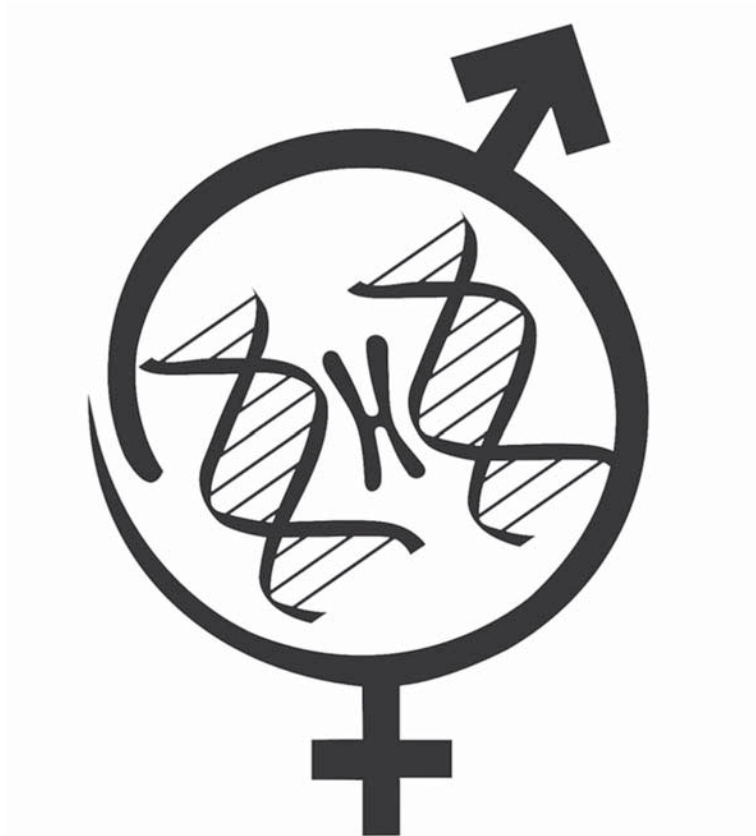


Abstracts of

Royan International Twin Congress

15th Congress on Reproductive Biomedicine
3-5 September 2014

9th Royan Nursing and Midwifery Seminar
3-5 September 2014



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

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Mohammad Hossein Nasr Esfahani

Dear Friends and Colleagues,

We are delighted to welcome you to Royan International Twin Congress, 15th Congress on Reproductive Biomedicine and 10th Congress on Stem Cell Biology and Technology in Tehran, September 3-5, 2014.

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

As ever, our scientific program relies on your contributions and new researches. We look forward to receiving your abstracts in all aspects of reproductive biomedicine.

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 scenes of Iran.

Best Regards
Mohammad Hossein Nasr Esfahani, Ph.D.
Congress Chairman
Reproductive Biomedicine

Abstracts of

15th Royan International Congress on Reproductive Biomedicine

3-5 September 2014



Royan Institute

Tehran, Islamic Republic of Iran

Invited Speakers

Andrology

I-1: The Andrological Counselling of the Infertile Men

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Background: Infertility is defined as the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year. About 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility. One out of eight couples encounters problems when attempting to conceive a first child and one in six when attempting to conceive a subsequent child. In 50% of involuntarily childless couples, a male-infertility-associated factor is found together with abnormal semen parameters. A fertile partner may compensate for the fertility problem of the man and thus infertility usually becomes manifest if both partners have reduced fertility. Male fertility can be reduced as a result of many diseases such as congenital or acquired urogenital abnormalities, urogenital tract infections, varicocele, endocrine disturbances, genetic abnormalities and immunological factors. In 30-40% of cases, no male-infertility-associated factor is found ("idiopathic male infertility"). The purpose of this presentation is to underline the importance of the evaluation of the infertile male in order to predict the spontaneous time-to-pregnancy to couples and to offer them the best chance to achieve a pregnancy, spontaneously or with assisted reproduction techniques.

Materials and Methods: We decided to review the literature in order to understand what are the newest advances in the evaluation of the infertile male.

Results: In the evaluation of infertile men, medical history, physical examination and semen analysis are standard assessments. If the results of semen analysis are normal according to WHO criteria, one test is sufficient. If the results are abnormal in at least two tests, further andrological investigations are indicated in order to diagnose, if possible, one of the conditions that could decrease male fertility. Sperm DNA fragmentation has emerged as a potential cause of fertility impairment and its assessment has been suggested as a useful adjunct to the laboratory evaluation, especially before the application of assisted reproduction technology (ART). Scrotal ultrasound is needed to detect the presence of varicocele and its characteristics (useful for deciding its surgical correction) and/or signs of seminal pathways obstruction; to demonstrate signs of testicular dysgenesis that are more frequent in infertile men and could lead to testicular malignancies. A low seminal volume can be due to distal seminal tract obstruction, where transrectal ultrasound is mandatory. Microbiological evaluation is useful in detecting infections of the male urogenital tract. Hormonal and genetic determinations are mandatory but only in specific situation (azoospermia, endocrinological diseases). Invasive diagnosis is indicated in patients with obstructive or non-obstructive azoospermia. In these cases, the procedures should be combined with extraction of testicular spermatozoa (i.e., TESE) for cryopreservation and subsequent ICSI and/or recanalisation surgery (in OA patients). Testicular biopsy is also important to detect carcinoma in situ, especially in men with non-obstructive azoospermia.

Conclusion: The andrological evaluation of the infertile male is extremely important even in the era of assisted reproduction technol-

ogy because it could lead to detect and treat diseases that could impair fertility and because the presence of malignancies among infertile patients is not so uncommon.

I-2: Varicocele and Male Infertility: A Clinical Dilemma

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Background: To date, impact of varicocelectomy on semen parameters is controversial mainly because clear preoperative selection criteria of patients to be submitted to varicocele surgery are still lacking.

A comprehensive review on varicocele pathophysiology, and indications to varicocelectomy according to controlled studies and meta-analyses from literature and the Guidelines of the Scientific Societies will be presented.

In our study we considered the characteristics of spermatic venous reflux, detected with Color Doppler Ultrasound (CDU), with the aim of finding a criterion for selecting infertile men candidate to varicocelectomy in order to significantly improve their semen quality.

Materials and Methods: Since 1983 our Group designed a prospective rigorous protocol study on infertile patients affected with varicocele, based on two preoperative semen analyses (basal and repeated three months later), a preoperative CDU in standing position to assess venous reflux along the spermatic and the pampiniform plexus veins, surgical varicocelectomy, a postoperative CDU (one month after varicocelectomy) for reflux recurrence exclusion, and two postoperative semen analyses (respectively 15 and 23 weeks after varicocelectomy). Semen samples were analyzed according to the WHO criteria in force at that specific time, and sperm concentration, forward motility and morphology were recorded.

According to the CDU, venous reflux was classified as basal continuous when, in standing position, a spontaneous reflux independent from respiration and increasing during Valsalva manoeuvre was registered, and basal intermittent, when, under the same conditions, a discontinuous reflux synchronous with breath movements was documented. At the end, out of 1.775 infertile patients, 360 met all strict inclusion criteria and were considered for the study: 319 patients showed continuous reflux (group A), whereas 41 had intermittent reflux (group B).

Results: Preoperatively, compared to the group A, group B showed both higher sperm concentration ($p = 0.03$) and morphology ($p < 0.0001$) without differences regarding sperm forward motility.

After varicocelectomy, compared to the baseline, neither sperm concentration, nor sperm motility, nor sperm morphology improved in the group B, whereas all sperm parameters improved significantly 15 and 23 weeks after varicocelectomy in the group A ($p < 0.0001$).

Conclusion: Preoperative semen parameters were found worst in infertile varicocele patients with a basal continuous reflux, and varicocelectomy improved significantly sperm quality only in this group of men; based on our results, infertile patients with a discontinuous reflux on CDU should not be routinely submitted to varicocelectomy.

I-3: New Advances in Surgical Sperm Retrievals for ICSI in NOA Patients

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Background: Nonobstructive azoospermia (NOA) is due to different forms of spermatogenic failure and affects the majority of azoospermic patients. In many NOA cases spermatogenesis was shown to be focal with a "patchy distribution". Different surgical sperm retrieval techniques have been developed to obtain gametes from the testicles of azoospermic men seeking fertility treatment. Nowadays, testicular sperm extraction (TESE) and microsurgical testicular sperm extraction (MicroTESE) are the most widely used. TESE is a standard open surgical biopsy: single or multiple specimens can be extracted from the same incision, or from different incisions (Multiple-TESE). In MicroTESE sperm extraction is done with the assistance of an operating microscope: testicular parenchyma is widely exposed and magnification can enable the search of seminiferous tubules that exhibit larger diameters (which are more likely to contain germ cells and eventually normal sperm production) in comparison to non-enlarged ones.

A comprehensive review of the literature on this topic shows: a. overall Sperm Retrieval Rate (SRR) is reported to be significantly higher using Micro-TESE in comparison with conventional TESE, ranging from 16.7 to 45% in the TESE vs. 42.9 to 63% with the MicroTESE; b. MicroTESE is reported to be the better technique in men with Sertoli cell only syndrome (SSR variable from 22.5 to 41% vs 6.3 to 29% in TESE group), and hypospermatogenesis (SSR variable from 81 to 100% vs 50 to 84% in TESE group); c. in case of a failed conventional TESE, a salvage Micro-TESE can offer a positive SRR of 45%.

The aim of our study was to define which is the more efficient sperm retrieval technique in NOA.

Materials and Methods: Patients: 558 infertile patients with NOA, aged 18-63 years (mean 36.8 Y), with normal testosterone level, normal karyotype and no Y-chromosome microdeletions were assigned to either TESE (N=356) or MicroTESE (N=202) on the basis of their clinical picture

Results: Testicular volume was comparable among groups, while FSH serum level was significantly higher in patients of MicroTESE group compared to TESE group (22.4 + 12.3 vs 19.64 + 14.8, p=0.025). Histology revealed that Sertoli cell only syndrome (SCO) was a more frequent finding in MicroTESE patients (79.2% vs 60.6%, p=0.00007), while hypospermatogenesis (HYPO) was a more common feature of TESE patients (23.87% vs 12.3%, p=0.01). SRR was comparable in both groups of patients (44.4% in TESE group and 39.6% in MicroTESE group, p=0.27), regardless of the histology findings.

Binary logistic regression showed, however, that MicroTESE provided significantly higher SRR compared to TESE in a subgroup of patients with FSH level > 12 mIU/ml, testicular volume < 7.1 ml and histology different from HYPO.

Conclusion: When looking at patients clinical characteristics, MicroTESE could provide better results in patients with higher FSH level and lower testicular volume.

I-4: A Simple and Safe Technique for Varicocele Surgery

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Varicocele is the most common pathologic finding in men in infertility study programs and is the most treatable cause of male infertility. Its correction is performed by different surgical techniques, none of which is yet acknowledged as the "gold standard." Some of them, especially the microsurgical ones, are very time consuming and thus expensive; furthermore, all varicocele surgery is not completely free from some complications and recurrences.

With this short video we present our antegrade subinguinal sclerotization technique.

Our variation of the antegrade subinguinal sclerotization technique is derived from our experience in using the Tauber's technique.

