ± 3.6 years. BMI was 5.7 ± 24.1 indicating obesity among men was studied. The greatest number of people who were self-employed, the majority of people in their jobs were dealing with chemicals. However, this study found that the risk of diabetes disease, mumps, varicocele among workers who were worker. The sperm analysis showed that The most common Problem related to decreased sperm motility in infertile men. So that more than 162 participants in the study were 40% lower sperm motility.

Conclusion: In this study the relationship between of factors such as obesity, smoking, BMI, occupational status and previous disease and sperm parameters in infertile men was not significant.

Keywords: Semen Analysis Parameters, Infertility, Sociodemographic Characteristics

P-219: GDF9, BMP15 and Their Receptors Expression During *In Vitro* Culture of Mouse Vitrified Ovarian Tissue Derived Preantral Follicles

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Background: *In vitro* culture (IVC) of isolated preantral follicles from cryopreserved ovarian tissue might be an efficient method for enhancing mature oocytes in patients who are exposed to infertility.

Materials and Methods: Ovaries of 13-day old NMRI mice were removed and randomly placed into control,

needle immersed (NIV) and solid surface vitrification (SSV) groups. For vitrification, ovaries were transferred into equilibration [7.5% (EG and DMSO)] and vitrification medium [15% (EG and DMSO) and 0.5 M sucrose], then they immersed in liquid nitrogen after loading by acupuncture needle in NIV group and cooling on pre cooled steel surface in SSV group. Thawing was done in 2steps (1 and 0 M sucrose solution). Mechanically isolated preantral follicles were cultured in α-MEM supplemented with (FSH, LH, ITS, FBS) for 12 days. The expression rate of maturation genes (GDF9 and BMP15) and their receptors (BMPR2, ALK5 and ALK6) in all experimental groups were evaluated quantitatively by real time PCR after 24 hour, 6, 10 and 12 days of culture.

Results: No significant difference was observed in the expression of maturation genes and their receptors between vitrification (NIV and SSV) and control groups and also between NIV and SSV groups. It must be noted that the expression patterns of mentioned genes in two vitrification groups were similar to the control one and assimilate the *in vivo* pattern in somehow.

Conclusion: Although the cooling rate in SSV method is delayed compared to NIV method, the pattern of maturation genes expression during IVC was similar in both groups and it seems that preantral follicles compensate cryoinjuries during IVC period.

Keywords: Preantral Follicles, *In Vitro* Culture, Maturation Genes, Ovarian Tissue

P-220: Vitamin E Protects Atrazine-Induced Toxicity in Testes of Mature Male Rats

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Background: According to several reports there is a positive correlation between the lowered quality of semen and any exposure to toxic compound specially those particularly disrupt endocrine functions. Like other herbicides atrazine (ATR) induces endocrine disruption and consequently interferes with sexual hormones physiological functions. Therefore current study was designed in order to evaluate the effect of long-time exposure to ATR on spermatogenesis, spermiogenesis, leydig cells distribution, sperm chromatin condensation and DNA integrity.

Materials and Methods: To follow-up present study 18 mature male rats were used. The animals assigned in to three groups as control-sham (0.2 mg/kg, orally, once a day, for 48 days) ATR-administrated (300 mg/kg, orally, once a day, for 48 days) and ATR+vitamin E (150 mg/kg, orally, once a day, for 48 days) groups. The percentage of tubules with normal spermatogenesis and spermiogenesis were evaluated in all groups. Moreover the aniline-blue staining was performed for analyzing sperm chromatin condensation and DNA integrity was evaluated using acridine orange staining technique.

Results: Histological observations demonstrated that, the percentage of tubules with arrested spermatogenesis and spermiogenesis significantly increased in ATR-induced rats. Following ATR-exposure, the percentage of sperms with damaged DNA and percentage of sperms with impaired chromatin condensation enhanced into about 57- and 59- folds more than control-sham respectively, while the Vit E-received groups manifested with 5 and 6% reduction in comparison to ATR-administrated ones.

Conclusion: Our findings showed that in the case of chronic exposure to ATR, the Vit E administration will be capable to prevent the ATR induced detrimental affects by lowering spermatogenesis and spermiogenesis arrest, and as well by reducing the DNA damages and nuclear immaturity.

Keywords: Atrazine, Spermatogenesis, Spermiogenesis, DNA Damage, Chromatin Condensation

P-221: Decision for Disclosure: The Experiences of Infertile Women Undergoing Assisted Reproductive Donation Procedures

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Background: Controversy surrounding disclosure among the recipients of assisted reproductive donation procedures is escalating worldwide, whereas little research has been conducted in this area .The purpose of this qualitative study was to explore the experiences of infertile women undergoing assisted reproductive donation procedures in relation to their decision for disclosure.

Materials and Methods: In an exploratory qualitative study 15 infertile women who were candidate to use donor egg, donor embryo or surrogacy were purposively selected from Montaserieh Infertility Research Center at Mashhad, Iran and interviewed using semi-structured method. Data were analyzed using conventional content analysis. Member check and peer debriefing were used to ensure trustworthiness.

Results: Two broad themes were identified: The recipient's decision to inform family members and friends and the recipient's decision to inform the resulting child. Most recipients decided not to inform family members and friends from using of donation procedures. The reasons for this decision were: negative values and beliefs of society, people's secret talk, social stigma, concern about potential harm of disclosure, fear of other's judgment, absence of compelling reason to disclose, and other's advice. Few women decided to disclose the issue due to family support, being released from internal pressure, and spouse view. Regarding informing the re-

sulting child, most recipients decided not to inform the child from reproductive process. The rationales for this decision included welfare of the child, child's protection and acceptance of the child by others, concern about the child's possible reaction, and absence of compelling reason for disclosure. Women with potential decision for disclosure indicated that changes in vision of society and improvement of the social climate with passage of time may direct them to disclose the issue to the child.

Conclusion: The main reason for secrecy was the concern of the societal negative views about assisted reproductive donation procedures. This worry itself deprives the women from family and friends' support and as a result causes tolerating the psychological pressure of using such procedures. It is therefore suggested to make people informed of donation procedures as a therapeutic approach for infertility through media in order to alter public views and also enhance the social recognition of these procedures, which will mediate infertile women's concerns.

Keywords: Disclosure, Infertile Women, Assisted Reproductive Donation Procedures, Qualitative Study

P-222: The Effect of Vitex Agnus Castus Essential Oil on Seminiferous Tubule Structure in Mice

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Background: Plant materials continue to play an important role in the maintenance of human health since antiquity. Phytoestrogens are naturally occurring plant substances that can either mimic or antagonize the action of endogenous estrogens. This study was conducted to investigate the effects of vitex agnus as a phytoestrogenic plants, essential oil on structure and morphology of seminiferous tubule in mice.

Materials and Methods: In this study, 25 adult male mice were divided in to five groups: group 1 and 2 as control and sham respectively, group 2 received 75 mg/kg, group 3 received 1000 mg/kg and group 4 received 1500 mg/kg essential oil via gastric gavage for seven consecutive days. After this time, animals sacrificed by cervical dislocation. Then both testes were taken immediately and fixed in Boein's solution and processed by standard and routine paraffin embedding. 5-7µ thickness serial sections were stained by Hematoxylin and Eosin. They were studied under the light microscope and photomicrographs were taken.

Results: Histological studies revealed that vitex essential oil can be damaged Spermatogenic germinal epithelium of seminiferous tubules and reduced height of germinal epithelium. In low doses, changes include appearance of vesicles in the germinal epithelium, changing the structure and morphology of Sertoli cells and changes in form and density of spermatogonia nucleus. In group 3, Sertoli cell height reduced significantly and also decreasing of germinal epithelium height was observed. In doses of 1000 and 1500, lumen diameter of seminiferous tubule was increased significantly. Severe damage of germinal epithelium and arrangement of Spermatogenic cells were detected.

Conclusion: Finally, it can be concluded that vitex agnus essential oil affect seminiforus tubule and its damaging is in dose dependent manner.

Keywords: Mice, Seminiferous Tubule, Vitex Agnus Castus

P-223: Analysis of Synaptonemal Complex Gene Disorders Involving in Recurrent Spontaneous Abortion

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Background: Spontaneous abortion (SAb) is the most common complication of early pregnancy. Numerous risk factors are associated with an increased risk of pregnancy loss such as: Maternally age, previous spontaneous abortion, prolonged ovulation to implantation, Gravidity, Interval Prolonged time to pregnancy, Balanced chromosomal translocations and Genetic disorders. The aim of this study was to determine the frequency of balanced chromosomal translocation in couples with a history of recurrent spontaneous and possibly genetic disorder which may be involved in synaptonemal complex during the posses of meiosis.

Materials and Methods: Sixty eight couples with the history of spontaneous abortion were selected and introduced into this survey during 2007-2012 (department of Medical Genetics department of Mashhad University). Giemsa banding technique was used to searching for chromosomal balanced translocation. However it should be mentioned that, this techniques will not be able to detect balanced chromosomal micro rearrangements which are behind the 4 mega base pair. PCR technique was used in order to diagnose of some synaptonemal complex involving genes disorders in spontaneous abortion.

Results: Results of our research are gathering and doing with PCR for diagnosis in synaptonemal complex gene disorders as soon as possibility.

Conclusion: It would be reasonable to suggest that single gene determinants may play an important role in such complication pregnancy than synaptonemal complex in chromosomal disorders.

Keywords: Spontaneous Abortion, Chromosomal Rearrangement, Synaptonemal Complex

P-224: The Effect of Alpha Lipoic Acid on ROS Production and TAC Levels of Cultured Isolated Pre-antral Follicles Drived from Mice Vitrified Ovaries

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Background: Ovarian cryopreservation is an efficient way to preserve fertility of cancer patients before chemotherapy. This study was designed to evaluate the effect of apha lipoic acid (ALA) on reactive oxygen species (ROS) production and total antioxidant capacity (TAC) of cultured isolated pre-antral follicles derived from vitrified and non-vitrified ovarian tissue.
Materials and Methods: Immature mouse ovaries were vitrified by cryotop method. Pre-antral follicles were isolated and cultured in α- minimal essential medium supplemented with 100 um of ALA. Follicular growth, oocyte maturation were evaluated. Separately, ROS and TAC were measured after 0, 24, 48, 72 and 96 hours of culture with spectrofluorometery and ferric reducing/antioxidant power (FRAP) assay, respectively.
Results: The rates of survival, growth, antrom formation and MII oocytes were significantly lower in vitrified groups, whereas in the presence of ALA were significantly higher in both vitrified and non-vitrified groups ($p<0.05$). ROS production of pre-antral follicles in vitrified and non-vitrified groups was increased, and TAC levels were decreased, while in the presence of ALA, ROS production was decreased and TAC levels were increased after 96 h of culture.
Conclusion: ALA improves the in vitro development of pre- antral follicles isolated from vitrified and non-vitrified ovaries through decreasing ROS concentration and increasing TAC level of during the culture period
Keywords: Ovarian Vitrification, ALA, ROS, TAC

P-225: The Reactive Oxygen Spieces Production of Mice Vitrified Isolated Pre-Antral Follicles Using Cryotop Methods

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Background: Cryopreservation is an important option for preserving the fertility. We examined the reactive oxygen species (ROS) production, total antioxidant capacity (TAC) and developmental competence, of vitrified isolated mice pre-antral follicles as an alternative method for restoring fertility.
Materials and Methods: The 140-160 µm pre-antral follicles mechanically isolated from ovaries of 14-day-old mice were exposed to equilibration solution consisting of 7.5% ethylene glycol (EG) and 7.5%dimethyl sulfoxide (DMSO) and 20% FBS for 5min and then exposed to vitrification solution composed of 15%EG, 15%DMSO, 20%FBS and 0.5M sucrose for 30 seconds and then were vitrified by using cryotop methods. Cryoprotectants were removed by equilibrating vitrified follicles in "warming solutions" consisting of descending concentration of sucrose and cultured for 12 days followed by an ovulation induction protocol at the end of this period to initiate oocyte maturation. In parallel, after 0, 24, 48, 72 and 96 hours of culture, ROS and TAC were analyzed by spectoflorometry and FRAP methods respectively.
Results: The growth rates of follicles in the second and fourth days of cultivation were higher in control group than vitrified group ($p<0.05$). The rates of Survival and antrom formation of control groups were significantly higher ($p<0.05$) than those of vitrified isolated follicles. Significantly fewer mature oocytes developed from vitrified-warmed pre-antral follicles than those of fresh controls (49.2 vs. 14.2%, $p<0.05$). ROS production, and TAC levels of culture of pre-antral follicles in vitrified and non-vitrified groups were decreased after 24 hours up to 96 hours, at iniate time of cultivation ROS production of vitrified follicle was significantly higher and TAC levels was significantly lower than non-vitrified samples ($p<0.05$)
Conclusion: Vitrification and *in vitro* culture of pre-antral follicles inducing the reactive oxygen species production.
Keywords: Pre-Antral Follicles, Vitrification, Cryotop, ROS, TAC

P-226: American Ginseng Effects on Damage Induced by Cyclophosphamide in The Nuclei of Rat Sperm

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Background: Oxidative stress occurs when the oxidative homeostasis is damage. Excessive ROS are generated and this reaction has been proposed to produce marked damage to the structure and function of sperm.
Materials and Methods: This study was designed to investigate the protective effects of of American Ginseng against cyclophosphamide - induced damage in the rat sperm. For this experiment, the mature male rats randomly divided into four groups, seven rats in each and were treatment with cyclophposphamide (6.1 mg/kg body weight) and ginseng (500 mg/kg body weight) for 50 days. The animals were killed one day after the last treatment. Samples of spermatozoafrom the cauda epididymidis were dissected immediately. Acridin orange and Aniline blue staining used for DNA denaturation and chromatin quality assay. Significance of difference between two groups was evaluated using Tukey test.
Results: In CP-treated group percentage of double stranded DNA and mature sperms decreased in the epididymal sperm as compared with the control group, while co-administration of ginseng decreased these alterations induced by CP.