A subinguinal incision is performed with the exposure and subsequent suspension of the spermatic cord between two elastic bands; the internal and external spermatic fasciae are opened to identify the venous vessels afferent to the internal spermatic veins in the fat of the spermatic cord; the latter is temporarily clamped by using the two above mentioned elastic bands (one upstream and one downstream) at 7-10 cm distance, a 25-G short Butterfly needle is inserted into the largest vessel and 1.5-3 mL of 3% aethoxysclerol mixed with 0.5 mL air is injected into it; after sclerotization the vein at the injection site is ligated to prevent loss of the sclerosing agent outside the vessel.

With our technique varicocele persistence (= finding again a continuous venous reflux at the 3-month ultrasonographic follow-up) is very low (1.9%). The most common complication (< 2%) is an isolate and small transient penile lymphangitis, the cause of which is unclear. Major complications, such as orchitis, orchio-epididymitis, orchio-funiculitis, and testicular atrophy, were never observed in our series of more than 1500 filed cases.

Colpi's antegrade sclerotization is easy to be performed and safe. Given its favorable results in terms of complications and recurrences, this technique can be used not only for the first-line treatment of varicocele, but for treating recurrences following other surgical techniques.

I-5: Alteration in B-Defensin 126 Genes Associated with Unexplained Male Infertility and ART Outcomes

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High expression of β -defensin 126 is reported in epididymis, which coats the plasma membrane of sperm during epididymal transit. This protein is considered as an important component of the primates and human sperm glycocalyx and supplies sperm to penetrate into the cervical mucus. Previous studies found DEFB126 variation would affect the sperm function and male fertility rate.

In this study, DEFB126 variation was investigated in 40 fertile men and 190 men with unexplained infertility including 35 patients who did not undergo any ART cycles, 76 male partners of unexplained couples whose wives had undergone IUI and 79 male partners of unexplained couples who had tried IVF and ICSI procedures. Standard PCR and Single-strand conformation polymorphism (SSCP), Tetra PCR and Sequencing were used to confirm the results of gene mutation. ELISA and Immunochemistry were performed for the assessment of this protein expression on sperm cells. Analysis of genetic data revealed 28.8% homozygote deletion in unexplained infertile men while this

deletion was detected in 7.5% of controls. The deletion frequency was statistically higher in infertile patients than that of in control group ($p < 0.05$). Results of the IUI showed that 24.4% of men, whose wife showed a negative result for IUI, were homozygote for this mutation ($p \leq 0.05$) while couples with a positive IUI result showed no mutation. However, no significant differences were found between homozygote mutation and wild type carriers in fertilization rates, implantation rates and clinical pregnancy of IVF as well as ICSI. The protein expression was less in men with del/del genotype in comparison to other genotypes ($p < 0.005$). Our results revealed a significantly higher rate of homozygote mutation in unexplained infertile compared with control fertile men. The frequency of this mutation was also higher in men with failed IUI compared with successful IUI, although IVF and ICSI results did not show any association with this mutation.

I-6: Fertility Preservation in Male Patient with Cancer

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Studies suggest that the ability to have biological children is of great importance to many people. The cancer and its treatment often pose a threat to fertility. Rates of permanent infertility and compromised fertility after cancer treatment vary and depend on many factors. Male infertility can result from the disease itself (best documented in patients with testicular cancer and Hodgkin's lymphoma), complications of surgery for cancer (e.g., retrograde ejaculation or anejaculation), primary or secondary hormonal insufficiency, chemotherapy, and radiation exposure can all result in temporary or permanent damage to sperm production, and fertility may be compromised soon after treatment begins. The effects of chemotherapy and radiation therapy depend on:

1. Drug or size/location of the radiation field
2. Dose, dose-intensity
3. Method of administration (oral versus intravenous)
4. Disease
5. Age
6. Sex
7. Pretreatment fertility of the patient.

Fertility preservation in these patients includes:

1. Minimization of adverse effect of treatments
 2. Fertility preservation options for men undergoing cancer treatment
 3. Methods of fertility restoration after completion of cancer treatment
- We will discuss these measures in adult, adolescent and children.

Animal Biotechnology

I-7: Using Gene Expression Data for Differentiating Stem Cells and Compound Repurposing

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I-8: A Preliminary Report on Production of Organ Deficient Goats at Royan Institute of Biotechnology

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Shortage of transplantable organs available for people with end-stage diseases has become goal of scientific project worldwide to find new approaches for providing the needed organs. These projects have mainly focused on complementation of pig embryo genetically manipulated or designed to produce an organ deficient embryo. Considering the fact that pig is not the common domestic animal in Middle East, therefore, we have launch a project to produce pancreatic deficient goat embryos via handmade cloning previously established in Royan Institute. In this project we have design a construct with optimization expressing Hes1 gene under control of Pdx-1 promoter in caprine fibroblast cells. It is known, overexpression of Hes1 halts pancreiogenesis. Then selected transfected cells will be used for handmade cloning technique and reconstructed embryos will be transferred to recipient goat in hope of producing an apancreatic embryo. Following, confirmation of this procedure in the next step we will complement the reconstructed blastocyst with human derived stem cells, in hope of producing a goat with "humanized pancreas". We and other researchers believe this procedure may lead to production of "humanized organ". Considering, our previous experience in producing transgenic goat and production of fibroblast lines containing the aforementioned construct, we hope in near future to produce apancreatic goat embryos.

Embryology

I-9: Sperm Trans Fatty Acids: An Under-treated Issue in Men Infertility

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Background: Mammalian spermatozoa are characterized by a high proportion of polyunsaturated fatty acids (PUFA) and the membrane structure of spermatozoa plays a crucial role in fertilization. Moreover, dietary fatty acid (FA) influence sperm FA profiles in several species. This study focused on responses to dietary omega-3 fatty acids and comprehensive fatty acid profiles analysis in men as well as trans fatty acids in previous studies in ruminant and human.

Materials and Methods: Sperm FA profiles of human and ruminants consists of: C22:6n-3 (docosahexaenoic acid: DHA) is the predominant PUFA and C16:0 (palmitic acid) is the predominant saturated FA in the sperm of these species. Sperm structure is associated with sperm movement and acrosome reaction. Whether the higher levels of DHA are concentrated in the sperm head or tail is variable among different species. There is a striking similarity between humans and ruminants whose sperm head contain higher

concentration of DHA.

Results: Dietary FA influence sperm FA profiles in human and ruminants. Improvements in sperm parameters are a typical response to fish oil after more than four weeks of supplementation in diets. Tams fatty acids are a missing link in biological systems such as sperm.

Conclusion: It appears that regulation of sperm FA mean melting point (MMP) is the key factor causing responses by dietary manipulation. Interestingly, some hypotheses which explain milk fat depression (MFD) in dairy cattle by dietary fatty acids seem to be applicable and sperm fat depression (SFD) may be suggested in infertile men.

Key words: Sperm, Trans Fatty Acid, Men Infertility

I-10: Vitrification of Baboon Ovarian Tissue: Will Vitrification Replace Conventional Freezing for Ovarian Tissue Cryopreservation?

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Background: The aim of this study was to evaluate the efficacy of a vitrification protocol developed by our group to cryopreserve ovarian tissue. For this, we used ovaries from non-human primates in order to have an animal model close to the clinical setting.

Materials and Methods: Ovarian biopsies from five adult baboons were vitrified, warmed and autografted. After five months, follicle survival, growth and function were assessed. The quality of stromal tissue and influence of the vitrification procedure on the cooling rate were also evaluated.

Results: Our results showed that after vitrification, warming and grafting, follicles were able to grow and maintain their function, as illustrated by Ki67, anti-Müllerian hormone and growth differentiation factor-9 immunostainings. Corpora lutea were also observed, evidencing successful ovulation in all animals. Stromal tissue quality did not appear to be negatively affected by our cryopreservation procedure, as demonstrated by vascularization and proportions of fibrotic areas, which were similar to those found in fresh ungrafted ovarian tissue.

Conclusion: Our results indicate that baboon ovarian tissue can be successfully cryopreserved using our vitrification protocol. However, before applying this technique in a clinical setting, we need to validate it by obtaining pregnancies.

I-11: Cryopreservation and Utilization of Ovarian Tissue: When, Where and How?

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In women, around 10% of cancers occur in those younger than 45 years old. Although aggressive chemotherapy/radiotherapy and bone marrow transplantation can cure more than 90% of girls and young women affected by disorders requiring such treatment, the ovaries are very sensitive to cytotoxic drugs, especially to alkylating agents. Therefore, these treatments can result in premature ovarian failure, depending on the follicular reserve, the age of the patient and the type and dose of drugs used. Several options are currently available for the preservation of fertility in cancer patients: embryo cryopreservation, oocyte cryopreservation or ovarian tissue cryopreservation. The choice of the most suitable strategy for preserving fertility depends on different parameters: the type and timing of chemotherapy, the type of cancer, the patient's age and the partner status. Fertility preservation needs to be completed before chemotherapy and/or irradiation is started and might take 2-3 weeks with established techniques such as embryo or oocyte cryopreservation. Cryopreservation of ovarian tissue is the only option available for prepubertal girls and woman who cannot delay the start of chemotherapy. So far, more than 30 live births have been obtained after orthotopic transplantation of cryopreserved ovarian tissue. Apart from the candidates for ovarian tissue cryopreservation, we will also discuss the procedures to freeze ovarian cortex and where we should transplant such pieces of tissue. Finally, results and possible future approaches will be reviewed.

I-12: Optimal Strategy toward Fertility Preservation: In Vivo and In Vitro Post-Thaw Options in Gamete, Embryo and Ovarian Tissue Cryostorage

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Background: Oocyte, embryo and ovarian tissue cryopreservation are proposed for fertility preservation to cancer patients who hope to be mothers after getting rid of the disease.

Materials and Methods: Embryo cryopreservation is not possible for unmarried single girls without sperm partner and oocyte retrieval is a long time procedure. Thus ovarian tissue cryopreservation is suggested for fertility preservation. The main goal of ovarian cryopreservation is getting back of tissue in to the body in order to fertility and hormonal cycle restoration. Different cryopreservation methods have been performed including vitrification of biological samples. In the recent years, whereas many studies have focused on using of different carriers, we used Cryopin, as a novel tool, so as to plunge the ovarian tissue into the nitrogen. Although successful ovarian tissue re-implantation could help the patients who want to be a mother, the malignancy returning to the original place is still remained a concerned issue. To find a solution, ovarian tissue (organ) or isolated follicle culture and xeno-grafting are safer and possible methods to obtain a mature oocyte ready for fertilization.

Results: In seven years experience, vitrification mostly could help us to reach to our scientific aims in fertility preservation. To obtain enough healthy vitrified-warmed embryos, 93.89% were considered as surviving embryos. Re-vitrifying 4-cell mouse embryos using closed

pulled straw (CPS) was not discernibly detrimental to embryos. In the case of blastomere damage after vitrification, both laser assisted hatching (LAH) and necrotic blastomere removal (NBR) techniques could reduce the incidence of embryo cell death, but have no significant effect on development and cell number. In comparison of different ovarian vitrification methods, because of the better primordial follicular preservation and more survivability, it appears that the combination of EG + DMSO with sucrose is better suited for vitrification of human, sheep, monkey and rat ovarian tissues, particularly at the initial stage could relatively restore ovarian function after vitrification and autotransplantation. Additionally, fewer cell death incidences occurred after 2-step dehydration procedure as compared to the 4-step vitrification method and using of therapeutic ultrasound may accelerate and increase re-angiogenesis and can help to promote ovarian follicular growth. Ultrastructural changes of the vitrified ovaries using EG + DMSO with sucrose, were considerably compared to the control but this result did not differ in comparison to the sucrose-free group. Both of the vitrified and non-vitrified ovarian autotransplantation caused restoration of the hormone cycle and ovarian function; these results approximated the controls after gonadectomy. In the last groups the percentage of follicular maturation and ultrastructure of transplanted ovaries were in better condition. Also the rate of expression of angiogenic factors in all of the transplanted ovaries, were comparable with the control ones (non-published data).

Conclusion: Although vitrification is a reliable method for cryostorage of gamete, embryo and ovarian tissue, now it is a challenge that: can vitrified oocyte, embryo or ovarian tissue lead to a completely healthy delivery? And this is the main future vision in the field of fertility preservation.

Keywords: Fertility Preservation, Cancer Patient, Vitrification, Transplantation, Culture

I-13: Transcriptome Dynamics of Human and Mouse Preimplantation Embryos Revealed by Single Cell RNA-Sequencing

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Background: Mammalian preimplantation development is a complex process involving dramatic changes in the transcriptional architecture. However, it is still unclear about the crucial transcriptional network and key hub genes that regulate the proceeding of preimplantation embryos.

Materials and Methods: Through single-cell RNA-sequencing (RNA-seq) of both human and mouse preimplantation embryos, we performed a comprehensive analysis of transcriptome dynamics from oocyte to morula embryos. Using the tool of bioinformatics such as weighted gene co-expression network analysis (WGCNA), we define the genetic programs and regulatory networks in preimplantation development.

Results: We found that each developmental stage can be concisely delineated by a small number of functional modules of co-expressed genes that are involved in the pathways of cell cycle, gene regulation, protein translation, and metabolism and mitochondrial function, respectively. Cross-species comparisons reveal that the majority of human stage-specific modules (7 out of 9) are remarkably preserved, only to diverge in developmental specificity and timing in mice. Our results shed light on the gene regulatory mechanism underlying progressive development of mammalian early embryos.

Conclusion: We have identified conserved key genes in human

and mouse embryos that drive mammalian preimplantation development. When compared to exome or genomic sequencing of individual blastomeres, RNA-seq has the advantage of quantifying gene expression defects due to either genetic or epigenetic alterations. We suggest that single cell RNA-seq of a blastomere would be a valuable approach in parallel with other well established preimplantation genetic diagnosis methods

Keywords: Gene Transcription, RNA-Seq, Preimplantation Embryos, Network Analysis

I-14: Vitrification of Human Immature Oocytes in IVM Program

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The cryopreservation of immature GV oocytes by vitrification is offering attractive perspectives, since they represent an important pool of germ cells for women undergoing IVM cycles, or at risk of infertility for OHSS or PCOs, particularly those poor responders to gonadotropin stimulation, or in case of cancer. Therefore, it becomes necessary to optimize both cryopreservation technique as well as in-vitro maturation condition for GV oocytes.

Our recent study showed that GV appears more suitable to vitrification than MII, as indicated by the good ultrastructural preservation of important structures that are present only in GVs, like the nucleus and migrating cortical granules (CGs). Also, with PI/Hoechst immunostaining technique, the oocyte viability rate was shown to reduce in vitrified GV (56.0%), when compared with fresh samples (86.8%). In addition, it has been revealed that upon supplementation of the IVM culture media with GDF-9 as an oocyte-secreted GF, resulted in 66.1% of maturation rate and 55.3% of good embryo development. It, however, has to be noted that both stress and apoptosis related genes as assessed by real time PCR, increased post vitrification of human immature oocytes. The differential expression of these genes profile may be useful to evaluate the outcome of these germ cells after cryopreservation.

The optimization of a clinical protocol allowing immature oocytes retrieval, that can be matured *in vitro* after successful vitrification, would offer a significant opportunity for fertility preservation.

I-15: Effects of Oxidative Stress on Sperm Function

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Semen analysis is the frontline and old reliable test in the assessment of male fertility, but it failed to provide absolute prediction of sperm function and its fertilizing ability. A wide variety of internal and external factors affect sperm function without discernible and significant effects on semen analysis. Oxygen toxicity and oxidative stress (OS) are inherent challenge of living cells such as spermatozoa under aerobic conditions. It is proved that OS affects sperm function and has a key role in the etiology of male infertility. A wide range of exogenous factors such as culture media, oxygen tension, metal ions, pollutants, temperature, visible light and also defective Spermatozoa and leukocytes are potentially sources of reactive oxygen

species (ROS) and subsequently OS.

OS impairs sperm function through inducing lipid peroxidation, DNA fragmentation and apoptosis. Lipid composition of sperm membrane is susceptible to peroxidation and consequently failures of membrane structure and function (ions and metabolites transport and gradient, signal transduction). ROS significantly increase DNA damage in the form of changes in all bases, creation of base-free sites, deletions, frame shifts, DNA cross-links and chromosomal arrangements. Spermatozoa show a lot of apoptosis features such as activation of caspases 1, 3, 8, and 9, annexin-V binding. Exposure of human spermatozoa to ROS triggers apoptosis process through caspase 3 activity. Thus the above effects of OS are demonstrated as asthenozoospermia, teratozoospermia, and apoptotic spermatozoa that accompany with poor to failed fertilization and embryo development, repeated ART failures and recurrent pregnancy loss.

OS can be overcome through many strategies such as antioxidant supplementation, treatment and prevention of pathogenesis result to OS, change in life style, in vitro culture under low oxygen tension and several other strategies. Finally measurement of OS markers is under sever inter and intra-laboratory variations. Therefore an integrated method with comprehensive measurement of the OS markers should be devised in future.

I-16: Assessment of The Vitrified Ovarian Tissue in Long Term Culture

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In vitro culture of human ovarian tissue the following cryopreservation is proposed for follicular development. There are no techniques that guarantee successful maturation of the follicles within the excised tissue. The viability of cultured human ovarian tissue improved by adding some growth factors to the culture media.

The efficiency of vitrification as the cryopreservation method for human ovarian tissue has been supported by several studies. It is the ultimate cellular response to suboptimal conditions and different kinds of stress that cells and tissues might encounter during their cooling and warming steps. Apoptosis may be initiated by cryopreservation due to physical alteration and during *in vitro* culture of ovarian tissue. Thus, for the first time the effect of LIF on the human ovarian tissue follicular development and incidence of apoptosis using several complementary techniques were assessed and apoptosis related genes expression at the mRNA levels by real time RT-PCR in long term culture of human ovarian tissue were analyzed. Our results showed LIF could improve the survival and development of the *in vitro* cultured follicles in vitrified and non-vitrified human ovarian tissue by decreasing apoptosis and expression of pro-apoptotic genes.

I-17: Failed Fertilization: from Molecular, Diagnostic and Clinical Perspectives

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Low and/or total failed fertilization following ICSI is a disappointing effect that some couples may face during their treatment which may have severe social and economic consequences for these infertile couples. This phenomenon has been mainly attributed to failed oocyte activation post ICSI. Researchers have regarded this failure due to the absence or deficiency of sperm associated to oocyte activating factors (SAOAFs). SOAFs are believed to be present in the perinuclear theca of sperm where it fuses with oolemma and upon its entrance into the oocyte. It activates phospholipase C zeta (PLC ζ) which converts phosphatidylinositol-4, 5- biphosphate (PIP₂) to Inositol1,4,5-triphosphate (IP₃) and Diacylglycerols (DAG). IP₃ elicits calcium release from endoplasmic stores leading to calcium oscillations which finally leads to oocyte activation. Although PLC ζ is recognized as a prominent factor involved in oocyte activation, another potential candidate is PAWP. To overcome the social and economic consequences of low and/or total failed fertilization in infertile couples undergoing ICSI, assessment of fertilization potential of a semen sample may have an empirical value in assisted reproductive techniques. We showed that quantitative assessment of these two candidates at protein and RNA levels can to certain extent predicted the fertilization potential of a semen sample. Upon such a diagnosis, the couples become nominee for artificial oocyte activation following ICSI. Artificial oocyte activation (AOA) can be achieved by mechanical, electrical or chemical means which leads to calcium raise. The latter is considered as the most common approach to induce AOA and overcome low and/or total failed fertilization. Several chemicals have been used to induce AOA including Ionophore and Ionomycin. In a series of study we showed that Ionomycin can be used as a suitable agent for AOA and in pilot study, assessing children health following AOA, we observed no adverse effect for this compound.

I-18: Avian Chimeras and Germ Cell Migration

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Background: In avian species, the germ line stem cell population arises outside of the embryonic gonad and proceeds on a circuitous migration to the germinal epithelium. Specifically, in the avian embryo, the process of germ line stem cell migration proceeds through a series of active and passive migratory phases. The germline stem cells or primordial germ cells (PGCs) located in the epiblast of the unincubated embryo translocate to the hypoblast between stages X-XIII. Subsequently, during gastrulation the PGCs are passively carried by the hypoblast to the germinal crescent at about stage 4. As blood islands develop and the embryo becomes vascularized, the PGCs are passively carried through the blood stream between stages 13-16. Subsequently, the PGCs leave the blood vessels and actively migrate to the gonad. Recently, it has become possible to culture germline stem cells using PGC populations from the embryonic blood. The practical application of PGC technology is that it is possible to perform genetic modifications of PGCs *in vitro*, and transfer the cells to recipient embryos to generate germ-line chimeras. This study evaluates migration of cultured PGCs.

Materials and Methods: Three lines of primordial germ cell cultures were established, and continually cultured for over 18 months. To examine the ability of the cultured germ cells to migrate PGCs were loaded with a vital fluorescent dye (PKH-26) and injected into the stage X embryos. At stage 28-30, the gonads were removed and examined for the presence of fluorescent PGCs. Furthermore,

injected embryos were cultured through hatching, and grown to sexual maturity. Subsequently, the birds were evaluated from the presence of germline chimerism; and frequency of germline chimerism

Results: Variability in the ability of cultured PGCs to migrate to the gonads was discovered. The range of germline chimerism was between 12.5 and 92% for the three cell lines; and the frequency of germline chimeras for any one cell line was between 1.5 and 17%.

Conclusion: The results of this study confirm that cultured avian PGCs can retain their ability to migrate to the gonad and produce germline chimeras after long-term culture.

I-19: Surrogate Egg Shell Culture for The Analysis of Avian Stem Cell Fate

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Background: The chick embryo is a classical model to study embryonic development. However, most researchers have not studied the effect of embryonic manipulation on chick hatchability.

To determine the effect of egg orientation and type of sealing film on the hatchability of cultured embryos, and to determine the fate of adult stem cells injected into the blastoderm.

Materials and Methods: Windows were made in the sharp end of recipient surrogate chicken eggshells, and donor embryos were placed into the recipient eggshell for the first three days of incubation. Survival over the first three days was maximized when windowed eggs were positioned with the window-end down regardless of cling-film type. Three-day-old cultured embryos were transferred into recipient turkey eggshells, sealed with cling-film, and cultured until hatch. Avian adult stem cells expressing a reporter gene were injected into the developing embryo, and their fate was evaluated in various tissues using PCR, and immunochemistry

Results: Water weight loss of the surrogate eggshell cultures regardless of cling-film types was not significantly different from control intact eggs. The embryos cultured in turkey eggshells and sealed with Handi Wrap® exhibited ($p < 0.05$) higher hatchability ($75 \pm 10.2\%$) than cultures sealed with Saran Wrap® ($45.2 \pm 13.8\%$). The hatchability of control intact eggs was ($86.4 \pm 5.3\%$). The hatchability of eggs sealed with Handi Wrap® was not significantly ($p > 0.05$) different from control eggs suggesting that Handi-Wrap® was an excellent sealant for chick embryos cultured after 3 days of incubation.