Conclusion: Alkaliting agents destroy the DNA and chromatin structure and function in sperm nuclei and this damage could be reversed by American Ginseng.

Keywords: Cyclophosphamide, Acridin Orange Staining , Aniline Blue Staining, DNA Damage,Sperm,Rat

P-227: Effect of Cigarette Smoking on Seminal TAC and MDA Levels in Fertile and Infertile Men

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Background: Defective sperm function is now recognized as one of the most important causes of male infertility. Lipid peroxidation (LPO) is known to cause various impairments of sperm cells and may play a major role in the etiology of male infertility. Malondialdehyde (MDA) is one of the final products of lipid peroxidation in seminal plasma. Seminal plasma possesses a rich source of different enzymatic and non-enzymatic antioxidants that protect spermatozoa against oxidative stress as one of the mediators of infertility causing sperm dysfunction and low sperm quality. In this study we determined the relationship between cigarette smoking as an exogenous source of ROS in increased levels of MDA and decreased TAC to estimate the intensity of oxidative stress.

Materials and Methods: MDA concentration and the TAC levels of seminal plasma were measured in fertile cigarette smokers (n=15), fertile non-smokers (n=15), infertile cigarette smokers (n=15), infertile non-mokers (n=15) by TBARs and FRAP methods, respectively.

Results: The mean TAC in the seminal plasma of fertile non-smoker men was significantly higher than fertile smokers ($p<0.001$) and infertile non-smokers was significantly higher than infertile smokers ($p=0.008$). In contrast, MDA levels in fertile non-smoker men was lower than fertile smokers ($p=0.89$) and infertile non-smokers was lower than infertile smokers ($p=0.23$) but this difference wasn't significant.

Conclusion: It is suggested that antioxidant deficiency and LPO may contribute to reduction in sperm fertilization potential. Therefore cigarettes smoking is a risk factor for increased seminal oxidative stress in infertile men.

Keywords: Cigarette Smoking, TAC, MDA, Fertile and Infertile Men

P-228: Low Dose HCG Adjunct to r-hFSH/ GnRH Antagonist for Controlled Ovarian Stimulation in Assisted Reproductive Technology: A Prospective and Randomized Trial

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Background: Deep suppression of LH in GnRH antagonist cycles may have detrimental effect on reproductive out come. It was hypnotized that adding LH activity via the administration of low dose HCG in late follicular phase shortened the duration of stimulation, decreased consumption of gonadotropin and increased estrogen levels. This study was designed to evaluate the effect of adding low dose HCG in GnRH antagonist / r-hFSH protocol on the outcome of assisted reproductive technology in the patients undergoing IVF/ICSI cycles.

Materials and Methods: This study was a prospective, randomized clinical trial conducted in Tehran University of medical Sciences. One hundred and twenty patients aged 21-39 years with an indication for IVF/ICSI were recruited. All patients received r-hFSH, when leading follicle reached 13-14 mm, a daily dose of cetrorelix (0.25 mg) was initiated. In addition, a daily injection of low dose HCG 200 IU was begun in a random fashion. The study was approved by the ethics committee of Teheran University of Medical Sciences.

Results: There was no difference in the number of retrieved oocytes, mature oocytes, endometrial thickness, fertilization rate, top quality embryo and total number of embryo between the two groups. Low dose HCG did not decrease the duration of treatment (11.88 ± 1.40) days in the study group vs (12.19 ± 1.31 day) in the control group, but significantly increase peak E2 (2565.40 ± 1758.60 pg/ml) in the study group vs (1788.35 ± 1298.02 pg/ml) in the control group. Although not significant, it was observed lower pregnancy rate in low HCG group (25% vs. 30%).

Conclusion: Our finding showed adding low dose HCG to GnRH antagonist in IVF/ICSI does not have positive effect on the outcome of the cycle.

Keywords: Assisted Reproductive Technology, GnRH Antagonist, Low Dose HCG

P-229: Uterine Transplantation, Is It A Promising Technique in Future?

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Background: Uterine factor infertility (UFI) refers to the refractory causes of female infertility stemming from the anatomical or physiological inability of a uterus to sustain gestation. Today, uterine factor infertility affects 3-5% of the population. Traditionally, although surrogacy and adoption have been the only viable options for females affected by this condition, the uterine transplant is currently under investigation as a potential medical alternative for women who desire to go through the experience of pregnancy. Although animal models have shown promising results, human transplantation cases have only been described in case reports and a successful transplant leading to gestation is yet to occur in humans .So, uterus transplantation for absolute uterus factor infertility is moving closer to human trials. Patients with the Mayer-Rokitansky-Kuster-Hauser (MRKH) syn-

drome, patients having had hysterectomy for benign or malignant uterine/cervical diseases and patients with intrauterine adhesions are the major groups of patients, who could benefit from this procedure.

Materials and Methods: In this review article it was decided to search in internet about this important surgery and evaluate if is it possible to achieve this technique in human in future? The search was performed with key word of uterine transplantation and there were several papers in regard to uterine transplantation. There was lots of research on uterine transplantation has been performed in appropriate animal models and three cases in human. Therefore, these studies were extracted and discussed.

Results: The results of searches showed that in 1896, Knauer published the first study of ovarian autotransplantation documenting normal function in a rabbit and that lead to the investigation of uterine transplantation in 1918. Erslan, Hamernik and Hardy, in 1964 and 1966, were the first to perform a dog autotransplantation of the uterus and subsequently deliver a pregnancy from that uterus in 2011 Ramirez, et al. demonstrated that a pregnancy can be carried in a sheep transplanted uterus under the influence of immunosuppressive therapy. In humans: in 1931, Lili Elbe died from organ rejection three months after receiving one of the world's earliest uterine transplants. In Saudi Arabia in 2000, a uterine transplant was performed by Dr. Wafa Fagee from a 46 year old hysterectomy patient into a 26 year old recipient whose own uterus had hemorrhaged after childbirth. The transplanted uterus functioned for 99 days, Post-operatively, the patient had two spontaneous menstrual cycles, followed by amenorrhoea; exploratory laparotomy confirmed uterine necrosis. The procedure has raised some moral and ethical concerns. A 21-years-old Turkish woman, who was born without a uterus, has become the first woman in the world to receive a womb from a deceased donor. The operation performed on 2011 by Dr. Ömer Özkan and his team at Akdeniz University Hospital in Antalya, Turkish Rivera has been a success. She has had 6 menstrual periods post-surgery and it is said that the uterus is fully functioning.

Conclusion: It needs to have more trial of this procedure in human until to do this technique successfully and after that many patients with lack of uterus could benefit from this procedure.

Keywords: Uterine Transplantation,Uterine Factor Infertility

P-230: Low Pregnancy Rate in Female Rats Mated by Male Rats Exposed to Acyclovir

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Background: Acyclovir [9-(2-hydroxyethoxymethyl)guanine; ACV], an acyclic nucleoside analogue, has shown a potent antiviral activity. It is known to inhibit the replication of herpes viruses including herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicellazoster virus, and Epstein-Barr virus in cell cultures and in animals. ACV is khown to be toxic to gonads,then the aim of the present study was to evaluate effect of ACV on potential fertility in rat.

Materials and Methods: In this study thirty-two adult male wistar rats (220 ± 20 g) were randomly divided into four groups (n=8 each) with one group serving as control sham (distilled water i.p.), in the drug treated groups Acyclovir administered (4, 16, 48 mg/kg/day i.p.) for 15 days . 11 days after the last injection four males from each group was placed in an individual cage with two super-ovulated same strain females. The presence of vaginal plugs and as well observation of sperm in the smears in the following morning was an indication, that mating had occurred and this was designated day 1 of gestation. The number of pergnent female rats bred to ACV treated male rats and untreated rats and also the number of offspring was recorded.

Results: Female mated by male rats exposed to ACV showed significantly lower pregnancy rate. There were no significant changes in the mean number of the offspring at any dose-level of ACV in comparsion with those of control sham group.

Conclusion: Taking collectively, present study suggests that ACV plays negative roles on reproductive system and function in male rats.

Keywords: Acyclovir, Pregnancy Rate, Antiviral Drugs, Rat

P-231: The Effect of Ceftriaxone on Testicular Tissue of Adult Male Mice

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Background: Ceftriaxone is an antibiotic used for treating respiratory,skin,bone and kidney infections. Our purpose was investigating effect of ceftriaxone on testicular tissue.

Materials and Methods: 40 mice with 6 weeks old were used in this study. The mice randomly were divided into 2 groups, control- sham and test groups. The test group was subdivided to IP1 and IP2 which received Ceftriaxone with 20 mg/kgBW and 50 mg/kgBW dose for 45 days respectively. Testicular weight (TW), tubular diferentiation index (TDI), repopulation index (RI), thickness of testicular capsul (TTC), thickness of interistitial seminiferous tubuls (TIST), seminiferous diameter (SD) and number of leydig cells were evaluated.

Results: Histological investigations in test groups revealed a significant reduction in percentage of leydig cells. Light microscopic showed atrophy of seminiferous tubuls. Ratio of spermatogonia B to A (TDI) decreased. TDI positive decreased in test group.

Conclusion: these data revealed that ceftriaxone had adverse effect in testicular tissue

Keywords: Ceftrixone, Testicular Tissue, Mice

P-232: Evaluation of Cilostamide Efficiency in Comparison with Forskolin on The Two-Step *In Vitro* Maturation of Mice Germinal Vesilce Oocytes

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Background: The effects of cumulus cells and the effectiveness of a PDE3 specific inhibitor (Cilostamide) in comparison with an adenylate cyclase activator (Forskolin) on the maturation rate and developmental competence of mouse germinal vesicle (GV) oocytes were evaluated

Materials and Methods: Germinal vesicles (GV) oocytes of PMSG-primed mice were divided into cumulus denuded oocytes (CDOs) and cumulus oocyte complexes (COCs) groups. GV oocytes were cultured in TCM199 (I) without any treatment only for 24 hours (control); (II) supplemented with 10 uM Cilostamide for 48 hours and (III) supplemented with 50 uM Forskolin for 48 hours. Metaphase II oocytes achieved following 24 hours (control; one-step manner) and 48 hours (biphasic culture; with meiotic inhibitors from 0 to 24 hours, no meiotic inhibitors from 24 to 48 hours) were subject to *in vitro* fertilization (IVF) and embryos were cultured.

Results: Immature oocytes cultured with Forskolin and Cilostamide in biphasic manner showed a significantly higher ($p<0.05$) maturation, fertilization and two cells embryo rates in COCs and CDOs compared to their control groups, while no significant difference was observed between Forskolin and Cilostamide in both COCs and CDOs groups ($p>0.05$). Despite the significant difference ($p<0.05$) between COCs and CDOs in the control group, this difference was not observed in the two-step culture in the presence of Forskolin or Cilostamide.

Conclusion: *In vitro* maturation with Cilostamide and Forskolin in biphasic manner positively influences GV oocyte developmental competence and are more effective in CDOs than COCs groups

Keywords: Cilostamide, Forskolin, IVM GV Oocyte

P-233: The Effect of Cumulus Cells in Biphasic *In Vitro* Maturation of Mice Germinal Vesicle Oocytes in The Presence of Alpha Lipoic Acid

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Background: The efficiency of *in vitro* maturation (IVM) has remained low despite extensive effort to understand the culture conditions. This study investigated the effect of cumulus cells and meiotic inhibitors on oocyte developmental competence in the presence of Alpha Lipoic acid (ALA)

Materials and Methods: Mouse germinal vesicle oocytes were considered in cumulus-denuded oocytes (CDOs) and cumulus-oocyte complexes (COCs) groups. GV oocytes from each main group were randomly distributed between two subgroups: IVM in the presence or absence of ALA. Each subgroups were divided into two groups as follow: (I) IVM without inhibitors only for 24 hours (control) and (II) IVM with 50uM Forskolin in combination with 10 uM Forskolin in two step manner: first step, GV oocytes were arrested meiotically in TCM199 containing Forskolin+Cilostamide and then, in the second step were matured without those for 24 hours. Obtained metaphase II oocytes were subjected to *in vitro* fertilization (IVF).