Conclusion: Surrogate egg shell culture is an effective way to culture avian embryos through hatching; and an effective methodology to evaluate donor stem cell fate.

Ethics and Reproductive Health

I-20: Towards The Transparent Embryo: Dynamics and Ethics of Comprehensive Preimplantation Genetic Screening

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Background: To study the ethical aspects of comprehensive preimplantation genetic screening (PGS) through microarrays and whole genome sequencing

Materials and Methods: In order to pinpoint ethical issues regarding comprehensive embryo screening we have first investigated the technical and moral issues by organizing a campus meeting with experts and by a literature study. Subsequently we have investigated the opinions of professionals regarding the possible introduction of comprehensive screening techniques in the IVF clinic and the ethical implications in a focus group study. We have also investigated the feasibility and analytical and clinical validity and clinical utility of whole genome sequencing for embryo selection. Finally, based on our previous conclusions, we have performed a theoretical reflection on professional responsibility regarding the use of micro-arrays in order to perform comprehensive chromosome screening.

Results: The increasing complexity and amount of information yielded by comprehensive testing techniques will lead to challenges to the principle of reproductive autonomy and the right of the child to an open future. Furthermore, it may complicate the responsibility of the clinician regarding the welfare of the future child, and may lead to conflicts with prospective parents. Moreover, the analytic and clinical validity and the clinical utility of whole genome sequencing as a tool for embryo selection is unproven. An alternative testing approach is preconception carrier screening for a number of disorders, combined with targeted PGD for couples at high risk. This may avoid some of the ethical problems of comprehensive embryo screening. Obviously, the ethics of this alternative needs further ethical scrutiny as well. Moreover, there is a need for studies querying the opinions of infertile couples undergoing IVF/PGS regarding the desirability of embryo screening beyond aneuploidy.

Conclusion: The introduction of comprehensive screening techniques for embryo selection is premature at best and calls for further ethical reflection that is grounded in empirical work.

Keywords: PGS, Embryo Screening, Whole Genome Sequencing, Micro-arrays, Embryo Selection

I-21: Medical Ethics Education: Advancements in Iran

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Background: History of medical ethics shows a great attention toward morality and ethical behavior in practice of Iranian physicians. Traditional medical ethics with its conventional methods of teaching have been included in medical education by Iranian physicians over centuries. However, modern academic methods of medical education have also been introduced in medical schools. We aim to summarize the main activities and achievements in medical ethics education in Iran in recent decades. Overall trends in medical ethics education and its academic status will be reviewed.

Materials and Methods: This is a review of published and non-published information on medical ethics education in Iran in recent three decades.

Results: Iranian physicians have put great emphasis on the issues of medical ethics and ethics education. In 2002, the Ministry of Health and Medical Education (MOHME) introduced a strategic plan

for medical ethics. The second main goal was focused on medical ethics education. One of the main achievements over recent decades has been the reconstruction of the system of medical ethics education at different levels.

Educational opportunities in medical ethics, including a wide variety of academic courses, conferences, and seminars, have increased considerably through decades. The movement of educational methods towards new and more interactive approaches has paved the way for introducing medical ethics courses as innovative and attractive programs.

Considering the lack of qualified teachers in Iran, plans for training specialists in medical ethics received special attention by establishment of MPH course (with a medical ethics focus) and doctoral course (PhD) in medical ethics.

Conclusion: Medical ethics education in Iran has experienced a big change in recent decades. Despite great advancements, there are some shortcomings in medical education. Integrated curricula across all levels of education should be strengthened to develop critical thinking skills and critical analysis in students. Longitudinal themes of professionalism and ethics education would be very helpful in this way. Moreover, electronic education makes possible teaching materials to be openly available for all those who are interested in the issues.

Keywords: Medical Ethics, Bioethics, Ethics Education, Medical History

I-22: A Critique on "Consent to Medical Interventions" In Penal Code of Iran

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Informed consent is central to the doctor-patient relationship and especially to respect the autonomy of the patient that is one of the four principles of biomedical ethics. According to article 190 of Civil Code "consent of both parties is an essential provision for a contract to be made" and according to the definition of contract under Article 183 of the same code, it seems that the doctor-patient relationship is a contractual relationship regardless of its kind. In addition to these statements and despite the fact that article 158 (e) of Penal Code Amendment 2013 of Iran has referred to consent to medical interventions, article 495 section 1 states that: "if a doctor has not been negligent or has not committed malpractice, he is not responsible for the harm, even if he has not obtained the "relief from responsibility" (bara'a) form from the patient. In essence, such statement invalidates the consent in medical interventions. Treating a patient per se (with or without the patient's consent) is recognized as an exemption on responsibility of a doctor.

Any medical intervention without patient's consent, especially when the patient is competent to give consent or refuse a treatment, is not ethically defensible. This is violating the patient's autonomy. Thus, some considerations need to be made about consent in medical interventions in order to protect the patient's autonomy in laws of Iran.

Female Infertility

I-23: The Role of Hysteroscopy in Sub Mucosal Myoma

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Hysteroscopy is a method through which endometrial cavity can be observed and manipulated transcervically.

The association of sub serous fibroids and intramural fibroids with infertility is controversial. But the role of sub mucous fibroids which are reported in 5%-18% of patients as a causal factor for infertility is likely. Sub mucous fibroids are postulated to cause menorrhagia, sub fertility and abortion. The study was conducted during a period of 4 years from April 2008 to March 2012. This was a prospective study. Overall 197 women underwent hysteroscopy were evaluated in three groups: with AUB 146 cases, with infertility 34 cases and with recurrent abortions 17 cases. The diagnostic tool was transvaginal ultrasound.

The overall success rate was recorded and analyzed after six months in order of indication of hysteroscopy. The procedure of surgery with general or conductive anesthesia for inpatient management was done with misoprostol premedication 200 microgram sublingually 2 hours before surgery the surgical equipment was rigid STORZ Hysteroscope with zero and 30 degree lenses and the loop monopolar cutting devices. The mean patient age was 34 years (range: 26 to 42 years). The most common symptoms were menorrhagia which was present in 30% of patients, premenstrual spotting and infertility or abortion with endometrial mass in trans vaginal ultrasound.

Conclusion: Hysteroscopic myomectomy is a safe procedure to enhance fertility especially in cases with symptomatic sub mucosal myoma.

Keywords: Hysteroscopy, Myoma, Infertility

I-24: The Role of Interleukins, Integrins and Other Proteins in Recurrent Implantation Failure

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Embryo implantation represents the most critical step of the reproductive process in many species. Inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures. The cell adhesion molecule (CAM) such as integrins, cadherins, selectins and immunoglobulins intervene to ensure adhesiveness between the embryo and the endometrium. Some recent studies indicate that women with unexplained RPL had significantly reduced integrin expression compared to that of control group. Co-expression of osteopontin (OPN) and b3 integrin is a biological marker for good endometrial receptivity and that both proteins play a critical role in blastocyst implantation. Homeobox A10 (HOXA10), a key transcription factor, does a vital part in endometrial receptivity by regulating the expression of downstream target genes, such as β 3-integrin (ITGB3). Findings clearly support roles for hCG-regulated factor, and fibroblast growth factor 2 (FGF2), in the blastocyst-endometrial cross-talk important for endometrial receptivity and blastocyst implantation.

Regarding the key role of interleukines, an altered expression of leukemia inhibitory factor (LIF) and IL-15 was shown in the endometrium of women with RIF. Changes in endometrial IL-1R throughout early gestation *in vivo*, suggests a deep modulation of endometrial receptivity. It is also found that human endometrial stromal cells (HESCs) rapidly release IL-33, which controls the temporal expression of receptivity genes. Recently, evaluation of the bone marrow mesenchymal stem cell (BMSC), revealed their positive role in improving the regeneration of endometrium and endometrial receptivity.

Researches on *in vitro* fertilization indicate that failure to achieve a clinical pregnancy after the transfer of at least four good-quality embryos in three transfer cycles, is not associated with abnormal endometrial integrin expression. In addition, the expression of integrins $\alpha 1$, $\alpha 4$, and $\alpha v\beta 3$ appears to bear no prognostic value in subsequent IVF treatment. It is also found that lower follicular fluid IL-6 levels in IVF patients are associated with the increased likelihood of clinical pregnancy.

The endometrial receptivity array (ERA) consists of a customized array containing 238 genes expressed at different stages of the endometrial cycle, and is coupled to a computational predictor that is able to identify the receptivity status of an endometrial sample and diagnose the personalized windows of implantation. The accuracy of the ERA test is superior to endometrial histology, and results were reproducible in the same patients 29-40 months after the first test.

I-25: New Steps towards The Artificial Ovary

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Background: In recent years, transplantation of cryopreserved ovarian tissue has proved to be a promising approach to restore fertility in cancer patients. However, such a procedure cannot be carried out in women at risk of ovarian involvement due to the threat of reintroducing malignant cells. The aim of this study was therefore to create an artificial ovary as an alternative to restore fertility in these patients.

Materials and Methods: We tested two fibrin matrices containing low concentrations of fibrinogen (mg/ml) and thrombin (IU/mL) (F/T): F12.5/T1 and F25/T4. Preantral follicles and ovarian (stromal and endothelial) cells (OCs) were isolated from mouse ovaries, encapsulated in the fibrin matrix and autografted to the peritoneal bursa.

Results: After one week, the follicle recovery rate ranged between 30.8% (F25/T4) and 31.8% (F12.5/T1). With both fibrin formulations, all follicles were found to be alive or minimally damaged, as demonstrated by TUNEL assay, and at the growing stage (primary, secondary and antral follicles), confirmed by Ki67 immunostaining. Isolated OCs also survived and proliferated after grafting, as evidenced by fewer than 1% of apoptotic cells and a high proportion of Ki67-positive cells. Vessels were found in both fibrin formulations and the global vascular surface area varied from 1.35% (F25/T4) to 1.88% (F12.5/T1). Numerous CD45-positive cells were also observed in F25/T4 and F12.5/T1 combinations.

Conclusion: Our results indicate that fibrin is a promising candidate as a matrix for the construction of an artificial ovary. Xenotransplantation of isolated human follicles and OCs is the necessary next step to validate these findings.

Keywords: Fibrin, Artificial Ovary, Isolated Follicles, Cancer, Fertility Preservation, Ovarian Tissue

I-26: The Role of Endometrial Injury in Improvement of The Pregnancy Rate in Patients Undergoing ART

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Background: Because implantation failure is more frequent, many methods have been suggested to improve the implantation; however, their results have been inconsistent. One of the most hopeful methods is local endometrial injury. To explore the possibility that local injury of the endometrium increases the incidence of implantation and pregnancy rate in the subsequent IVF cycle in patients having previous failed IVF outcome

Materials and Methods: A literature search was conducted on PubMed. The regular protocol is used; endometrial samples in patients were taken by a biopsy catheter in the follicular phase in the luteal phase or during both phases.

Results: Some patients undergoing IVF treatment fail to conceive despite several attempts made with good-quality embryos and no identifiable reason. Studies have shown that inducing injury may help improve the chances of achieving pregnancy. Basic and clinical science data about the association between endometrial biopsy and improved implantation rate are limited. Nevertheless, recent evidence suggests that endometrial biopsy before IVF among women with previous repeated IVF failure is associated with improved rates of implantation, clinical pregnancies, and live births. Researchers hypothesized that the biopsy inflicted on the endometrium could lead to a massive secretion of cytokines and growth factors during the process of wound healing which could help in implantation.