Results: The COCs maturation, fertilization and two cells embryo rates were higher than CDOs in control group ($p<0.05$). Immature oocytes cultured in biphasic manner showed a significantly higher ($p<0.05$) maturation, fertilization and two cells embryo rates in COCs and CDOs compared to their control groups, while no significant difference was observed between relevant COCs and CDOs in the presence or absence of ALA ($p>0.05$)

Conclusion: Supplemented maturation medium with Cilostamide+Forskolin could improve the developmental competence of GV oocytes. While, Adding ALA could not improve developmental competence. Meiotic inhibitors were more effective in CDOs than COCs

Keywords: IVM, Cumulus Cells, Meiotic Inhibitors, ALA

P-234: Expression of Human Chorionic Gonadotropin (hCG) Hormone Using Chinese Hamster Ovary Cells

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Background: Human chorionic gonadotropin (hCG) is a member of glycoprotein hormones family consist of two different non-covalently heterodimeric chains: alpha and beta subunits with 92 and 145 amino acids respectively. This hormone plays an important role in human reproduction and physiology especially for maintenance of the corpus luteum during the first months of pregnancy

Materials and Methods: The aim of our study was to production of recombinant hCG in Chinese hamster ovary (CHO) cells. In this regard, cDNA encoding alpha and beta subunits were amplified by PCR and after digestion by XhoI/SalI cloned into the pcDNA3.1 (+) expression vector. The recombined plasmids were transformed into the TOP10F/ strains of E.coli. After that, cloning was confirmed by PCR and sequencing methods. Then two gene constructs were linearized by PvuI and subsequently co-transfected into the CHO cells using the

electroporation Technique. The transfected cells were examined by PCR
Results: Positive cell lines were subjected for expression analysis of recombinant hCG by SDS-PAGE and Western blotting methods
Conclusion: Many researches confirm that production of recombinant protein in eukaryotic expression systems such as CHO cells is a reliable method for producing of therapeutic human chorionic gonadotropin
Keywords: CHO Cells , Recombinant hCG, Expression

P-235: Ceftriaxone Effects on Sperm Quality and DNA Damage in Adult Mice

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Background: Ceftriaxone is a third-generation Cephaloporin antibiotic. It has broad spectrum activity against gram-positive and gram-negative bacteria. There are some reports about its adverse effects on sperm quality and quantity but there is a need for more investigations so we did it.
Materials and Methods: 40 adult male mice were divided into 3 groups as followings: a control group, high dose and low dose group receiving of saline normal, 20 mg/kg and 50 mg/kg Ceftriaxone respectively. Half of the treatment groups were analyzed after one week of daily injections and the other half after 45 days. Sperm quality factors such as total sperm count, sperm viability, DNA damage and percentage of immature sperms was evaluated.
Results: Ceftriaxone reduced total sperm count and sperm viability in treatment groups and also increased percentage of immature sperms and sperms with DNA damage in treatment groups specially in high dose in comparison with the control group.
Conclusion: These data revealed that Ceftriaxone can decrease sperm quality and quantity.
Keywords: Ceftriaxone, Sperm Quality, DNA Damage, Adult Mice

P-236: The Role of Trace Elements in Treatment of Ovariectomized Osteoporotic Rats

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Background: Osteoporosis is one of the uncomfortable postmenopausal symptoms. The risk of imbalanced nutrition especially traces elements and vitamins are high during post menopause and may lead to osteoporosis due to reduction of content of mineral bone. The aim of this study was to investigate the potential consequence of Selenium (Se) treatment in ovariectomized rat model with osteoporosis in preventing bone loss.
Material and Methods: 30 mature female Wistar rats weighing approximately 200g were selected and ovariectomized. In control group rats were given distilled water and in experimental group were gavaged with different doses of Sodium selenite, (0.1 -0.6 mg/kg). It was administrated for two months. The femur bone of the animals was collected and tissue bone investigated under a light and electron microscope level.
Results: The ovariectomized rats showed a significant decrease in bone mass density of femur in comparison with the intact control group. Ovariectomized group not treated by Se showed inappropriate lamellar structure and a large uncalcified bone matrix. Administration of Se rat reversed bone loss and prevented destruction of bone tissue and bone trabecular formation and arrangements were observed in the treated group and had similar structure with control group compared to the ovariectomized group. Serum biochemical assays revealed that Se prevent osteoporosis induced in ovariectomized group ($p<0.05$).
Conclusion: Se prevents structural alternation in bone after osteoporosis. Bone resorption was considered as an effective therapeutic approach. These data clearly suggest that Se has the potential effect as an alternative treatment for prevention of bone loss in postmenopausal osteoporosis.
Keywords: Trace Elements, Treatment, Selenium Ovariectomy, Osteoporosis, Postmenopause

P-237: Chronic Alcoholism Induced Male Infertility: Impairment of Sperm Quality

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Background: Prolonged alcohol abuse in men can cause testosterone deficiency and shrinkage of the testicles (testicular atrophy). This can lead to impotence, sterility and 'feminisation'. When alcohol is broken down in the body, it may also interfere with normal sperm structure and movement. This study was designed to determine the effects of administration of alcohol on sperm quality, sperm maturation and DNA damage
Materials and Methods: Twenty adult male mice were divided in 2 groups: control sham (salin normal) and test group received ethanol (3 g/kg body weight as 25%, v/v) was given by gastric intubation daily for 45 days. After 45 days all mice were sacrificed and cauda epididymis were removed and placed in 1ml HTF+BSA 4mg/ml and incubated for 30 min in Co_2 incubator (5% Co_2, 37°C) to allow the spermatozoa to swim out ($p<0.05$). The spermatozoal suspension was analyzed for sperm motility, concentration in the cauda epididymis, viability and sperm chromatin quality and DNA integrity was assessed by Aniline Blue and Acridine Orange staining following sperm sample.
Results: This study showed that treatment with ethanol

caused significant decrease in sperm concentration in cauda epididymis, motility, and viability, while abnormal sperms increased as compared to control. These changes were associated with significant increase in DNA damage and chromatin abnormality in the cauda epididymal spermatozoa as evidenced by Acridine Orange (AO) and Aniline Blue staining respectively ($p<0.05$)

Conclusion: The present study demonstrates that chronic consumption of ethanol has toxic effect on spermatozoa and impairs fertility in male mice. Although it is hypothesized that chronic consumption of ethanol induced testicular injury with adverse effect on sperm production, sperm DNA integration and nuclear maturation.

Keywords: Alcohol, Sperm, Viability, DNA Damage, Mouse

P-238: Evaluation of The Effect of Ethanol Consumption on Testis Tissue in Mice

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Background: Alcohol affects reproduction in both men and women in a number of ways. Chronic exposure to ethanol may results in pathophysiologic changes in cellular function. The present work was designed to investigate the morphology of testis submitted to experimental ethanol ingestion.

Materials and Methods: Twenty adult male mice were divided in 2 groups: control sham (salin normal) and test group received ethanol (3 g/kg body weight as 25%, v/v) was given by gastric intubation daily for 45 days. After 45 days all mice were sacrificed. their testis were immediately removed, washed in normal saline and placed in fixative formalin. After routine histological processing and embedding in paraffin, sections of 5 µm thickness were cut and stained by Hematoxylin-Eosin. Prepared specimens were examined and photographed by light microscopy.

Results: Abnormal testicular changes occurred and semeniferous tubules destructions were also observed. The number of leydig cell,spermatogonia cells,sertoli cells were decreased.

Conclusion: The present study demonstrates that chronic consumption of ethanol has toxic effect on spermatozoa and structure of testis.

Keywords: Ethanol, Mice, Structure of Testis, Sermatogonia

P-239: Effect of Grass Kebar (Biophytum Petersianum Klotszch) to Increase Uterus Fertility and Increasing Number of Children on Female White Rat (Rattus norvegicus)

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Background: Grass Kebar (Biopytum petersianum Klotzch) is one of Indonesian medicinal plants, which can only be found in Kebar sub-district, Manokwari, Irian Jaya. This plant is mainly used by the community Manokwari to maintain the fertility of women of reproductive organs. This study aims to determine the effect of extract Grass Kebar dose on the fertility of the uterus and the number of progeny of white rats (Rattus norvegicus) are produced.

Materials and Methods: To determine the effect of plant extracts against the fertility of the uterus, performed Completely Randomized Design with six replications. Female rats fed grass Kebar orally at doses of 0.51 ml, 1.02 ml, 1.53 ml and without grass Chebar (control). Effects of extracts of the progeny is done through the provision of Grass Kebar in female rats at doses of 0.51 g / ml, 1.21 g / ml, 1.53 g / ml, 2.04 g / ml and 2.55 g / ml with simple random sampling.

Results: The results showed that the increase in endometrial thickening is proportional to the dose given Kebar grass, with the optimal dose of 1.51 ml. Provision of grass Kebar also showed a significant effect on increasing the number of white female pups. Effective dose Kebar grass in increasing the number of children of white rats (Rattus norvegicus) female was 2.04 g / ml.

Conclusion: Kebar grass proved to affect the fertility of the reproductive organs are indicated by the thickening of the endometrium and an increase in the number of children female rats.

Keywords: Kebar Grass, Fertility, Endometrium, Progeny

P-240: Does Ciprofloxacin Exert Severe Oxidative Stress in Testicular Tissue?

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Background: Ciprofloxacin was shown to have cytotoxic effects on testicular germ cells. Its mechanism of cytotoxic action is not fully understood. To investigate the possibility of the involvement of an oxidative stress induction in this mechanism, total antioxidant power (TAOP) in the testis was evaluated.

Materials and Methods: A number of twenty four mature male NMRI mice were used.The animals assigned into control and test groups. The test groups subdivided into low dose (206 mg/kg) and high dose (412 mg/kg) CPFX treated groups. Control animals received carboxymetyl cellulose. All animals were treated orally for 45 days. At the end of the study the animals were sacrificed and testicular tissues were homogenized in PBS. TAOP of the testis was determined by measuring its ability to reduce Fe+3to Fe +2.The complex between Fe +2 and TPTZ

gives a blue color with absorbency at 593nm.

Results: Although TAOP in the testis of low dose and high dose treated mice slightly increased but this change is not significant among three groups.

Conclusion: Oxidative stress induction by CPFX is not remarkable during 45 consecutive days. Therefore the direct action of CPFX may be involved in its mechanism of action on testicular germ cells. Meanwhile long exposure to CPFX (More than 45 days) may result in extend damages of germinal epithelium in testicular tissue which may in turn result in remarkable alterations in TAOC.

Keywords: Ciprofloxacin, Oxidative Stress, Testis, Mice

Abstracts of

7th Royan Nursing and Midwifery Seminar

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Tehran, Islamic Republic of Iran

Invited Speakers

I_{nm}-1: Pre-Gestational Diabetes Mellitus (Pre-GDM)

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Despite progress in diabetes care and treatment, pregnancies in women with either type 1 or, type 2 DM are still associated with poorer outcomes with respect to healthy non diabetic women. Pregestational DM complicates 0.2 - 0.6% of pregnancies, 35% had type1 and 66% had type 2 DM. In contrast to GDM, pre GDM is more serious because the potential effects of uncontrolled glycemic levels begins at fertilization and implantation, continue throughout pregnancy and remain as a postpartum threat during breast feeding.
To prevent excess complications in mother and fetus, diabetic care and education must begin before conception. This is best accomplished by a multidisciplinary team that includes a diabetologist (internist), an obstetrician familiar with management of high risk pregnancy, diabetes educators including: Nurse, dietitian and social worker and other specialists as deemed necessary. Pregnant women with type 1 and type 2 diabetes should talk with a diabetes nutritionist, to determine their goals for daily calories, carbohydrates, nutritional balance in foods, and timing of eating throughout the day.
The goals of preconception care are: involve and empower the patient in the management of her diabetes, achieve the lowest HbA_{1C} test without excessive hypoglycemia, assure effective contraception until stable and acceptable glycemia is achieved and identify, evaluate and treat long-term diabetes complications such as retinopathy, nephropathy, neuropathy, hypertension and coronary disease.

I_{nm}-2: New Approaches in Cryopreservation

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Cryopreservation of embryos and gametes has become an indispensible service for infertility treatment. Vitrification is now a prevalent technique for cryopreservation in assisted reproductive technology (ART) replacing, in many cases, conventional slow cooling methodology.
Fertility preservation in female patients has become a very topical issue. Various malignant and nonmalignant diseases have been successfully treated with high-dose chemotherapy or radiotherapy. Although many young patients receiving these treatments are at risk of developing reproductive failure, a number of fertility preservation options ranging from embryo cryopreservation to ovarian tissue cryopreservation are now available. Embryo cryopreservation is a well established technique for preserving fertility. The success rate with oocyte cryopreservation has been on the rise. Both oocyte and embryo freezing require ovarian stimulation and novel ovarian stimulation regimens utilizing aromatase inhibitors which have been developed for ovarian stimulation in women with estrogen sensitive cancer. Although ovarian tissue cryopreservation is a novel technology, it is the only fertility preservation option for children and the only treatment strategy that can restore ovarian function. *In vitro* maturation is a promising technology and can be applied in conjunction with ovarian tissue cryopreservation. There are 2 main methods for transplantation of frozen-thawed ovarian tissues: orthotopic and heterotopic transplantation. These technologies are investigational, although rapidly evolving, and their list of appropriate indications may expand in the future.