Conclusion: Endometrial injury may have a beneficial role in the rates of embryo implantation; improve the clinical pregnancy rate and live birth in ART. In addition, there is insufficient evidence regarding the effect of endometrial injury on miscarriage or multiple pregnancies and none on adverse events such as bleeding and pain. However, there are still several unanswered questions regarding patient selection, timing, technique and number of endometrial biopsies needed.

Keywords: Endometrial Injury, Biopsy, Implantation Failure, Pregnancy

I-27: Management of Poor Responders: Current and Past Recommended Strategy

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The management of poor responders in IVF cycles is extremely controversial due to their inadequate response to controlled ovarian hyperstimulation. A low ovarian response may be idiopathic or may be associated with many different factors including age, diminished ovarian reserve, endometriosis and prior ovarian surgery, and a reported incidence 9-24% of IVF patients.

Several strategies have been proposed for the treatment of poor responders. The most commonly used strategies to improve oocyte yield in poor responders include an increased daily dose of gonadotropins (300-450 IU per day) with GnRH agonist (long, stop or micro-dose flare protocols) or GnRH antagonist protocols.

There are a number of newer procedures in assisted reproduction that may find a role in the management of poor responders. These include *in vitro* maturation (IVM), cryopreservation of oocytes or embryos from multiple retrieval cycles, as well as cryopreservation of donor oocytes allowing for commercial egg banking as well as in sperm banking.

Despite these recommended strategies, managing poor response cycles, however, continues to present challenges for the repro-

ductive endocrinologist. In this review, we will review the available options, including adjuvants prior to cycle starts, those used at the initiation of gonadotropin therapy, GnRH analog flare protocols, GnRH agonist microdose flare protocols, agonist versus antagonist protocols, clomiphene flare protocols, hMG versus recombinant FSH, the role of LH in stimulation protocols for poor responders, minimal stimulation, natural, clomiphene or letrozole only IVF, the available gonadotropin regimens and doses, as well as IVF laboratory options, in handling low oocyte yield cases.

We will also discuss that over the next coming years, some of the above options will become standard care in assisted reproduction, while others will no doubt be discarded when efficacy cannot be established.

I-28: Anti Müllerian Hormone and Time to Pregnancy in A Fertile Population

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Background: To investigate the relevance of Anti-Müllerian-Hormone levels as predictor of Time to Pregnancy in normal fertile population and secondary to investigate whether the age of the women had an impact on the Time to Pregnancy.

Materials and Methods: Interview studies of Time to Pregnancy combined with measurement of Anti-Müllerian-Hormone levels. Setting: Tertiary reproductive medicine unit.

328 pregnant women recruited via antenatal clinics in Poland, Ukraine and Greenland. Methods: Each pregnancy trimester were categorized into quartiles according to the AMH level and the association between AMH quartile and TTP was tested in a Cox regression model with female age, sperm concentration and sperm DNA fragmentation index of the partner as covariates. Main outcome measures: Time to Pregnancy, Anti-Müllerian Hormone levels.

Results: By comparing TTP in the four AMH quartiles, the mean values were 4.1, 3.7, 3.7 and 2.9 months, respectively. A statistically significant difference of 1.2 months (Crude Hazard Ratio (HR) 0.70, 95% CI: 0.51; 0.96, Adjusted HR 0.70, 95% CI: 0.49; 0.99) in TTP was found between 1st and 4th quartile. The women in the first AMH-quartile with the lowest AMH levels, had an odds-ratio of 8.1 (95% CI: 1.8; 37.0, $p=0.007$) for experiencing a TTP exceeding 9 months.

Conclusion: Lower serum AMH concentrations were associated with delayed time to pregnancy even after adjustment for male fecundity. Female age was not related to time to pregnancy in the study population.

I-29: Menstrual Cycle Dependent Variation in Serum Levels of Anti-Müllerian Hormone Relation to Age and Antral Follicle Count

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Background: To investigate, through three menstrual cycles, intra-individual variation in levels of Anti-Müllerian Hormone (AMH) and to elucidate if this variation is age dependent. Furthermore, to

find the level of correlation between AMH concentration and antral follicle count in healthy females.

Materials and Methods: In this prospectiv trial study, levels of AMH were measured on days 5, 10, 15, 20, 25 and 30 during three consecutive menstrual cycles. Antral follicle count was determined with ultrasound by day 5 in same cycles. Setting: Fertility clinic of a tertiary university hospital.

A total of twenty-six healthy non-smoking women, sixteen below 30 years and ten above 35 years, with regular menstrual cycles and no use of hormonal medication were recruited through advertisement. Inter- and intracycle variations of serum AMH levels and its correlation to the antral follicle count.

Results: A substantial and statistically significant longitudinal variation in AMH level was seen ($p < 0.001$) which in absolute terms was calculated to a mean of 13.4 pmol/L (95% CI: 10.1- 16.8) corresponding to relative mean of 87.4 % (95% CI: 75.9- 98.9%). In the three cycles, cycle-day 10 ($p < 0.001$) and 15 ($p = 0.03$) revealed a significantly higher AMH value compared to cycle-day 5; and cycle-day 30 ($p=0.04$) a significantly lower value. The differences between menstrual cycle-days were only seen in the young study-group. AMH levels correlated significantly to small antral follicle count.

Conclusion: Longitudinal measurements of AMH confirm variation to an extent that question one recording as sufficient for decision making related to prognostication and treatment strategies.

I-30: Human Endometrial Receptivity: from The Basic Research to Clinical Translation

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The endometrium is a hormonally regulated organ that is non-adhesive to embryos throughout most of the menstrual cycle in humans. Endometrial receptivity refers to a hormone-limited period in which the endometrial tissue acquires a functional and transient ovarian steroid-dependent status allowing blastocyst adhesion. Functional genomic studies of human endometrium in natural cycles have demonstrated that endometrial receptivity is an active process involving up- and down-regulation of hundreds of genes, for review see.

Personalized medicine is a well-accepted concept in reproductive medicine except for the endometrial factor that is still neglected. Our group has developed the endometrial receptivity array (ERA), a customized array of 238 genes coupled to a computational predictor capable of diagnosing a functionally receptive endometrium regardless of its histological appearance. The accuracy of the diagnostic tool ERA has been demonstrated to be superior to endometrial histology and results are completely reproducible 29 to 40 months later.

Clinical results obtained will be presented to demonstrate the diagnostic and therapeutic efficiency of the ERA test in patients with implantation failure (IF), through personalization of the day of embryo transfer (pET). As well in our laboratory we are involved in new non-invasive diagnostic methods using endometrial fluid (EF). The aim of our new study is to use lipidomics to unravel the specific composition of EF through menstrual cycle, identify lipid biomarkers for endometrial receptivity and to demonstrate their clinical diagnostic value. Our results open the assessment of PGE2 and PGF2, as the basis of a non-invasive diagnostic method to be performed 24 hours prior to embryo transfer, to diagnose the endometrial receptive status.

I-31: Reconstruction of Human Endome-

Trium from Somatic Stem Cells

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Background: During reproductive life, the human endometrium undergoes around 500 cycles of growth, breakdown and regeneration. This outstanding regenerative capability is the basis for cyclic endometrial preparation and its dysfunction is involved in pathological disorders. Endometrial regeneration is mediated by the existence of a specialized endometrial stem cell (SC) population recently identified. This presentation will review the evidence available to date for the existence of endometrial SC in human endometrium detailing the functional approaches that have been used to identify their regenerative capacity and the translation to clinic in on-going clinical projects.

Materials and Methods: Identification and localization of SSC is hard and laborious given the lack of clear differential morphological features and molecular markers. Two techniques based on general SSC properties, such as slow cycling and an ATP-binding cassette membrane, are available for this purpose. First, labelling with DNA marker 5-bromo-2-deoxyuridine (BrdU) has been used to identify the existence of SSC in animal models. BrdU signal progressively decreases in each division, and the retaining of the labelling either indicates no division or a very low division rate, one of the most important characteristics of SSC. Second, the Side Population (SP) method was initially created to identify SC in human hematopoietic tissue. In this technique, the cell-permeable DNA-binding dye Hoechst 33342 is loaded into the cell population of interest; SC have the ability to pump out this dye via an ATP-binding cassette membrane mechanism, resulting in a low-fluorescence side SP "tail" by flow cytometry.

Results: We have proven the existence of endometrial SSC in mice endometrium by identifying the low cycling cell population using BrdU technique in combination with the co-localization of typical markers of undifferentiation¹. In humans, we have demonstrated the existence of this endometrial SSC subset using the SP technique and further reconstructing the human endometrium from injecting isolated endometrial SP cells² or SP cell lines³ into the NOD-SCID mice model. We have isolated, identified and characterized the SP corresponding to the stromal and epithelial compartments identifying its gene signature, characteristic immunophenotype and telomerase pattern. We analyzed their clonogenic activity under hypoxic conditions and the differentiation capability *in vitro* to adipogenic and osteogenic lineages. Finally, we demonstrated the functionality of endometrial SP to develop human endometrium after subcutaneous or subcapsular kidney injection in NOD-SCID mice. We have also demonstrated the contribution of bone marrow to the reconstruction of the human endometrium and the SP subpopulation⁴.

Conclusion: Based on the information gathered from these studies we have isolated the cell population responsible for the regeneration of the human endometrium and clinical trials using this technology in severe cases of Asherman syndrome and thin endometrium are in process.

I-32: Prevention Protocols in Order to Prevent OHSS in COS of PCO and Non PCO Patients in ART Cycle

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Background: The affordable protocol to prevent the severe OHSS in two groups of patients PCO syndrome and non PCO high responder.

Methods and Materials: PubMed and Medline were searched from January 2000 till end of May 2014.

This Study investigates whether the mild protocol is associated to prevent severe OHSS in infertile female.

Results: According to this survey, 9 studies were included and data analysis showed that a significant reduction of oocytes pick up but optimal embryo implantation and lower rate of severe OHSS in PCOS individuals and non PCO high responder.

Conclusion: In presence of PCO ovary and previous OHSS, We should be aware of severe OHSS (that is life threatening). Mild stimulation can significantly reduce the incidence of severe OHSS; otherwise, this protocol is affordable and patient friendly and leads to low cost and patient drop out.

Keywords: PCOS, High Responder, OHSS

I-33: Role of Hysteroscopy in the Diagnosis and Infertility Treatment

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Abnormalities of the uterine cavity can be a contributing cause of subfertility, and assessment of this factor has been suggested as a routine investigation in the evaluation of subfertile women.

Hysterosalpingography has been used as the most common procedure in the investigation of infertility cause. Saline infusion sonohysteroscopy can show uterine cavity abnormalities, which is highly sensitive and specific in identifying intrauterine abnormalities.

Hysteroscopy is considered as the definitive diagnostic procedure to evaluate abnormalities that have been showed on hysterosalpingography, vaginal sonography or sonohysteroscopy during routine infertility workup.

Hysteroscopic myomectomy can increase the chance of pregnancy in women with unexplained subfertility and submucosal myoma.

The hysteroscopic polypectomy shown on ultrasound significantly increases the clinical pregnancy rate. Hysteroscopic septolysis or intrauterine adhesions lysis in women with unexplained subfertility might significantly increase the rate of pregnancy.