I_{nm}-3: Reduction: Reasons and Consequences

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During the recent decades with increasing the use of assisted reproductive techniques including IVF, IUI and ovulation induction, the rate of multiple pregnancies has been increased worldwide. More than 30% of ART pregnancies are twins or higher-order multiple gestations (triplets or greater) a frequency 15- to 20-fold greater than with spontaneous conceptions. Because of the high cost of treatment and the relatively low success rate of such treatments (which is improving every day), a decision is sometimes made to implant several fertilized eggs.
Many studies indicate that multiple pregnancies have negative impact on families' emotional wellbeing and are associated with social and financial burden on couples. From 1980s, multifetal pregnancy reduction has been introduced as an efficacious method to reduce fetal number and improve the survival of remaining fetuses. Since most of the fetal loss subsequent to embryo reduction occurs several weeks after the procedure, an inflammatory response to dead fetoplacental tissue with releasing of cytokines and stimulation of prostaglandins might leads to fetal loss, uterine contractions and preterm labour. In a large multicentre study, improved outcomes were observed by multifetal pregnancy reduction done by expert hands in terms of fetal loss and early prematurity. The most important complication of high order multifetal pregnancies (more than two) is preterm delivery ranging from 32% to 86% in different studies. In a recent study in Royan Institute report that fetal reduction of triplets to twins leads to 4% increase in miscarriage rate and couldn't be demonstrated any correlation with the initial number of fetuses and other outcomes such as preterm birth, gestational age at delivery and birth weight of neonates. In this study the extreme prematurity was more common in non reduced group compared to reduced group. As a consequence, the mean gestational age at delivery for reduced group was significantly higher than expectantly managed group and the average gestational length was 4 weeks longer in the reduced group. Furthermore, in the current study, the mean birth weights of neonates were higher in the

reduced group than control group and the percentage of low birth weight infants (<2500 g) was significantly higher in the expectantly managed group. As prematurity is the most important cause of neonatal and perinatal deaths and subsequent handicap infants, prolongation of gestational age at delivery due to fetal reduction could less the rate of deaths and handicap among survivors. But as ultrasound equipment improved and doctors gained technical expertise, the procedure triggered fewer miscarriages. Genetic diagnosis before reduction is becoming more common, safe in experienced hands.
The ultimate goal in prevention is to significantly reduce the likelihood that any multifetal pregnancy will occur, including twins. Reduction is hardly the only area in which reproductive innovation has outpaced cultural consensus. The justification for eliminating some fetuses in a multiple pregnancy was always to increase a woman's chance of bringing home a healthy baby, because medical risks rise with every fetus she carries. Not only does the danger to the mother increase with more fetuses, but also the risk of miscarriage, ending the lives of all of the fetuses increases. Ultimate decision of patients depends on: extent of their religious and antiabortion sentiments, whether they medical-scientific careers, how proactive the advice from physicians has been.

Key words: Fetal Loss, Fetal Reduction, Multifetal Pregnancies, Preterm Delivery

I_{nm}-4: Genetic Aspects of Male and Female Infertility

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Genetic causes can be directly responsible for various clinical conditions of male and female infertility and genetic variability may affect the ability to reproduce. This review aims to summarize current research on genetic diagnosis and genetic causes of reproductive disorders. Chromosome abnormalities account for 60% of all spontaneous abortions, and the most common type, trisomy, is closely associated with advanced maternal age. Three percent of couples have a balanced chromosome abnormality. With regard to the chromosomal alterations, the predominating anomalies are those associated with the sex chromosomes. However, a wide range of structural autosomal anomalies has been identified such as Robertsonian and reciprocal translocations, inversions, duplications and deletions, which can be associated with infertility. Single-gene defects are most likely to be found among patients with hypogonadotropic hypogonadism, which may be due to defects in the Kallmann's syndrome (KAL) genes or the gonadotrophin-releasing hormone receptor genes in women. With premature ovarian failure there is an increased risk of having a pre-mutation of the FMR1 gene. Complex genetic inheritance may explain the variable familial links in polycystic ovary syndrome and endometriosis, but no definitive genetic pathways are as yet known. With recurrent miscarriage, genetic defects causing thrombophilias are 2-fold more likely. It is known that 10-15% of cases of azoospermia and severe oligozoospermia are genetically-based, represented mostly by Klinefelter's syndrome (KS) and by the microdeletions of the Y chromosome. The microdeletions of the AZF region, the second most common cause of male infertility, determine a severe primitive testiculopathy with consequent azoospermia or severe oligozoospermia. Lastly, mutations in single genes can be directly responsible for male infertility, such as: CFTR (cystic fibrosis), KAL, AR and INSL3-RXFP2 (associated with anomalies in the descent of the testicles, as in cryptorchidism).The high frequency of genetic alterations in infertile couples demands appropriate and correct diagnosis of these patients in order to reduce the risk of transmitting genetic anomalies to the offspring. Moreover, understanding of the methods used for genetic diagnosis and research is becoming a standard requirement for the clinical practice of reproductive medicine.

I_{nm}-5: Consultation and Ethic Points in ART

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Background: Infertility is a complicated and psychologically threatening and challenging crisis. The rapid development of ART has raised many ethical and legal issues and lead to various emotional changes (anxiety, depression, stress, aggressiveness, etc.) as it may involve considerable time, expense, and loss of privacy. It can be both dangerous and expensive to enter the world of assisted reproduction without an experienced guide to lead the couple through not only the complex technology, but also a whole host of consumer issues.
Materials and Methods: Articles were obtained from PubMed and Google databases and other relevant journals and reference lists.
Results: There are different kinds of attitude regarding donation for helping another couple or research issues(such as deciding the fate of unused or freeze eggs, sperm, and embryos) even using ART(including all ART options, PGD, sex selection, abortion and reduction in multiple pregnancy) or adaption among people by different religion, culture and their law which make it difficult to prepare an international or even national agreement and guideline for ethical and moral problems in ART as well as principles of selection for key decisions to provide a fair and suitable procedure for infertile patients. Iran is the only Muslim country in which gamete and embryo donation is practiced. Although embryo donation is accepted by law and gamete donation by clergy leaders, there are difficulties in acceptability of these procedures by this nation. It is widely accepted that if IVF is to be developed, ongoing embryo research is inevitable. In so far as human embryos cannot themselves consent to be the subjects of research, no research of any kind may be carried out without the consent of parents or legal guardians. In many jurisdictions, subsequent to IVF or ICSI, it is common practice for surplus embryos to be used, with the consent of the natural parents, to provide children for

other infertile couples. The Guide to Ethical Conduct and Behavior specifically requires that "any fertilized ovum must be used for normal implantation and must not be deliberately destroyed."Fertility clinics should address the psycho-social and emotional needs along with ethical, legal and moral issues of infertile couples as well as their medical needs. The content of counseling may differ depending on the concerned couple and the existing treatment options. Many physicians would hesitate to provide infertility treatments to women with drug addiction, serious developmental delay, or severe psychiatric illness because they believe the woman would not be a good parent. It usually involves treatment implication counseling, emotional support counseling, and therapeutic counseling. Different studies have shown the beneficial effects of psychiatric and psychological treatments not only in adapting to unsuccessful treatments but also in reducing stress and bringing about successful pregnancy.

Conclusion: The woman should be given sufficient time to process information about the pros and cons of the procedure, and given time to ask questions before arriving at a decision to accept or refuse planned care. Patient information is generally held under legal and ethical obligations of confidentiality. Information provided in confidence should not be used or disclosed in a manner that might identify a patient/client without her consent. Clinicians and Infertility specialists should assess level of grief and adjust counseling accordingly, Counsel how to tell family and friends of their problem, include the patient's partner in psychological care, Provide comfort, empathy, and ongoing support, acknowledge and reassure about the future. Psycho-cognitive teachings such as opening the situation for infertile couples can probably help them in overcoming and controlling the natural emotional distress brought about by treatment failure and acceptance of the probability of being left childless.

Keywords: Assisted Reproductive Techniques, Infertility, Cons

I_{nm}-6: Abortion and Infertility

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Background: The maximum probability of conceiving during a menstrual cycle is only about 40%. One third of conceptions do not result in the delivery of a baby recurrent miscarriage, defined as the loss of 3 or more pregnancies before 20 weeks post-menstruation that affects 1% of all women. Approximately 20 percent of pregnant women will have some bleeding before 20 weeks' gestation, and roughly one half of these pregnancies will end in spontaneous abortion.

Material and Method: This review summarized reports found on Pubmed database about the miscarriage.

Results: Parental chromosomal anomalies, maternal thrombophilic disorders and uterine anomalies (congenital anomalies, adhesions, leiomyoma) have been directly associated with recurrent miscarriage; however, in the vast majority of cases the pathophysiology remains unknown. Useful investigations include parental karyotyping(family history of miscarriages, should be taken into consideration when deciding who should be karyotyped), an ultrasound scan of the pelvis and Routine hysterosalphingography as a screening test for uterine anomalies, a testing for lupus anticoagulant (LAC) and anticardiolipin antibodies (aCL), known collectively as antiphospholipid antibodies (APA), to exclude an antiphospholipid syndrome (APS) , Screening for bacterial vaginosis and inherited thrombophilic defects, screen for maternal endocrine disease (Chronic maternal diseases: poorly controlled diabetes, celiac disease, autoimmune diseases, PCOS, thyroid function tests and HbA1C measurements), Screening for Low mid-luteal phase serum progesterone or an endometrial biopsy, however the majority of RM cases following investigation are classified as idiopathic. High level of homocysteine (hyperhomocysteinaemia) can be associated with RM too.

Therapeutic strategies will depend on the underlying cause found. Progesterone has been administered orally, intramuscularly and vaginally. Aspirin and/or heparins have become routine treatment for women with APS and inherited thrombophilias and a history of RM. miscarriage resulting from trisomic conceptuses increases significantly after age 40. Supportive care has a beneficial effect on unexplained recurrent miscarriage. The association between miscarriage and ionizing radiation, organic solvents, alcohol, mercury and lead is confirmed, whilst an association to caffeine, hyperthermia and cigarette smoking is suspected. In women with missed spontaneous abortions, expectant management has a variable but generally lower success rate than medical therapy; In contrast, medical therapy for missed spontaneous abortion results in high success rates for completion of a spontaneous abortion without surgical intervention. Women experiencing spontaneous abortion with unstable vital signs, uncontrolled bleeding, or evidence of infection should be considered for surgical evacuation. Sexual activity also does not elevate risk in women with uncomplicated pregnancies.

Conclusion: Conceptions and misconceptions was born of the need for clear, understandable, and unbiased information about the complex and fast-growing world of assisted reproduction. Physicians should realize the importance of providing care that is sensitive to the medical and psychological aspects of a couple who experiences spontaneous abortion in addition to address the issue of feelings of guilt, the grief process, and how to cope with friends and family.

Key words: Miscarriage, Pregnancy

I_{nm}-7: Cord Blood Banking Potential and Prospective

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The term "cord blood" is used for blood that is drawn from the umbilical cord and the placenta after a baby

is born. Up until recently this afterbirth was discarded as medical waste. Cord blood contains stem cells that may be frozen for later use in medical therapies, such as stem cell transplantation or regenerative medicine.

The umbilical cord and placenta are rich sources of stem cells. These are different from both the embryonic stem cells in a fertilized egg and any stem cells obtained from a child or adult person. The stem cells in cord blood can grow into blood and immune system cells, as well as other types of cells.

Stem cells can transform into other types of cells in the body and create new growth and development; they are the building block of the immune system. This transformation of cells provides physicians with a way to treat leukemia and some inherited disorders. Cord blood stem cells have the same ability to treat disease as bone marrow does however, there is significantly less rejection.

The cord blood collection process is simple, safe, and painless. It is usually completed in less than five minutes by health care provider. Cord blood collection does not interfere with delivery and is possible with vaginal or cesarean deliveries.

Cord blood can either be donated for the public cord or stored privately for the family. Most parents who want to save the cord blood must choose between those two options, and must make the decision a few weeks before the birth.

When you privately bank your baby's cord blood stem cells, you have a stem cell unit that is a genetic match to your baby, should a doctor determine that a transplant with the stem cell unit should ever be needed. It may also be possible that the cord blood stem cells will be a suitable match for a family member who is a blood relative, including siblings, your spouse, or even yourself as determined by a doctor.

Cord blood is an accepted source of stem cells for any of the diseases where stem cell transplants are a standard therapy. This covers a long list of oncology and hematology diagnoses. Cord blood transplants have been in use since 1988, and by the end of 2009 there had been about 20,000 cord blood transplants world-wide (1). Yet, it was not until late 2011 that the US FDA finally licensed cord blood transplants for most of the diseases on the standard therapy list. While the FDA licensure does not change clinical practices at transplant centers, it does open up access to cord blood transplants for those patients whose health insurance providers had previously used the lack of licensure as a reason not to cover the transplants.

Emerging cell therapies is a catch-all phrase for any new applications of cord blood stem cells that are currently in clinical trials. Success stories from these trials have received a great deal of media attention. But not every patient is a success story, and until follow up studies against controls are completed, it is not clear what fraction of the patients benefited from the therapy and the measure of benefit they received.