I-34: Comparison between Hysteroscopy and Sonohysteroscopy in Uterine Cavity Assessment

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Intrauterine pathologies have been shown to be present in 25% of infertile patients. Structural abnormalities of the uterine endometrial cavity may affect the reproductive outcome adversely, by interfering with the implantation or causing spontaneous abortion.

Intrauterine abnormalities may visualize using a variety of tech-

niques. Including hysterosalpingography (HSG), transvaginal sonography (TVS), sonohysterography (SHG), and hysteroscopy. It has shown that three - dimensional saline contrast sonohysterography (3D-SCSH) is a reproducible method for the quantification of the percentage of a submucous fibroid protruding into the uterine cavity. Although hysteroscopy is currently accepted as the gold Standard for preoperative classification of submucous fibroids, it has several disadvantages: using hysteroscopy is possible to measure the size of the fibroid and the degree of fibroid protrusion into the cavity based on the Subjective impression of the operator, rather than on an objective measurement. Further more, hysteroscopy can be technically difficult in women with large and multiple submucous fibroids. In conclusion SCSH is a minimally invasive procedure that allows accurate visual assessment of the cervical canal and uterine cavity. The procedure of SCSH is acceptable and well tolerated by almost all patients. Therefore, SCSH should be part of the infertility work up before ART, even in patients with normal HSG and/or TVS, to ensure normality of the uterine cavity before ET.

I-35: Individualization of Treatment in ART

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I-36: The Role of LH in Controlled Ovarian Stimulation

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It is known that the follicular selection and final stages of follicular maturation are equal if not more dependent on low circulating levels of LH. In addition to stimulating production of thecal androgens as substrate for estrogen synthesis, LH stimulates granulosa cell via LH receptors induced by FSH and estrogen in larger but not smaller follicles. The data from numerous assisted reproduction studies confirm that most patients will respond to gonadotropin preparations containing only FSH. However, 10-12 % of patients may fail to respond adequately to this type of ovarian stimulation. Presumably these patients lack adequate concentrations of endogenous LH after pituitary down-regulation and would benefit from the addition of LH to ovarian stimulation.

Specific subgroups of women may benefit from LH activity supplementation, patients > 35 years, in those showing an initial abnormal ovarian response to r-hFSH monotherapy, and in low prognosis women treated with GnRH antagonists and clearly patients with hypogonadotropic hypogonadism require exogenous LH to achieve optimal assisted reproduction outcomes.

Exogenous LH supplementation was consistently with higher peak estradiol concentrations. The use of hMG in long GnRH agonist cycles was associated with a 3-4 % increase in live birth rate. The advent of recombinant gonadotrophins brought significant changes in fertility therapy. Over a decade, recombinant LH (rLH) has been used for clinical trials and the amount of peripheral LH that is necessary for optimal follicular growth, oocyte maturation, subsequent embryo development and assisted reproduction outcomes during ovulation induction can now be better evaluated.

The assisted reproduction practitioner should always bear the impor-

tance of adequate LH stimulation in mind. Future research should focus on good method for identifying patients most likely benefit from LH supplementation.

I-37: Controlled Ovarian Stimulation in Cancer Patients

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Recent advances in the technology of vitrification of human oocytes and embryos have increased the opportunities for fertility preservation in cancer patients. Ovarian stimulation in this group of patients is associated with some unique challenges. A controversy in this field rotated around the hypothesis that the transient elevations of estrogen during an IVF cycle may stimulate tumor growth; therefore, increasing the risk of treatment failure or cancer recurrence. However, this is a hypothetical jeopardy and no studies have reported that a single IVF cycle exacerbated the risk of poor oncologic outcomes. Some centers attempt to alleviate the peak estrogen levels during stimulation, using a modified IVF protocol developed by Oktay et al. This modified protocol uses anti-cancer hormonal treatment during an "Antagonist Cycle". Ideal protocol of superovulation for this group of patients includes a compromise between obtaining a relatively large number of oocytes for vitrification, to maximize chances of later pregnancy, and the absolute need for avoidance of ovarian hyperstimulation syndrome (OHSS) in a patient who will shortly begin chemotherapy. These studies, in general, demonstrate that COS cycles for women with cancer are similar to those of women without cancer. A GnRH antagonist protocol with moderately high dose of FSH, possibly determined by pre-treatment AMH, may be an appropriate option. Most studies report using an antagonist protocol, as this allows the quickest start of stimulation. However, some patients used a protocol with GnRH-agonist or some centers used tamoxifen or letrozole for women with hormone-sensitive cancers. Some centers tended to be more conservative (lower starting dose) for cancer patients to minimize the risk of OHSS, other centers used standard starting doses, or even slightly higher doses, given that this was likely the couple's attempt at COS, and they wanted to minimize the risk of poor response. For women with cancer, the overall gonadotropin usage was found to be similar in majority of previous studies. For patients planning to commence chemotherapy, timing of fertility preservation techniques can significantly influence the decision making process. However, if there is no time, or for women with hematologic disorders, there may significant pressure to avoid delays in chemotherapy; in this situation it is possible to commence fertility treatment in either the pre-ovulatory or luteal phase by using a GnRH antagonist for 2-5 days to produce a profound decrease in gonadotropins and then commence FSH stimulation. There is no data to suggest the ideal method of fertilization, some centers use standard ICSI criteria, while others use ICSI for all embryo banking cases to avoid the risk of fertilization failure.

I-38: Individualized Controlled Ovarian Stimulation: Matching Protocols with Patient Profile

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The aim of all assisted reproductive techniques (ART) is a live birth of a single healthy baby. Many clinical and laboratory strategies can influence the ART clinical outcomes. In this lecture we try to explore the clinical and laboratory strategies to maximize success in ART.

Three are the main issues: 1. optimize the number of oocyte retrieved with an individualized controlled ovarian superovulation standard protocol. This protocol must be defined having pre-defined the response to FSH (with AFC and AMH); only after that, we could consider a moderating approach in high responding women (avoiding the OHSS); simple maximizing approach in normal responding women and a lower treatment burden in the reduced responding women.

Moreover, To avoid the risk of ovarian hyperstimulation syndrome the ovulation should be triggered with GnRH agonist in a GnRH antagonist stimulation protocol. All the embryos obtained should be cryopreserved with vitrification and then transferred in a natural cycle (cycle segmentation). The correct number of oocyte to be retrieved to maximize live birth rate seems 15. With this number we increase the chance to find the most competent oocytes to be fertilized and the number of embryos. 2. Single embryo transfer possibly at the blastocyst stage to reduce multiple pregnancies and cryopreserve with vitrification the supernumerary cleavage stage embryos or blastocysts or oocytes, or all the embryos in case of cycle segmentation. Cryopreservation offers a very good contribution to the cumulative live birth rates. 3. Improve the efficiency of IVF - number of babies born per transferred embryos - lowering the number of transfers, the abortion rate, the abnormal pregnancies and the number of multiple pregnancies by transferring one single euploid blastocyst after genetic screening of 24 chromosomes. At the moment, these strategies can maximize the live birth rates in patients undergoing IVF.

I-39: Facts and Myths in IVF Treatment

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Many myths are still present today in assisted reproductive techniques and particularly in infertility management. Most of them exist because we do not want to really look at the facts for various reasons. In this lecture we will review 7 of these myths: 1. HP-HMG improves pregnancy rates, 2. HMG prevents progesterone rise in follicular phase, 3. Clinical importance of progesterone rise in follicular phase, 4. Ovarian stimulation increases the risk of breast cancer, 5. Oocyte vitrification does not give good live birth rates, 6. IMSI improves live birth rates, and 7. pre-implantation genetic screening improves live birth rates. For each of these myths the literature will be objectively reviewed trying to outline the real facts.

Genetics

I-40: Male Genome Programming, Infertility and Cancer

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Background: During male germ cells differentiation, genome-wide re-organizations and highly specific programming of the male genome occur. These changes not only include the large-scale meiotic shuffling of genes, taking place in spermatocytes, but also a complete "re-packaging" of the male genome in post meiotic cells, leading to a highly compacted nucleo-protamine structure in the mature sperm cells. This process is essential to protect the male genome for its journey out of the male body through the hostile environment of the female organism, as well as to prepare its rapid activation after fertilization. Although it is a critical step for successful reproduction in most species, nearly nothing is known on the molecular basis of this process. Our objective is to identify molecular actors driving male genome programming and functionally characterize their roles.

Materials and Methods: A combination of strategies, including the generation of several mouse models (RousseauxSBRM11_pmid21208144), molecular, structural and proteomic approaches, as well as genome-wide analyses of male germ cells at different stages using chromatin immunoprecipitation (ChIP) and new generation sequencing (NGS) are used. The latter also involve the design and usage of dedicated bioinformatics analyses, which we are developing.

Results: Early post-meiotic cells, round spermatids, inherit a haploid genome associated with histones, in a somatic-like nucleosome based chromatin, which then undergoes a genome-wide reorganization associated with the replacement of histones by protamines. Our work during the last 12 years has unravelled several key molecular mechanisms involved in this formidable remodelling of the genome. Our initial investigations were based on two observations. First, several specific histone variants are expressed and incorporated into the chromatin of male germ cells. Second, a genome-wide histone hyperacetylation wave occurs in elongating spermatids (HazzouriEJCB2000_pmid11152286; FaureMHR2003_pmid14614037), which precedes their removal and replacement by transition proteins and protamines. Combining proteomic and bioinformatics screens (RousseauxS-BiRM2012_pmid22788531), we identified several candidate factors. In depth characterization of these factors involved structural and molecular approaches, as well as the use of genetically modified mouse models. One of our major findings regarding the role of histone acetylation is that Brdt, a double bromodomain containing testis specific member of the BET family, guided by acetylated chromatin, is playing essential roles in the stage-specific programming of the male genome (Pivot-PajotMCB2003_pmid12861021; MoriniereNature09_pmid19794495; GaucherEMBOJ12_pmid22922464). Recently, our investigation of the role of the histone acetyl transferases (HAT) p300 and CBP in post-meiotic cells, using a double conditional KO mouse model, highlighted their role in the control of a late post-meiotic gene expression program involved in the metabolic remodelling of male germ cells (Boussouar_Andrology14_pmid24522976). We also unravelled the role of testis-specific variants in the programming of pericentric regions, as well as in specifically preparing chromatin for post-meiotic packaging, and for post-fertilization events (GovinJCB07_pmid17261847; WuJRD08_pmid18703863; MontellierGenesDev2013_pmid23884607). Our lab is also involved in collaborative studies with Pr Y.M. Zhao's lab, leading to the discovery of a large panel of new histone modifications, hugely enlarging the so-called "histone code", and increasing our understanding of their role in male genome programming (TanCell11_pmid21925322; DaiNCB14_pmid24681537).

Conclusion: We are now in a position to propose the first molecular models explaining male genome programming (Goudarzi_JMB2014_pmid24613302). Medical applications are described below in "Project application and usage".

Keywords: Male Genome, Epigenetics, Infertility, Chromatin, Spermiogenesis

Reproductive Imaging

I-41: Pelvic MRI at Infertility

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Compared with ultrasound, MRI has limited indications in evaluation of Female Infertility.

Laparoscopy is the gold standard for the diagnosis of pelvic endometriosis.

MRI is helpful in determining the extent of deep infiltrating endometriosis, especially when laparoscopic inspection is limited by adhesions.