Oral Presentations

O_{nm}-1: The Association of Apoprotien E Polymorphisms with Recurrent Miscarriage in Iranian Women

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Background: Thrombophilia has been viewed as a multigenic disorder rather than a monogenetic clinical phenotype and Apo E has been shown to play an important role in lipid metabolism in pregnancy. As individuals carrying the E4 allele of the ApoE gene have the highest risk for thrombosis, we evaluated the frequency of the Apo E4 genotype among women suffering from recurrent pregnancy loss.
Materials and Methods: 81 women with a history of two or more consecutive spontaneous abortions were examined for their APO-E genotypes based on restriction fragment length polymorphism analysis of PCR-amplified fragments including amino acid positions 112 and 158. The observed genotypes were compared with negative controls.
Results: Women experiencing recurrent pregnancy loss had a significantly higher prevalence of Apo E3/4, E4/4 genotypes (19.75%) compared with control women (2.46%) (p=0.00001).
Conclusion: Apo E4 polymorphism may contribute to the thrombophilic risk factors contributing to recurrent pregnancy loss.
Keywords: Apo E4 Polymorphism, Apolipoprotein E, Recurrent Pregnancy Loss

O_{nm}-2: The Role of Ultrasound in Infertility

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Ultrasound plays an increasing role in the evaluation, monitoring, and treatment of infertility. About 15% of all couples attempting to have children are affected by infertility. As women delay conception due to career choices and age, the frequency of infertility increases. Infertility is defined as no conception after one year of unprotected intercourse. Ultrasound plays a remarkable role in infertility since it permits the direct visualization. With the increasing utility of the transvaginal ultrasound, the female pelvis became more and more accessible; a wider variety of gynecological pathology was open to diagnosis and treatment. Real time conventional scan and Doppler analysis are now part of the routine pelvic exam and an accurate evaluation allows gathering a number of anatomical and even histological details. Transvaginal sonography (TVS) has multiple roles in the treatment and evaluation of fertility disorders in women:

- Assessment of follicular maturity and abnormalities of ovulation.
- Assessment of endometrial development.
- Assessment of ovarian/endometrial blood flow
- Evaluation of tubal patency
- Detection and evaluation of uterine malformations
- Guided follicular aspiration
- Detection of adhesions, submucosal fibroids, and polyps with sonohysterography
- Evaluation of complicated early pregnancy, multifetal pregnancy.

The male factor alone is seen in about 30% of patients. Ultrasonography is being used with increased frequency in the evaluation of the infertile male. With the newer advances in technology, scrotal and transrectal sonography are now well established in assessment of male factor disorders. In conclusion, infertility evaluation and treatments are relying increasingly on ultrasound. Which provides information about the infertile patient.We can still conclude that sonography, in the hands of a skilled practitioner, with a good technological basis, is one of the best diagnostic tools in gynecology, successfully replacing more invasive methods.

O_{nm}-3: To Determine The Prevalence and Risk Factors of Domestic Violence Against Women with Female-Factor Infertility in An Iranian Setting

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In this cross-sectional survey conducted from August 1, 2009 to January 31, 2010, 400 women with primary infertility attending Valiasr reproductive health research center in Tehran, Iran, were interviewed using the Revised Conflict Tactics Scales questionnaire (CTS2). This instrument was developed to investigate the presence domestic violence.
A total of 247 women (61.8%) reported having experienced domestic violence because of their infertility. The most common type of violence was psychological (n=135, 33.8%), followed by physical (n=56, 14%) and sexual (n=32, 8%), with 24 women (6%) reporting injuries. All women reported their husbands to be the perpetrators.
In conclusion, domestic violence against infertile women is a considerable yet unreported problem. Clinicians should identify the abused women and provide them with medical care and supportive counseling.

O_{nm}-4: Physical Activity, Body Mass Index, and Fertility Status

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Background: Body mass index and physical activity may affect the reproductive system. The purpose of this study was to determine the fertility status and parity and its relationship with physical activity and body mass index in a community in Babol, Iran.
Materials and Methods: The research design of the present study was a population based cross-sectional study, and their current physical activities were measured using the original International Physical Activity Questionnaires Short form. A face to face household interview was conducted using a special designed interview questionnaire. A total of 1140 women aged 20-45 were selected by a standard cluster sampling method.
Results: Significant difference observed in level physical activity (p=0.000), walking (0.045), and body mass index (0.0001) between these women, either with (1, 2 and 3 or more child) or without children. The chai-square test did not reveal any statistical significant difference in Met-minutes of sedentary, intensity of walking, moderated, vigorous and total physical activity between these women, either experienced or no infertility. The adjusted and unadjusted OR showed no significant association between fertility problems and level of physical activity. The adjusted OR for fertility problem in women with underweight and normal weight was significantly lower than in those with overweight and obese women (OR=0.60; CI=0.37, 0.95 and OR=0.30; CI=0.10, 0.92, respectively).
Conclusion: Increased risk of experienced difficulty conceiving at same stage of life was reported only for women over weight and obese awareness of the highlighted. Therefore, it is necessary to emphasize on the benefits of weight loss.
Keywords: Physical Activity, Epidemiology, BMI, Infertility, Women

O_{nm}-5: Successful Weight Loss and Its Relationship with Some Demographic Factors in Infertile Polycystic Ovary Syndrome Patients

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Background: Polycystic ovarian syndrome (PCOS) is one of most common endocrinology problems of infertile women. Since lifestyle modification and weight loss is the most effective treatment and associated with failure in many cases, this study evaluated the factors impact on weight loss of PCOS women.
Materials and Methods: This cross-sectional study was conducted on infertile women with PCOS referred to Fatemezahra infertility research center in 2010-11. The questionnaire contains demographic information, clinical, laboratory findings and ultrasonography of all subjects, and then anthropometric measurements were taken. After completing the data, diet for weight loss and exercise were recommended and all patients received Metformin. The samples were followed for 4 times monthly.
Results: 142 women in age 17-40 years entered the study. Of them 91.5% of were overweight and the majority of them were housewives (90.2%). Most of them had under high school education (83.5%) and (54.9%) lived in urban areas. The mean weight loss was (5.32 ± 4.8) up to maximum 17 kg, which related directly within creasing women age. The average of weight loss in women over 30years old and under 20 years old were 7.4 and 3.6 kg, respectively. According to education households' with post high school education level had higher values of weight loss in comparison with under high school case (7.7 kg vs. 5.4 kg). Finding showed that a non-significant relationship between the duration of infertility and place of life.
Conclusion: The results revealed weight loss in women with PCOS had a positive association with level of education and their age, while place of life and duration of infertility were not correlated in the process of weight loss in these patients.
Keywords: Weight Loss, PCOS, Metformin, Education, Age

O_{nm}-6: Social Consequences of Infertility in Iranian Infertile Women: A Qualitative Study

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Background: Infertility as a life crisis is accompanied by a wide range of social, psychological, physical and financial problems. These problems are varied- both in nature and extent- in different locations according to specific cultural- social contexts. Infertility is a source of social suffering for infertile people, especially women. As infertility prevent individuals from achieving the specific role of parenthood, infertile women have to cope with social consequences of their inability in addition to the usual and personal psychological outcomes. This study aims to examine the social consequences of infertility among infertile women.
Materials and Methods: This study follows a qualitative approach, using a content analysis method. 15 infertile women were selected using purposive sampling and consideration of maximum diversity. Data were collected using semi-structured interviews and were analyzed by a conventional content analysis method.
Results: The findings of this study consist of 7 categories and five main themes including psychological violence, instability in life, social isolation, social self-alienation and feeling of social exclusion. One of the major subcategories of psychological violence was stigma that also includes the subcategory of decrease in social status.
Conclusion: Social support of infertile women should be a priority for health workers in developing countries. The nature of this social support should be specified by identification of consequences of infertility in the context of every country and aims at empowering infertile people to reform the current value system and increase their

self-confidence.
Keywords: Social Consequences, Infertility, Infertile Women

O_{nm}-7: Safe Pregnancy after ART

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Background: The transition to parenthood is a period of intra- and interpersonal changes and may bring up unexpected problems. The first child's birth has a significant effect on marital relations because the relations change from dyadic to triadic. In general, marital relationships seem to be especially vulnerable during the transition to parenthood, and several studies have revealed a decline in marital satisfaction and an increase in marital conflict among parents. However, during recent years, it has been shown that there is a great variability in the way the marital relations change during transition to parenthood. It has been suggested that babies do not create distress between couples nor bring couples closer together but rather amplify already existing difficulties, which can be seen as deterioration of the relationship. Comparisons of marital relations between couples who conceived either spontaneously or via ART have shown great variation. Couples with successful ART have been reported to show greater marital cohesion (e.g. a mutual feeling of being a couple, sharing things and ideas), less marital distress, to experience their relationships with their partners in a more positive way and to have more stable relationships than couples experiencing spontaneous pregnancy.
Materials and Methods: During recent years, the development of assisted reproduction technique (ART) methods has made parenting possible for a substantial number of infertile couples. For the majority of infertile couples successful treatment outcome results in improvement in emotional wellbeing, but less is known about how a previously infertile couple, as a unit, is affected during the transition to parenthood. Social, psychological and infertility-related issues as well as gender may be of relevance in determining the impact of infertility on marital relationships. The ability to reproduce is intimately tied to sexuality, self-image and self-esteem. Some studies have revealed no difference in marital satisfaction between ART and control couples and between ART couples and those couples who had children through adoption. In contrast, there are also reports of more marital conflicts among ART parents than controls.
Results: Generally, mental health problems during the transition to parenthood are also affected by various social factors; for example low economic status, stressful life events and lack of social support from spouse, friends or family are known to be risk factors for postpartum depression. In addition, child-related factors such as health of the child and worry about the child are important determinants of parental mental health Differences in the experience of pregnancy and birth after ART compared to spontaneous conception are of interest for the developmental psychologist. Individual experiences with infertility in those affected have often led to states of anxiety, low self-esteem and low confidence, but this does not seem to persist in pregnancy. Higher anxiety symptoms occur primarily. Increased symptoms of anxiety are found in pregnant women and their partners who have undergone the high stress of infertility. Pregnant women after ART are less concerned about the physical changes during pregnancy. Couples with previous ART mediated more harmony on the first impression, however, on the other hand being less communicative. A long duration of infertility (7 years or more) was associated with the expression of a large birth anxiety. Postpartum disorders such as depression are influenced by the interaction of several risk factors, particularly prematurity, multiple births and caesarean sections reinforce the emotional problems. However, where fears about the fetal survival are higher, postpartum difficulties are greater and self-esteem is lower after ART. Fears about the fetal survival and postnatal educational difficulties were greater after the application of assisted reproduction and the self-esteem lower. Pregnancy and parenthood after assisted reproduction may be idealized and is associated with a difficult transition to parenthood.
Conclusion: It has been presumed that pregnancy and parenthood experienced after infertility and assisted conception will be unproblematic and gratifying. However, theorized that past infertility and ART conception might be associated with elevated anxiety about pregnancy loss; delayed mother-infant attachment; diminished maternal confidence; hyper vigilant and overprotective parenting and idealized expectations of parenting capacity and the infant.
Keywords: Assisted Reproduction Technique, Pregnancy, Parenthood

O_{nm}-8: Psychiatric Problems in Infertile Women Referring to Infertility Clinic of Hamadan Fatemyeh Hospital

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Background: Infertility is an emotional crisis with physical, economic and psychosocial challenges, because it interferes with all personal life aspects. This event is a sever tension of life and produces strong emotional shock in the infertile couple. This study is done with the aim of determination the psychiatric problems of infertile women referring to infertility clinic of Hamadan Fatemyeh Hospital.
Materials and Methods: This is a descriptive study. Sample size was 200 infertile women which were selected by convenience sampling. Data was collected by a demographic questionnaire and SCL90, and analyzed through descriptive statistics.
Results: Our study indicates that infertile women experienced 9 categorize of SCL90 respectively: depression 22.5%, paranoidideation 20%, psychosomatic 14.8%

anxiety 6%, phobia 4%, obsession 4%, interpersonal sensitivity 2%, psychotics 4% and no experienced hostility.

Conclusion: It seems women experience more stress in personal, social and sexual life and women are more sensitive to infertility phenomena that probably is due to cultural problems, mental susceptibility and/or special forms of social communication

Keywords: Infertility, Psychiatric Problems, Female

O_{nm}-9: The Effect of Integrated Couple Therapy and Group Therapy with Cognitive-Behavioral Approach on Increasing Sexual Self-Concept and Accelerating Fertility

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Background: Women and men who have infertility problems, are faced with numerous psychological pressures, including changes in family environment and social networks, sexual problems and challenges of infertility stress which are associated with depression and psychological distress and can result in premature withdrawal of medical infertility treatments and unresolved feelings of loss and grief, as some researchers have found that the patients with infertility are equal to cancer patients in terms of depression and anxiety level and the infertile population suffer from lower self-esteem and less sexual satisfaction and matrimony satisfaction. (Peterson, 2011, Gipson, 2002).The present study aims to explore the effect of group therapy and couples therapy synthetically, with emphasis on cognitive- behavioral approach in order to increase sexual self-concept and to accelerate the effectiveness of medical treatments in the area of infertility.

Materials and Methods: The research sample was carried out from 120 infertile women (1 to 6 years), age: 22 to 45, education diploma to Ph.D., among whom 30 subjects were chosen randomly by available sampling method and were categorized into two groups (n=15) of experiment and control, pre-test and post-test using Beck depression inventory (BDI), sexual self-concept questionnaires (20 sexual components), routine experiments of spermogram and sonography of ovulation, cognitive-behavioral therapy was run for 12 sessions (6 sessions of couples therapy, 6 sessions of group therapy).

Results: According to the results of this research, this method of psychotherapy increased the self-concept infertile couple and reduced their stress and with research team efforts for 10 months, greater number of experiment group subjects were pregnant than control group subjects.

Conclusion: This method of psychotherapy has been used to reduce psychological pressures associated with infertility so that the treatment of medical infertility may be effective more quickly and also the affective, emotional and economic costs of these families and society may be reduced.

Keywords: Couple Therapy, Group Therapy, Cognitive-behavioral, Sexual Self-Concept, Infertility

Poster Presentations

P_{nm}-1: Applications of Hysterosonography for The Detection of Intracavitary Uterine Abnormalities

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Hysterosonography (SHG) is a new noninvasive technique that involves the slow infusion of sterile saline solution into the uterus during ultrasound imaging. Expansion of endometrial cavity by SHG allows optimal visualization and plays an important role in the evaluation of abnormalities related to the uterus. The most common indications for hysterosonography are: abnormal uterine bleeding both pre and post-menopausal,investigation on of infertility and recurrent miscarriage, endometrial assessment for patients on tamoxifen therapy, congenital uterine malformation, abnormal hysterosalpingogram, indirect assessment of tubal patency, retained foreign body such as IUD, post-abortal placental remnants, endometrial synechia, polyp, fibroids, endometrial neoplasia or hyperplasia. SHG is a relatively easy technique. The entire procedure usually takes about 5-10 minutes. In this technique after a baseline transvaginal ultrasound the cervix is exposed with a sterile speculum and gently cleaned with Betadin, balloon-tipped silicone urine fully catheter is advanced through the cervical canal and into the lower uterine segment. After distention of the balloon the speculum then is exchanged for an endovaginal ultrasound probe. This is followed by the instillation of 5-20 cc sterile saline through the catheter under direct ultrasound guidance until an adequate distention of the uterine cavity was obtained. The entire endometrial cavity to the cervical canal was investigated .Uterine abnormalities that can be detected at SHG include congenital anomalies and acquired endometrial anomalies which includes polyps, leiomyomas, hyperplasia and synechiae. SHG has known as a first line investigation modality in diagnosis of uterine abnormalities. According the literature hysterosonography offers adequate visualization and characterization of intrauterine lesions with high sensitivity (90-100%) and specificity (40-98%) compared to diagnostic hysteroscopy. SHG as a reliable, simple, feasible, time efficient, cost-effective and non-invasive method is currently regarded as the preoperative diagnostic procedure and can reduce indications for diagnostic hysteroscopy.

P_{nm}-2: The Stigma of Womanhood Thief: Polycystic Ovary Syndrome

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Background: In many ways, the experience of stigma has come out of the closet but yet it remains a seriously neglected issue in the global response to some chronic disease such as polycystic ovary syndrome (PCOS). PCOS is the most common hormonal problem in women which can be a precursor to many life threatening conditions including type II diabetes, hypertension, cardiovascular disease, and cancer. This means PCOS contributes to some of the leading causes of death or disability in women today! The symptoms of PCOS can be not only physically debilitating, but also emotionally and psychologically wrenching. Infertility, Irregular or absent menses, excess hair growth on face and body, male-pattern hair thinning, acne and obesity can make the stigma in both visible and hidden, physically and psychologically. For many women, learning that they have PCOS is shattering. It can distort their basic definitions of womanhood. Shame anger, guilt, denial, and blame are just some of the emotions she and her partner may be dealing with. Fertility treatment for PCOS can involves emotional, ethical, moral even religious dilemmas. PCOS and social stigmas around symptoms, adversely impact on self-efficacy, fail to conform to societal norms, decreased sexual self-worth, sexual satisfaction, emotional health, self-esteem and body image, loss of feminine identity and sexuality, impaired quality of life, maladaptive coping strategies such as avoidance of social interaction. Many people feel ashamed of their PCOS and often try to hide it as they can sense other people's judgment. These women also may react by internalizing and accepting negative attitudes about themselves, and may increase unhealthy behaviors. Stigma against the condition itself may keep people from admitting they need counseling or other help. Sometimes the discrimination feels worse than the illness itself.

Materials and Methods: This is a review about PCOS stigma.

Results: Therefore, stigma reduction efforts should help people understand what it is and what it does.

Conclusion: Clinicians should pay attention to the psychosocial dimension of PCOS, regardless of symptom severity or treatment response. Other efforts:

• Psychological counseling and participation in a PCOS support group in order to enhance a woman's sense of control and reduce shame and stigma.
• Disseminate information and facilitate knowledge sharing. It's also useful to know about their rights.
• Providing social support by their social communities, which may include spouses, family members, friends, and acquaintances.
• Foster collaboration between individuals and groups for the purposes of research, programming and advocacy.

Keywords: PCOS, Stigma

P_{nm}-3: The Effect of Ginger Hydro-Alcohol Essence on The Ovarian Hormons in the Brest Cancer Rats by Treatment Cyclophosphamide in Estrus Cycle

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Background: Cyclophosphamid as an anti-neoplastic medicine, is classified as a derivative of mustard nitrogen, which passes through brain- blood -barrier and it is known as causing significant poisoning affects with high stoppage effects on body. Through attaching two DNA chains of cell nucleus and breaking inter/intra-chain connection and inhibition RNA synthesis Cyclophosphamide causes high toxicity effects on body. Of main effect of the medicine is Amenorrhea, reducing hypothalamuses- hypophysis gonad hormones, ovaries fibrosis, infertility, arthritis, Thrombophlebitis, renal toxicity, bladder fibrosis, and cystic hemorrhagic.
Materials and Methods: The study is conducted over 40 rats of Wistar race with average weight of 45 g. in 5 group each of which with 8 members. First, samples were excited by IP injection of PMSG (10 IU) in follicular phase, then ovulation activated by administering HCG (10 IU), and then we injected to them 3 mg/Kg/daily DMBA in via gavage process and they received Brest cancer after 5 weeks. Control group received no medicine. While sham group only received medicine solvent and experiment group received cyclophosphamide with a dose of 5 mg/Kg/BW. After 21 days samples received ginger hydro-alcohol essence (gavage) with dose of 1 g/kg/BW with Cyclophosphamide.
Results: During this study, statistical tests kruskal-wallis on different concentrations of hypophysis gonadotropic plasma hormones estrogen, progesterone and different factors were examined. Rates in experimental group which received hydro-alcohol essences with Cyclophosphamid placed at $p<0.05$ level in comparison to the group which only received Cyclophosphamid and there was a significant increase in gonadotropic hormones.
Conclusion: This research showed us that ginger hydro-alcohol essence with dose of 1 g/kg/BW could inhibit pharmacology effects of Cyclophosphamide for fertility.
Keywords: Cyclophosphamid, Ginger Essence, DMBA, Brest Cancer, Ovarian Hormons

P_{nm}-4: Emotional Responses to Infertility. Nursing Interventions

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Background: One third of the women who sought infertility treatment had an impaired psychological well-being. The unfulfilled desire of millions of infertile couples worldwide to have their own biological children results in emotional distress. Infertility has mental, social, and reproductive consequences.
Materials and Methods: In this review article, electronic searches were undertaken in PubMed, Scholar Google and up to date since 2007.
Results: Couples and individuals vary in their responses to the infertility experience. Psychological interventions and improvements in the organization of care are essential to positively impact on outcome during diagnosis and treatment in this group of women. Appropriate nursing interventions are suggested for each specific stage. It can be useful to take cultural factors into account in evaluating the mental health of infertile women. Psychosocial interventions are more effective in reducing negative affect. Proper psychological care and counseling should be an integral part of infertility management.
Conclusion: The need for additional and continuous training for nurses employed in fertility settings is paramount. Health-care personnel treating women with fertility problems should be aware of the emotional response of their patients in order to recognize and treat possible psychiatric morbidity. It is crucial to mandate psychiatric counseling in all fertility centers in order to diagnose and treat infertile patients with psychiatric disorders.
Keywords: Emotional, Infertility, Nursing, Intervention

P_{nm}-5: Midwifery Intensive Cares in Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome is a common endocrine disorder with 3-7% prevalence in the general population and 10% in women of childbearing. This rate has increased in infertile women. The main composition of this syndrome includes anovulation or olygoovulation, clinical and laboratory signs of increased androgens and observation polycystic ovaries on ultrasound in evaluation. In this study the objective is expression of intensive midwifery cares in women with polycystic syndrome.
Materials and Methods: Current study through review of several papers pays to survey polycystic ovary syndrome and it midwifery intensive cares. Articles are used from 2004 onwards.
Results: Women with polycystic ovary syndrome show different metabolic disorders like abdominal obesity, insulin resistance, hypertension, dyslipidemia and increased levels of inflammatory markers. All this women are at risk of infertility with ovulatory origin, diabetes, cardiovascular disease, fatty liver, depression, osteoporosis, metabolic syndrome and some related cancers especially endometrial cancer. Due to various complications of polycystic ovary syndrome, several studies have been made toward reduction these complications. training and caring role of midwives in diet for weight loss, how to use drugs to stimulate ovulation, a regular menstrual cycle, exercise, in taking dietary especially foods with high carbohydrates and low in fat, the use of zinc, vit D and calcium for prevent osteoporosis, application acupuncture for improve insulin sensitivity and decrease glucose, screening women with this syndrome that are at risk for endometrial cancer be useful.

Conclusion: Despite the prevalence of PCOS in women of childbearing age and its Impact over the health of women and according midwives prominent role in women's health, nowadays midwives are seen faintly in the area of infertility.

Keywords: Infertility, Polycystic Ovary Syndrome, Midwifery Intensive Cares

P_{nm}-6: Satisfaction and Preferences of Cesarean Delivery in Nulliparus Women in Babol 2010: A Postpartum Survey

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Background: Despite international policy documents calling for reduction in medical interventions in birth. Cesarean section (CS) rates throughout the world, so as in Iran, have continuously rising. Many publications have examined the reasons behind the worldwide rising cesarean delivery rate. The purpose of this study was to investigate pregnant women's intentions for opting for CS and postpartum satisfaction.
Materials and Methods: This cross-sectional study was conducted at six medical centers (4 public-2 private) in Babol over a 1 year period including rural, semi-rural and urban women after the childbirth. Randomly 180 women were eligible to include this survey. Semi structured questionnaires were completed just a day after giving birth by CS. The questionnaire was developed on the basis of a review of published reports, a review of existing questionnaires, and consulting with obstetricians, midwife and epidemiologist. Data gathered and analyzed by SPSS version 18 software and Chi-square test.
Results: The mean age of the individuals was 25 ± 4.92. 59.7% were occupied in urban area. 53 (29%) subjects had chosen CS by their own demand and the others (71%) which most of them preferred vaginal delivery had to be performed CS because of specific medical indications. In the first group, the main reasons mentioned by mothers for choosing CS were respectively: fear of pain (62%), fear of fetal damage during vaginal delivery (37%) and relatives' suggestions (35%). On the other hand, in obligative group, the main indications by the physician's diagnosis were prolonged labor (27%); fetal endangered (24%) and cephalopelvic disproportion (16%). In selective group 92.5% were satisfied after labor which in the other group 62% of mothers express some degrees of satisfaction ($p<0.001$, OR=9.30).
Conclusion: As many studies revealed, if a woman chooses cesarean delivery in her first delivery, she is more likely to have subsequent deliveries by cesarean. Then specific and scientific based supportive care during first labor and delivery, and preparation or education on issues surrounding cesarean section, could play a pivotal role in making the true decision and improves women's satisfaction with birth.

Keywords: Cesarean Section, Nulliparus, Babol

P_{nm}-7: Sociological Study of Infertility and Its Consequences among Infertile Women

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Background: Infertility is a worldwide problem presented in various communities and both infertile women and men suffered its psychosocial impacts. But in societies like Iran- where the cultural norms and stereotypes, usually put more value on women as mothers and consider children as a part of women's status and identity and a source of power for them in family and in the society- the infertile women are far more vulnerable than their male counterparts. This study aims to examine the impact of infertility on different aspects of infertile women's lives.
Materials and Methods: This study reviewed the current literature on infertility in various databases including: PubMed, Google Scholar, Proquest, Elsevier, Springer (English articles) and Iran Medex, Magiran and Scientific Information Database (Persian articles). This review along library studies aims to present a sociological analysis of infertility and its consequences.
Results: Some of the most significant sociological factors in studying the phenomenon of infertility are the culture, gender and the treatment process. In Asian cultures fertility ensures stability and continuous marital relationship. In some communities sense of femininity is defined through motherhood, which is often the only way to improve women's status within the family and society. Therefore, there is a gender-related suffering in all infertility cases. Nowadays, with technological advancements in infertility treatment area, the infertile women face the challenges of treatment procedure in addition to previous bio-psychological and social consequences of their situation.
Conclusion: Several studies in the field of infertility and its impact on various aspects of women's lives show the necessity for infertile women's empowerment in various spheres of life.