MRI allows for much more anatomic detail as well as better measurements of uterine fibroids. In addition, MRI can determine which and how many Fibroids are degenerating, also pelvic MRI is an excellent modality at differentiating fibroids from adenomyosis.

MRI is excellent at evaluating the various uterine anomalies as well as any associated anomalies such as malformations in the ovaries and kidneys.

Due to its excellent tissue resolution and ability to differentiate between blood, fat, and other types of soft tissues, MRI is excellent at further characterizing masses within the ovary.

Possibilities would include a hemorrhagic cyst and endometrioma, various types of benign and malignant ovarian tumors, and some conditions which may mimic an ovarian mass such as a peritoneal inclusion cyst which can occur in a patient who has had a prior hysterectomy but still has one or both ovaries remaining.

Again, MRI is frequently utilized to differentiate hydrosalpinx from a more concerning ovarian mass.

I-42: Cardinal Manifestations of Endometriosis on Transvaginal Ultrasound

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Endometriosis is a common benign gynecologic disorder, defined by endometrial glands and stroma outside of the endometrial cavity. The three primary types of endometriosis are superficial peritoneal lesions, ovarian endometriomas, and deep infiltrating endometriosis (DIE). Although laparoscopy continues to be the gold standard for the diagnosis of endometriosis, transvaginal ultrasonography showed important role for the non-invasive diagnosis of endometriosis.

In this abstract the most common indirect ultrasound-based markers/ findings (pelvic adhesions, endometrioma, and echogenic lesions) would be assessed.

Although ultrasound is poor at detecting adhesions, it is able to dynamically assess mobility and fixation. The presence of one of the following findings can be considered to indicate the likelihood of pelvic adhesions: (a) blurring of the margins of the ovary (margins), and adhesion of the ovary to the uterus (b) 'fixation' and (c) distance of the ovary from the probe. The most common organs affected by endometriotic deposits are the ovaries. Although there is some variation in the sonographic appearance of endometriomas, the classic endometrioma is a homogenous,

focal lesions with low level echos, they may be uni or multilocular, containing thin or thick septations and may contain wall nodules. Ultrasound is also predominantly used to evaluate ovaries by detecting the pinpoint focal lesions with low level echos.

The use of simple ultrasonographic findings, e.g. adhesion of the ovary to the uterus, distance from the probe, presence of pinpoint focal lesions and the classic features of endometrioma allow appropriate patient counselling of further diagnostic or therapeutic interventions and to reduce the number of unnecessary laparoscopies. Our experience suggest that the inclusion of ovarian margins, mobility, and fixation as indirect ultrasound-based markers of pelvic pathology improves our ability to predict or exclude the presence of pelvic adhesion due to endometriosis lesions.

I-43: Uterine Artery Embolization(UAE), An Alternative Treatment for Fibroids

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Background: To evaluate the results (rates of success, complications and clinical outcomes), advantages or disadvantages of Uterine Artery Embolization (UAE).

Materials and Methods: We were able to follow 28 women with uterine fibroids, ranging age of 21 to 48 years (mean age 34 y), who underwent UAE in Mostafa Khomeini Hospital from May 2005 to June 2014, for the period of 6 months to 8 years.

Twenty patients (71.5%) were nulliparous, 6(21.5%) were primiparous and 2(7%) had two previous pregnancies.

UAE performed by femoral approach, local anesthesia and using the seldinger technique to achieve bilateral uterine artery embolization while using 355-700 PVA particles.

Results: Bilateral UAE was successful in 27 patients and unilateral embolization performed in one case.

Decrease in uterine volume was 32-58% and decrease in size of (dominant) fibroid volume was about 43 to 80%.

Post-embolization syndrome was seen in about 40% of patients with different degrees while they were well managed as out-patients. Improvement in menorrhagia, pelvic pain and bulk effect were seen in 90-98%. No major complications or need for re-admission or progress to hysterectomy was observed.

Conclusion: UAE is a valuable alternative treatment for symptomatic fibroids, especially in women who want to preserve their uterus (but when patients seek for certainty on the cessations of problems, hysterectomy remains the treatment of choice).

I-44: 3D Ultrasonography of Ovarian Tumors

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A prospective study was conducted at the OB/GYN department of Kasr El Eini hospital, Cairo University from Jan 2000 - Oct 2002. The study included 51 patients in the age group between 40-70 years (including pre and post menopausal women). All patients were sub-

jected to following after taking a consent from each patient: Complete clinical evaluation; including history taking and clinical examination, Ultrasonographic evaluation using Voluson 530d (GE - Kretz); Conventional pelvic 2-D scanning and 3-D ultrasonographic evolution, histopathological examination of tissue specimens after D & C and/or laparotomy according to the case. Ultrasonography was done in patients with ovarian lesions, they were described according to the following: Site (right, left or bilateral), Size, Nature (solid, cystic or heterogeneous), Echogenicity, Wall thickness, Wall for the presence of irregularities and Septa. 22 patients had adnexal masses, 6 were post-menopausal. The most sensitive U/S sign for malignancy in an ovarian mass is a thick wall (>3mm), while the most specific signs are thick septa (>3mm) and irregular wall. Combining 2D and 3D assessments achieves a higher accuracy than either alone; with 85% sensitivity and 80% specificity. Doppler assessment of vascularity is a useful adjunct to the evaluation and, combined with 3D assessment, could achieve the highest accuracy. Although surgery for an ovarian mass is currently the inevitable outcome, prior knowledge of the odds for malignancy can guide such aspects as timing, extent and availability of frozen section pathology. Exact knowledge of the mass size, surface and relation to surroundings may select cases for Laparoscopic surgery.

I-45: Important Points in Interpretation of Sonographic Images of Female Pelvis (Imaging Case Review)

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Ultrasonography represents the method of choice in the investigation of the female pelvis. An accurate interpretation of the images must take into consideration the specific features of the uterus, ovaries and fallopian tubes.

The present case review aims to demonstrate important points in interpretation and management of the female pelvis images.

I-46: Ultrasound Guided Aspiration of Ovarian Cyst

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Ultrasonography is the primary imaging tool for a patient considered to have an ovarian cyst. On a sonogram, simple ovarian cysts have a uniformly thin, rounded wall and a unilocular appearance that is either hypoechoic or anechoic. They usually measure 2.5-15 cm in diameter, and posterior acoustic enhancement (a hyperechoic area) may be visible deep to the fluid-filled cyst. Most commonly, they are functional follicular or luteal cysts or, less commonly, serous cystadenomas or inclusion cysts. Complex cysts may have more than 1 compartment (multilocular), thickening of the wall, projections (papulations) sticking into the lumen or on the surface, or abnormalities within the cyst contents. Malignant cysts usually fall within this category, as do many benign neoplastic cysts. According to a consensus statement by the society of radiologists in ultrasound, for simple ovarian cysts with no suspicious features on ultrasound, current

follow-up guidelines state.

In women of reproductive age.

In post-menopausal women.

Persistent simple ovarian cysts larger than 5-10 cm (especially if symptomatic) and complex ovarian cysts should be considered for surgical removal. The surgical approaches include an open incisional technique (laparotomy) and a minimally invasive technique (laparoscopy) with very small incisions. Sonographically guided therapeutic aspiration of symptomatic ovarian cysts is viable alternative to surgical extirpation; even in pregnant women. Fine needle aspiration under ultrasound guidance is simple, safe and useful in the management of ultrasonically benign unilocular ovarian cysts in pre-menopausal women. It is of particular use in pregnancy, and in patients unsuitable for laparoscopy or surgery. Surgery can be reserved for cysts which recur after aspiration, cysts with a hemorrhagic aspirate, cysts in post-menopausal women and those with ultrasonic criteria of malignancy. Transvaginal cyst aspiration has many advantages including short hospital stay, rapid recovery, excellent patient tolerance, and a low rate of procedure-related complications. Ovarian cyst recurrence following transvaginal drainage is a more significant problem than previously documented, especially if the cyst is on the left side. However, when recurrences do occur, repeat transvaginal aspirations may be considered in the symptomatic patient.

Oral Presentations

Andrology

O-1: Evaluation of Ethnic Patterns of Y Chromosome Microdeletions in Iranian Infertile Men with Azoospermia/Severe Oligospermia Referred to Royan Institute

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Background: Microdeletions of the long arm of the chromosome Y are the most common molecular genetic cause of severe infertility in men which affect three regions of AZFa, AZFb and AZFc (Azoospermia factor). These regions contain various genes involved in spermatogenesis. The effect of ethnicity on the patterns of Y chromosome microdeletions has not been extensively studied, particularly in Iranian population. The present study aimed to reveal the ethnic patterns of Y chromosome microdeletions in Iranian infertile men with azoospermia/severe oligospermia referred to Royan institute.

Materials and Methods: 1889 infertile men referred to Royan Institute with azoospermia/severe oligospermia were examined for Y chromosome micro-deletions from March 2012 to February 2014. We determined micro-deletions of Y chromosome in AZFa, AZFb and AZFc regions using multiplex PCR and six different STS (Sequence-Tagged Site) markers. The patients were categorized into different ethnic groups based on their birth origin and language of their three male generations.

Results: Among 1889 infertile men, we determined 103 cases of Y chromosome micro-deletions (5.4%). Of them, 38 cases were from Azeri origin (36.8%), 28 cases from Fars origin (27.1%), 13 cases from Kurd origin (12.6%), 6 cases from the North of Iran (Gilaki and Mazandarani) (5.8%), 8 cases from Lor origin (7.7%), and 2 cases were from Arab origin (1.9%). There were also 5 cases of non-Iranian (Afghani and Iraqi) origin. We could also determine 130 micro-deletions in AZF regions of 103 Iranian infertile men including 94 micro-deletions in AZFc region (72.3%), 29 in AZFb region (22.3%) and 7 in AZFa region (5.3%). This pattern of incidence was nearly similar to the reports from other countries.

Conclusion: The pattern of Y chromosome micro-deletions in Iranian infertile male population is similar to the other world regions with AZFc as the most common type, and the ethnic origin of the patients does not affect on this pattern.

Keywords: Male Infertility, Y Chromosome Microdeletion, Iranian Ethnicities, Oligospermia, Azoospermia

O-2: A Novel Antioxidant Formulation to Treat Male Infertility Emanating from Sperm Oxidative DNA Damage: Promising Preclinical Evidence from Mouse Models

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Background: Sperm DNA damage (SDD) is a significant male infertility factor, yet it is not routinely diagnosed or treated in couples undertaking fertility treatment by ART. Men with this condition are likely to experience sub-fertility or infertility, expose their female partners to greater risk of miscarriage and pass on de novo sporadic DNA mutations potentially compromising the health of their future generations. The present situation is gravely concerning as clinical studies confirm moderate to severe SDD in about 60% of all men visiting IVF centers and in about 80% of men diagnosed with idiopathic male infertility. To alleviate SDD in infertile men and effectively address this area of unmet clinical need, CelloXess scientists, after almost a decade of rigorous research, designed and developed a unique proprietary antioxidant formulation "Fertilix™". To get an early assessment of Fertilix™ efficacy in rodents, our academic collaborators in France and Spain used two independent mouse models of sperm oxidative stress to study the effect of Fertilix™ in 1. the prevention of damage to sperm DNA and 2. improving fertilization and pregnancy rates.

Materials and Methods: Two well-established mouse models of sperm oxidative stress from 2 independent laboratories were utilized to evaluate the efficacy of Fertilix. In both models, 12 male mice were provided with Fertilix in their drinking water for 2 weeks (Scrotal Heat Shock, SHS) or 4 weeks (Glutathione Peroxidase 5 knock out, GPX-5 KO) and compared with control groups for SDD and pregnancy rates.