Keywords: Infertility, Infertile Women, Sociological, Risk Factors, Consequences

P_{nm}-8: The Relation between Birth Weight and Obesity in Iranian Primary School Children

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Background: In developed countries, overweight and obesity in children and adults is considered as basic problems. Several surveys show increasing trend of childhood obesity in developing countries, particularly in the Middle East and because of its risky role on people's health, researchers aimed to assess the relations between birth weight and BMI in primary school children of Rasht/Iran.
Materials and Methods: This was a case-control study which was performed in8primary schools of Rasht. 320 samples including 80 in case group (BMI ≥ 85th percentile for ageandsex) and 240 in control group (BMI= 5th-85-th percentile for age and sex) were selected. Data gathering tool were questionnaires, scales and tape meter. Data were analyzed statistically by Chi-square, Mann-Whitney and Stepwise Multivariate Regression tests.
Results: Findings showed that mean and standard deviation of birth weight (g) in groups by Mann-Whitney test were statistically significant (p=0.000). Multi variate regression analysis indicated that birth weight, age, exclusive breast feeding and meal have significant effects on body mass index.
Conclusion: According to results, identifying children at risk for adolescent obesity provides physicians with an opportunity for earlier intervention with the goal of limiting the progression of abnormal weight gain.
Keywords: Birth Beight, Body Mass Index, Obesity, Children

P_{nm}-9: Infertility and Healthy Diet

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Background: Over the past twenty years, fertility problems have increased dramatically. At least 25 percent of couples planning a baby will have trouble conceiving, and more and more couples are turning to fertility treatments to help them have a family. Eating a fertility diet in preparation for pregnancy and to boost fertility is one of the most powerful health changes everyone can make. It is true, food can increase sperm production and save partner from indecently expensive infertility treatments when they want to start a family. Here are some good health tips for men to stay fertile and potent.
Materials and Methods: This study is a comprehensive literature review from 1990 to 2oo11 with keywords including infertility, healthy and diet, is carried out by searching Iranian and International databases including Iran Medex, IranDoc, Pubmed, Sience Direct and Ovid.
Results: Nutrients in food for increasing fertility are:
1. Selenium: This is an unusual mineral that increases the motility of sperms. Selenium rich food increase sperm's mobility and thus raises the chances of conception. Foods: Cottage cheese, eggs, chicken, turkey and nuts.
2. Zinc: This a very vital mineral required for regulating body's testosterone level. Testosterone as is the hormone responsible for male reproductive functions. Until and unless men have sufficient Zinc in their body, testosterone is not produced in adequate amounts which in turn reduce sperm count. Foods: Abundantly available in red meat, seafood and eggs. Vegetarians need to watch it. Have pumpkin seeds and oatmeal.
3. Vitamin A: The Vitamin A rich food increases the value of sperm production by making sure that the sperms that are produced are healthy and agile. If men have a deficiency of this vitamin then their sperms will be slow and lazy, not good enough for conception. Foods: Carrots are best but red chilli peppers, spinach, iceberg lettuce, broccoli and dry fruits like apricots too will be good for increasing fertility.
4. Vitamin C: We probably consume citrus foods for the protection against cold but the antioxidants in them help 'cleanse' the sperms. They get rid of toxins and purify the semen by extracting free radicals. Foods: Oranges, lemon, red and yellow bell peppers, tomatoes are foods that increase fertility.
5. Omega-3 Fatty Acids: These are special boosters that when present in the blood increases the blood circulation of the male reproductive organs hence giving energy and boosting potent sperm production. Foods: Found mainly in fish like Salmon and Sardines. Walnuts also could be an alternative for vegetarians.
Conclusion: Both partners should follow the dietary recommendations .Although it goes without saying that a healthy diet is crucial to a successful pregnancy and a healthy baby, many people are unaware of the fact that diet can help to correct hormone imbalances that may affect your ability to conceive. There are also certain foods and drinks that are known to lower fertility.
Keywords: Infertility, Healthy, Diet

P_{nm}-10: The Role of Nurses and Midwives in The Care of Women Volunteers Surrogacy

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Background: Various methods of ART have been able help to many women to have children. Surrogacy is one of these methods that is performed in Iran like other country. According to development of this method we need to survey of all, legal, religious, moral, psychological and sociological aspects.
Materials and Methods: A review of library.
Results: Although in most of volunteers of Surrogacy women who Self-giving are without financial motivations and just for improvement and continuation of other families to bear the risks of pregnancy and other household hazards associated with 9 months of pregnancy, childbirth and complications of Cesarean delivery but sometimes, women who are willing to do this due to financial needs to be done. Considering that every individual has the right. Doctors and midwifes are realm of competence and expertise to participate in notifying patient about the disease and consequences of the treatment process and outcomes and the direction of treating psychological

problems during and after pregnancy. Few studies have been made in exploration of the effects and risks of this approach, but complications such as hysterectomy after delivery has been reported. For this method have also expressed same symptoms for IVF procedure.

Conclusion: Therefore this technique as a method of surrogacy should be considered high risk pregnancy and Training courses should be considered for health care providers be able to best care for obstetric complications associated with the ethical, legal and social consideration.

Keywords: Surrogacy, Women, Pregnancy

P_{nm}-11: Care Plane of Anxiety Decrease on Hemodynamic Signs and Cortisol Level in Women Undergoing Elective Surgeries

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Background: Although surgery are now used as one of the treatment methods and save the patients from death that have some complications. The patients may be considered anxiety from the surgery as a threat of the natural process of the life. The aim of this study was assessment of the Impact of preoperative nursing care on hemodynamic signs and cortisol level in women undergoing elective surgeries.

Materials and Methods: This randomizes clinical trial study carry out in kosar hospital at Qazvin University of medical sciences. This study was clinical trial that 60 female patient's candidate for elective gynecologic surgery enrolled based on random allocation in two group case and control. The Statistical Package for the Social Sciences, SPSS 17 for Windows, was used for the analysis. Data analysis was performed by using descriptive (mean ± SD) and analytical statistics such as such as chi- square, fisher's exact test, t test and Wilcoxon. The significant level was set at less than 0.05 ($p< 0.05$).

Results: Two groups were homogeneous in age ($p=0.2$), marital status ($p=0.5$), education ($p=0.1$), employment status ($p=0.13$) and admission ($p=0.3$). With preoperative nursing care, mean score of cortisol level at morning in case group 314.23 (L/Nmol) was less than control group.

Conclusion: According to the results of this research preparation nursing care plan lead to decrease the level of cortisol in patients in pre elective operation. Therefore this program as a non pharmaceutical and effective plan is recommended for them.

Keywords: Nursing Care, Anxiety, Operative, Hydrocortisone

P_{nm}-12: Relaxation Technique Effect on Stress Score and Pregnancy Test of Infertile Women in Isfahan Fertility and Infertility Center (2010)

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Background: Infertility effects on psychological and emotional tension in couples and make severe stress in infertile men and women. Relaxation technique is one of the methods which reduce stress and balance human emotions. The purpose of this study was determination of relaxation effect on stress score in the infertile women and pregnancy test as outcome of their treatment.

Materials and Methods: This study was a semi experimental and clinical trial study. Participants were studied by randomize sampling in two groups. At first, the stress score was determined in both groups, by Newton's infertility stress questionnaire. Stress score was evaluated in participants and then relaxation technique was administered in the intervention group. This technique performed in 12 sessions. All questionnaires were completed under supervision of the researcher after embryo transfer to the uterus (after 2 weeks) and before pregnancy test administering.

Results: Independent t-test showed total stress score does not have significant difference in groups before intervention ($p>0/05$). Whereas, independent t-test indicated significant difference in stress scores between the two groups after intervention ($p<0/05$). Stress score was higher in the control group than the intervention group. Chi-square test also showed that positive pregnancy test was significantly higher in the intervention group ($p<0/05$).

Conclusion: Relaxation technique as a complementary and alternative medicine (CAM) method can reduce stress score in infertile women and effect on treatment outcome.

Keywords: Infertility, Relaxation, Stress

P_{nm}-13: Pregnancy Outcomes in Women with Polycystic Ovary Syndrome Compared with Normal Women

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Background: Polycystic ovary syndrome (PCOS) is a common reproductive disorder associated with many characteristic features, including hyperandrogenaemia, insulin resistance and obesity which may have significant implications for pregnancy outcomes and long-term health of the woman. It has an incidence ranging from 5 to 10%.

Materials and Methods: This study is conducted to evaluate the pregnancy and perinatal outcome in wom-

en with PCOS. Pregnancy outcomes in 208 women with PCOS and were compared with 240 normal women.
Results: Women with PCOS demonstrated a significantly higher risk of developing gestational diabetes (133 in PCOS groups Vs 98 in normal group, p=0.00), pregnancy-induced hypertension and preeclampsia (67 in PCOS groups vs. 21 in normal group, p=0.01) and preterm labor (22 in PCOS groups vs. 14 in normal group p=0.01). Furthermore, women with PCOS seem to experience increased risk of cesarean delivery than normal group. There was no increased risk of congenital anomalies.
Conclusion: Women with PCOS are at increased risk of pregnancy complications. Pre-pregnancy, antenatal and intrapartum care should be aimed at reducing these risks.
Keywords: PCOS, Pregnancy Outcome, Normal Women

P_{mn}-14: The Stigma of Womanhood Thief: Polycystic Ovary Syndrome

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Background: In many ways, the experience of stigma has come out of the closet but yet it remains a seriously neglected issue in the global response to some chronic disease such as Polycystic Ovary Syndrome (PCOS). Polycystic Ovary Syndrome (PCOS) is the most common hormonal problem in women which can be a precursor to many life threatening conditions including type II diabetes, hypertension, cardiovascular disease, and cancer. This means PCOS contributes to some of the leading causes of death or disability in women today! The symptoms of PCOS can be not only physically debilitating, but also emotionally and psychologically wrenching. Infertility, Irregular or absent menses, excess hair growth on face and body, male-pattern hair thinning, acne and obesity can make the stigma in both visible and hidden, physically and psychologically. For many women, learning that they have PCOS is shattering. It can distort their basic definitions of womanhood. Shame anger, guilt, denial, and blame are just some of the emotions she and her partner may be dealing with. Fertility treatment for PCOS can involves emotional, ethical, moral even religious dilemmas. PCOS and social stigmas around symptoms, adversely impact on self efficacy, fail to conform to societal norms, decreased sexual self-worth, sexual satisfaction, emotional health, self-esteem and body image, loss of feminine identity and sexuality, impaired quality of life, maladaptive coping strategies such as avoidance of social interaction. Many people feel ashamed of their PCOS and often try to hide it as they can sense other people's judgment. These women also may react by internalizing and accepting negative attitudes about themselves, and may increase unhealthy behaviors. Stigma against the condition itself may keep people from admitting they need counseling or other help. Sometimes the discrimination feels worse than the illness itself.
Material and Methods : This is a review about PCOS stigma.
Results: Therefore, stigma reduction efforts should help people understand what it is and what it does.
Conclusion: Clinicians should pay attention to the psychosocial dimension of PCOS, regardless of symptom severity or treatment response. Other efforts:
• Psychological counseling and participation in a PCOS support group in order to enhance a woman's sense of control and reduce shame and stigma.
• Disseminate information and facilitate knowledge sharing. It's also useful to know about their rights.
• Providing social support by their social communities, which may include spouses, family members, friends, and acquaintances.
• Foster collaboration between individuals and groups for the purposes of research, programming and advocacy.
Keywords: Polycystic Ovary Syndrome, PCOS, Stigma

P_{mn}-15: The Role of Lifestyle Modification in Management of Overweight Infertile Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a complex, heterogeneous disorder of uncertain etiology, with a prevalence of up to 10% and frequently associated with obesity, with at least 50% of women with polycystic ovary syndrome demonstrating overweight or obesity defined by body mass index >25 or >30 kg/m^2. The syndrome is associated with numerous morbidities, including infertility, obstetrical complications; type 2 diabetes mellitus, cardiovascular disease, and mood and eating disorders.
Material and Methods: Obesity, central obesity and insulin resistance are strongly implicated in its etiology and improving these factors should be a central treatment focus. However, extreme non-surgical efforts and short-term dietary improves both metabolic and endocrine complications of PCOS as well as clinical markers such as ovulation but are associated with high rate of weight regain
Results: Recent studies indicated that lifestyle modification through individualized treatment on diet, exercise, and modifying additional lifestyle factors such as psychosocial stressors and drug addictions for 24 weeks, and monthly individual or group sessions to follow up and reinforce the changes, improves psychological self-esteem, anxiety, mean depression and general health questionnaire scores and reproductive outcomes in overweight infertile women with PCOS. In a recent clinical trial lifestyle modification significantly reduced total androgen, lipid profile and waist circumference and had better pregnancy rate in comparison with clomiphene citrate and metformin treatment in overweight infertile women with PCOS
Conclusion: Lifestyle modification programs with an emphasis on behavioral management and dietary and exercise interventions without rapid weight loss leads to a reduction of central fat and improved insulin sensitivity, which restores ovulation in overweight infertile women with PCOS; however, the literature on assessment of ef-

fectiveness of lifestyle modification on improving pregnancy rate and reducing miscarriage rates, gestational diabetes has been sparse and structured recommendation for enforcing components of lifestyle modification programs should be assessed in our population.

Keywords: Lifestyle Modification, Overweight Infertile, Polycystic Ovary Syndrome

P_{mn}-16: The Effect of Environmental Contaminants on Testicular Function

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Male reproductive health has deteriorated considerably in the last few decades. Nutritional, socioeconomic, lifestyle and environmental factors (among others) have been attributed to compromising male reproductive health. In recent years, a large volume of evidence has accumulated that suggests that the trend of decreasing male fertility (in terms of sperm count, quality and other changes in male reproductive health) might be due to exposure to environmental toxicants. These environmental contaminants can mimic natural oestrogens and target testicular spermatogenesis, steroidogenesis, and the function of both Sertoli and Leydig cells. Most environmental toxicants have been shown to induce reactive oxygen species, thereby causing a state of oxidative stress in various compartments of the testes. However, the molecular mechanism(s) of action of the environmental toxicants on the testis have yet to be elucidated. This review discusses the effects of some of the more commonly used environmental contaminants on testicular function through the induction of oxidative stress and apoptosis.

Keyword: Apoptosis, Environmental Contaminants, Male Reproduction, Oxidative Stress, Reactive Oxygen Species, Testis, Spermatogenesis

P_{mn}-17: Support from Health Care Providers for Infertile Couples

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Undergoing treatment with assisted reproductive technology may be a traumatic experience that will contribute to a variety of psychological difficulties. This is chiefly important because the numbers of women looking for infertility services are increasing. Therefore the emotional and psychological support offered in their nursing care is dominant. The degree of emotional distress can be very high and it is necessary that this be known. Here we review the existing literature on this group of women, which are placed in one of three stages:
A. Planning/Hoping stage: a couple are optimistic about conception practices and plans
B. Doubting/Wondering stage: a couple may express feelings of decreased self-esteem
C. Worrying/Despairing stage: in this stage the signs and symptoms of anxiety and depression are more severe. Hopelessness and blaming of self or the partner, makes it difficult for couples to relate to each other and make future decisions. It is hypothesized that greater perceived support from health care providers is related to lower stress, depression, and anxiety for both infertile men and women. The focus of intervention for stage A is educative, for stage B is both educative and supportive and for stage C, based on the severity of symptoms, is referring to mental health professional. The aim of the proposed interventions is to stop progression to the next level of psychosocial symptoms. Verbal and written education about essentials of male and female anatomy and physiology, how the drugs act on their body and probable side effects is one of the primary roles of the care provider. Providing videos/DVDs and referring to specific support groups such as the Polycystic Ovarian Syndrome Association can be educational components of treatment which encourage couples to become active participants. Published evidence suggests that women who receive counseling and are in a support group are more likely to conceive than those who do not participate in active support. A follow-up visit should be planned based upon the amount of support the couple feels they need. These interventions, along with passage of time, typically improve common worries. Finally, since advanced nursing roles in fertility generate continuous communication between nurses and patients that may conclude to intimacy, managing the nurse-patient relationship and maintaining a safe bounded relationship which both nurses and patients are comfortable, are suggested.

Keywords: Infertility, Nursing Care, Education, Psychological Support

P_{mn}-18: The Effect of Ambient Air Pollution on Infertility

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Development disorders may be arisen from the unavoidable maternal exposure to particulate air pollution during the prenatal life that can be affected not only periconceptional period but also postnatal life. A kind of chemical material categorized as air pollutants are present in the urine, blood and semen of exposed men and may decline sperm count and affect sperm quality. Environment toxic can alter fertility on different pathways in cellular processes including mitotic interference, altered energy sources, enzyme inhibition, altered cell signaling, mutation, affect in gene expression and alterations in immune functioning and programmed cell death. There is some

evidence for an association between brief exposure to high levels of environmental contaminants during the prenatal life and early pregnancy loss that showed increase in the risk of miscarriage. Air pollution has been associated with fertility impairment in women, possibly due to a number of factors involved in female reproductive function. Exposed to air pollution in women result to early pregnancy loss and it could be related to changes in the maternal uterine vascular compartment or intrauterine environment prior to pregnancy, which can be due to propose compromised delivery of maternal blood to the placenta and an increase in the resistance to its flow. The sperm DNA damage that may result from the exposure to intermittent air pollution could be also linked to the increased rate of adverse pregnancy. Male idiopathic infertility may be due to exposure to environmental toxicants that alter the sex hormones, spermatogenic function or sperm maturation. More recently, the effects of exposure to particulate air pollution on reproductive and pregnancy outcome have garnered increased interest. However, a limited number of studies have been conducted to examine the association between male reproductive outcomes and ambient air pollution, specifically semen quality, which includes sperm concentration, sperm count along with morphological abnormalities. Finally, some studies confirmed the increased risk of early pregnancy loss previously and adverse pregnancy observed in experimental studies in women exposed to air pollution. In sum of, exposure to particular air pollution has been associated with a variety of health effects, ranging from subclinical outcomes to death.

Keywords: Air Pollution, Infertility, Pregnancy Loss

P_{mn}-19: A Comparison of Neonatal Outcomes following Transfer of Frozen and Fresh Embryo

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Background: Currently, frozen embryo transfers contribute an increasing rate of births from assisted reproductive technologies such as *in vitro* fertilization (IVF). However, very few studies evaluated the neonatal outcomes from frozen embryo transfer. This study aimed to investigate and compare the neonatal outcomes from frozen embryo transfer versus fresh embryo transfers.

Materials and Methods: This systematic review assessed more of 20 studies from books, articles and different sites of internet about the neonatal outcomes following transfers of frozen and fresh embryo in 1995-2012.

Results: Studies illustrated no significant difference between two kinds of embryo transfers in preterm birth rate and mode of delivery. However, the boys to girls ratio was 1.05 times higher in the frozen group. Low birth weight (LBW), small for gestational age (SGA) and prematurity were approximately twice higher in the fresh population. In another study compared intrauterine parameters of 910 singletons born after frozen embryo transfer (FET) with 9603 babies from fresh embryo transfer and 4656 naturally conceived (NC) children. The mean birth weight of the frozen babies was 134g higher compared to the fresh babies ($p<0.0001$). The rate of large for gestational age (LGA) babies was significantly different between the three groups with 16.9% for FET, 10.3% for fresh transfer and 11.4% for NC babies. The same applied to the rate of babies with birth weight of 4500gr or more (5.6%, 2.8% and 3.4% respectively).

Conclusion: Frozen embryo transfer did not seem to adversely affect neonatal outcomes including prematurity, LBW and SGA and even birth weight in FET was higher because in FET cycles hormone supplementation mimics the natural cycle, compared to the super physiologically high hormonal stimulation of women in fresh cycles, this similarity to natural cycle in FET may influence endometrial receptivity, early implantation and placental development in a positive way, leading to fetal growth and higher mean weight.

Keywords: Frozen Embryo Transfer, Fresh Embryo Transfer, Neonatal Outcome

P_{mn}-20: A Randomized Clinical Trial of The Efficacy of Acupressure on Nausea and Vomiting of Pregnancy

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Background: Considering nausea and vomiting are a common complaint of pregnancy. Most non-medication therapy and complementary and alternative medicine were preferred to reduce it in recent years. The aim of our study is to determine the effect of acupressure (on point KID21) on nausea and vomiting of pregnancy.

Materials and Methods : This single blind clinical trial study was performed on 80 women with nausea and vomiting in the first trimester of pregnancy. Women were randomly divided to two groups; intervention group with the acupressure on KID21 point and the placebo group with pressure on sham acupressure for 20 minutes per day in four consecutive days. The intensity of nausea was assessed by visual analogue scale (VAS) and vomiting frequency was evaluated by counting during these four days. The results compared with each other by Chi-square, t tests, Mann-Whitney, Friedman and Sign-rank test.

Results: The intensity of nausea between intervention

and placebo groups was shown statistically significant differences on the fourth day (p<0.001 U=228.5) and also the intensity of vomiting on the fourth day demonstrated significant difference between two groups (p<0.001, U=380). The intensity of nausea and vomiting in the intervention group was less significantly (p<0.05).
Conclusion: Acupressure with pressure on point KID21 is more effective than sham acupressure in reduction of nausea and vomiting in pregnancy, so it can be purposed to diminish intensity of pregnancy nausea and vomiting.
Keywords: Acupressure, KID21, Nausea, Vomiting, Pregnancy

P_{mn}-21: Inclusion of Sunflower Seed in Diet at Different Levels Has A Negative Influence on The Different Reproductive Morphology Parameters

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Background: Effect of feeding with different levels of sunflower seeds on Reproduction system in laying hens
Materials and Methods: A total of 160 white Bovens hens at 30 weeks of age were housed in cages in an open-sided building under a 16 h light: 8 h dark lighting schedule. Birds were randomly divided into four treatments and were fed, ad libitum, diets containing 0% (control), 3%، 6% and 9% sunflower seeds from 30 to 36 weeks of age. At the end of the experimental period, 8 females from each treatment were randomly chosen, anaesthetised and killed by decapitation. Ovary and oviduct samples were immediately weighted and ovarian follicles were classified
Results: No negative effects were detected in ovary and oviduct weights, expressed in both absolute terms and relative to body weight. The numbers and total weights of large yellow follicles (LYF) in the ovary were significantly affected by sunflower seeds supplementation (p<0.05)
Conclusion: it could be concluded that inclusion of sunflower seed in laying hen diets at different levels have a negative influence on the different reproductive morphology parameters in laying hens.
Keywords: Sunflower Seed, Reproduction System, Ovarian Follicles, Oviduct

P_{mn}-22: The Effect of Zinc on Semen Quality

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Background: Zinc in human semen seems to play an important role in the physiology of spermatozoa. Zinc deficiency leads to gonadal dysfunction, decreases testicular weight, and causes shrinkage of seminiferous tubules. The adult human body contains 1-3 g of zinc, about 0.1% of which is replenished daily. Excessively high amounts of zinc has been reported in human seminal plasma by many authors, the mean ranges from 78.9 to 274.6 mg/L. The major contributor of seminal plasma zinc is the prostate gland . This high concentration of prostatic zinc ions comes into contact with sperm after their functional maturation. On the other hand, the seminal zinc ions get diluted by the vaginal and cervical fluids immediately after ejaculation. Therefore, it is not clear as to how this high zinc level in seminal plasma affects the sperm function. Zinc has also been reported to be the primary factor responsible for the antibacterial activity of the seminal plasma. It has suggested that zinc may have a role in sperm production and/or viability, in the prevention of spermatozoa degradation, and in sperm membrane stabilization Although some beneficial effects of zinc on semen have been accepted, controversies continue regarding zinc levels between different subfertile groups as well as the relationship between zinc and semen parameters. The aim of this review study, therefore, was to examine the relationships between concentrations of zinc in blood and seminal plasma and sperm quality among infertile and fertile men.
Materials and Methods: This review article prepared by studdying of articles obtained from Google, pub med sites with key words such as : zinc ; infertility semen quality.
Results: Dissanayake (2010) studied semen parameters of 152 healthy men To assess the relationship between seminal plasma zinc and semen quality using two markers; zinc concentration (Zn-C) and total zinc per ejaculate (Zn-T). The results show Count, motility, viability, pH and viscosity are affected by variations of seminal plasma zinc. Zn-C was significantly high in the asthenozoospermics compared with the normal motile group; 138.11 µg/mL (83.92) vs. 110.69 11 µg/mL (54.59) (p<0.05). Zn-T was significantly low in samples with hyperviscosity compared with samples with normal viscosity; 220.06 µg (144.09) vs. 336.34 µg (236.33) (p<0.05). Conversely, Zn-T was high in samples with low viability compared with those with normal viability; 437.67 µg (283.88) vs. 305.15 µg (221.19) (p<0.05) there was a negative correlation between pH and Zn-C (r=-0.193, p<0.05) as well as Zn-T (r=-0.280, p<0.01). On the other hand, correlations were positive between Zn-T and sperm count (r=0.211, p<0.05) Zhao and Xiong (2005) studied 90 asthenozoospermic patients, 60 oligoasthenozoospermic patients and 20 fertile men with normal sperm quality and found that a positive relationship between the feebleness of sperm production and motility of asthenozoospermic and oligoasthenozoospermic patients and the lower content of seminal plasma zinc . Colagar et al. (2010) assess the relationship between Zn levels in seminal plasma with sperm quality in fertile and infertile men. Semen samples were provided by fertile [smoker (n = 17), nonsmoker (n = 19)] and infertile men [smoker (n = 15), nonsmoker (n = 21)]. Fertile subjects, smoker or not, demonstrated significantly higher seminal Zn levels than any infertile group (p<0.001). A trend was observed for a lower Zn levels in seminal plasma of smokers compared with nonsmokers. Seminal Zn in fer-

tile and infertile (smokers or nonsmokers) males correlated significantly with sperm count ($p<0.01$) and normal morphology of sperm ($p<0.001$).

Conclusion: According to the results, the andrological variables sensitive to seminal plasma zinc. There is a positive relationship between the feebleness of sperm production and motility of asthenozoospermic and oligoasthenozoospermic patients and the lower content of seminal plasma zinc .

Keywords: Zinc, Infertility, Semen Quality

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International Journal of Fertility and Sterility (IJFS)

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