Results: The 8-Hydroxy-deoxy Guanosine (8-OHdG) is a biomarker of DNA oxidation. The average background levels of 8-OHdG in WT mice is around 30%. This level doubles up to about 60% in transgenic mice deficient in the antioxidant enzyme GPX-5. Our results indicate that a 2 weeks pretreatment of GPX-5 KO mice with Fertilix provides a complete protection of sperm DNA against oxidation. In mouse models of Scrotal Heat Shock (SHS), only 35% (19/54) female mice got pregnant resulting in 169 fetuses. This is contrast to the Fertilix pretreated group where 74% (42/57) female mice got pregnant resulting in 427 fetuses. The role of chance in obtaining supporting results for the efficacy of Fertilix in both models is minimal.

Conclusion: Fertilix antioxidant formulation is highly efficacious in mouse models of sperm oxidative stress. Fertilix prevents SDD in GPX-5 deficient mice and restores pregnancy rates almost back to normal levels in mice subjected to SHS. These results, if confirmed in humans, will impact the normal practice at ART centers. Antioxidants will be an adjuvant therapy to a couple's fertility treatment prior to undertaking IUI or IVF procedures.

Keywords: Oxidative Stress, Sperm DNA Damage, Male Infertility, Antioxidant, Fertilix

Animal Biotechnology

O-3: Drug Repositioning by Merging Gene Expression Data Analysis and Cheminformatics Target Prediction Approaches

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The transcriptional responses of drug treatments combined with a protein target prediction algorithm was utilised to associate compounds to biological genomic space. This enabled us to predict efficacy of compounds in cMap and LINCS against 181 databases of diseases extracted from GEO. 18/30 of top drugs predicted for leukemia (e.g. Leflunomide and Etoposide) and breast cancer (e.g. Tamoxifen and Ouabain) were already proven effective. LC50 of a predict compound, Fenbendazole, on HL60 and BMSC cell lines was experimentally evaluated to be 127nM and 5066nM (48hrs). Novel predicted drugs for differentiating stem cells to cardiomyocytes such as Famotidine enhanced expansion rate and heart-beat strength of Embryoid Bodies and expression of cardiac precursor markers (Nkx2-5, Gata4, Mef2c) and cardiac markers (Actc1, Tagln, Myh6) considerably during and post cardiac progenitor cell (CPC) formation. Combining target prediction with gene expression analysis enabled us to predict effective compounds and targets for diseases and differentiation of stem cells.

O-4: The Interaction of Bioactive Glass Nanoparticles with Mesenchymal Stem Cells *In Vitro*

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Background: Bioactive glass (BG) nanoparticles are amongst the most promising class of biomaterials for hard tissue regeneration because of their distinctive properties of rapid bone bonding, controlled biodegradability and their ability to stimulate new bone growth. Despite the vast interest in BG scaffolds in medical applications, the synthesis of bioactive glass nanoparticles is still facing numerous challenges. In addition, despite the great potential of BG's as porous scaffolds for bone regeneration, concerns have arisen on their long-term fate in the body as small particles may be released after implantation. This could lead to undesirable reactions with the surrounding cells, hence investigations on such nanoparticles is crucial. BG nanoparticles, also have the potential to be injected directly into the defect site to allow healing and regeneration of bone tissue. As mesenchymal stem cells (MSCs) are precursors to osteoblasts, the effect of nanoparticles on their behaviour is critical. In this study 80S20C (80 mol% SiO₂ and 20 mol% CaO) mono dispersed BG nanoparticles have been synthesised and characterised for the first time. The combined utilization of nanoparticles and human MSCs in regenerative medicine requires particle uptake into the cells. Hence, the BG nanoparticle's uptake and distribution inside MSCs was assessed using confocal microscopy and transmission electron microscopy (TEM). The effect of the BG nanoparticles on cell viability, metabolic activity and proliferation as a result of particle uptake was also determined.

Materials and Methods: The Stöber process was applied

to produce sol-gel derived BG nanoparticles. To follow the internalisation and intracellular distribution of the BG nanoparticles inside MSCs (Lonza, UK) in 3D, cells were exposed to BG nanoparticles at a concentration of 100µg/ml in cell medium (Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin streptomycin (all from Invitrogen, UK) for 24 hours. For confocal microscopy the actin cytoskeleton was stained with Alexa Fluor conjugated phalloidin (molecular probes, UK) and particles were labeled with FITC. TEM was also used to monitor the uptake and distribution of the nanoparticles (100µg/ml) inside MSCs after 24 hours exposure. Cells were fixed, osmicated and the samples were embedded in resin and sectioned. The effect of the nanoparticles on cell viability and proliferation was determined by exposing MSCs to three different BG nanoparticle concentrations: 100, 150 and 200 µg/ml in cell medium (DMEM) for 24 hours and their response monitored over the period of 1, 4 and 7 days using LIVE/DEAD (Molecular Probes, UK), MTT (Sigma, UK), total DNA using Hoechst (Sigma, UK) and Lactate dehydrogenase Cytotox-one™ (Promega, UK).

Results: The processing route for the synthesis of the BG nanoparticles was successful as it resulted in spherical and dense particles with a composition of SiO₂ and CaO. The uptake and intracellular localization of the BG nanoparticles inside MSCs was confirmed by confocal microscopy and TEM. The uptake of the 100µg/ml of BG nanoparticles by MSCs was captured, for the first time, using confocal. The nanoparticles were then seen in the cell cytoplasm. Also, TEM demonstrated nanoparticles entrapped in endosomes after 24 hours exposure. Furthermore, the dissolution behaviour and break-up of the nanoparticles inside cells were also observed using TEM. No significant levels of cytotoxicity were observed for the nanoparticles at all concentrations following a range of cytotoxicity assays. At the concentrations of 100 and 150 µg/ml, the particles were seen to increase metabolic activity of human MSCs. The effect of the BG nanoparticles on MSC differentiation will also be presented.

Conclusion: Spherical bioactive glass nanoparticles of 80S20C composition with controlled size were produced. In this study we successfully showed that BG nanoparticles were able to internalize inside MSCs. The viability and proliferation assays confirmed that none of the BG nanoparticles concentrations tested induced any major toxicity to MSCs.

Keywords: Bioactive Glass, Nanoparticles, Stem Cells, Cytotoxicity, Cellular Uptake

O-5: Preparation of Multi-Component Drug Delivery Systems

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Background: Despite global interest, the development of single step processes for the preparation of effective drug delivery systems still faces numerous challenges. There is great demand for processing methods that are efficient, flexible, scalable and economical for the generation of wide range of encapsulated structures. Over the past few decades, electrohydrodynamic (EHD) processing has received significant attention as a new method for the preparation of structures for therapeutic applications. The technique enables the production of nano or micro scale particles with a controlled size distribution. Encapsulation studies using coaxial EHD, whereby two or more concentric liquid jets are formed simultaneously, present

great potential for delivery systems i.e. carrier vehicles and multi-layered capsules¹. Often biodegradable and biocompatible polymers are used in delivery vehicles to encapsulate or entrap therapeutic agents. These materials are widely used as they allow sustained and controlled release of the encapsulated drug¹. The current study utilizes the EHD method combined with a novel tri-needle device for the preparation of multi-layered structures using different polymeric materials. The ability to fabricate such multilayered particles contributes to great advances in biomedical fields, in particular pharmaceutical applications. In the current study the utilization of this technology for the preparation of encapsulated drug delivery systems for the treatment of urinary tract infections will be presented. Recent cell studies will also be presented to validate this method as suitable for further loading of bioactive components.

Materials and Methods: In the EHD technique, a liquid droplet is subjected to an electric field and the body of the liquid becomes charged. The electrostatic repulsion offsets the surface tension and a droplet forms at the end of the needle from which a fine jet emerges and breaks up to form smaller droplets¹. During the process, solvent evaporates from the highly charged droplets leading to particle formation. The resulting particles' morphology, size and dispersity are very much dependent on the processing conditions such as the applied voltage, liquid flow rate and also the distance between droplet formation and collection². All these factors have been thoroughly investigated in this study. The liquid suspensions were prepared using three different biocompatible polymers of PLGA, PCL and PMSQ dissolved in Dimethyl carbonate, Dichloromethane and Ethanol, respectively, at various concentrations. A new device consisting of three separate needle, coaxially arranged, was used. The outer needle, central needle and inner needle were perfused simultaneously with PLGA, PCL and PMSQ solutions, respectively, at fixed flow rates. A high power voltage supply was connected between the needles and a grounded electrode. The jet and droplet formation processes were monitored using a high-speed camera. Stable nanoparticles, were collected in a petri dish filled with ethanol. A range of techniques were applied to characterize the nanostructures including transmission electron microscopy (TEM), scanning electron microscopy (SEM) with focused ion beam (FIB) milling, fourier transform infra-red (FTIR), nuclear magnetic resonance (NMR), dynamic light scattering (DLS) and UV-Vis spectroscopy.

Results: The morphology, size and structure of the prepared nanoparticles that were studied using SEM and TEM are presented in 1. The figure, together with other results (not presented here), show that the processing route was successful as it resulted in the production of multi-layered nanoparticles with an average size of 350 nm (\pm 50 nm). The difference in density between the polymers used in this study allowed good visualization of the three distinct layers via TEM. All the results combined demonstrated the successful synthesis of mono-dispersed, spherical, multilayered particles using a single step process. Cell data showed the non-cytotoxic nature of the particles, making them further suitable for medical applications. Recent drug encapsulation studies will also be presented. In the current study the utilization of this technology for the preparation of encapsulated drug delivery systems for the treatment of urinary tract infections combined with a using gentamicine will be presented³. The capsules showed to kill the *Enterococcus faecalis* in a dose responsive manner.

Conclusion: The capability of the electro-hydrodynamic process, in combination with a novel tri-needle device, for the preparation of drug delivery carriers was shown to be successful.

Keywords: Drug Delivery, Multilayered Structures, Nanoparticles, Human Osteoblast Cells, Infection

O-6: Cryopreservation and Long-Term Main-

tenance of Bovine Embryo-Derived Cell Lines

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Background: The aim of this study was to develop methods for cryopreservation and long-term maintenance of putative bovine embryonic stem cells (ESCs).

Materials and Methods: Putative bovine ESC (bESC) lines (n=3) isolated in conventional medium were used to compare slow-freezing and vitrification.

Results: After warming, vitrified cells (96.9%) demonstrated significantly ($p < 0.05$) better survival than frozen-thawed cells (81.5%) and formed significantly more colonies with good morphology (vitrification: 93/93, 100.0%, slow-freezing: 74/106, 69.81%, $p < 0.05$). The effect of inhibitors of differentiation (PD184352, SU5402, CHIR99021) on ESC maintenance was assessed on putative bESC lines established in N2B27-3i medium (n=8) or conventional medium (n=1) after culture over 30 passages (>240 days). All cell lines expressed ALP, SSEA1, SSEA4, OCT4, REX1 and SSEA1. OCT4 expression was confirmed by relative real-time PCR and was upregulated in early passages of putative bESCs cultured in N2B27-3i (2.9 ± 89 -fold higher at Passage (P) 2-4), whereas the converse was observed later (P22-26; 2.2 ± 0.1 -fold increase in conventional medium). Putative bESC lines isolated in N2B27-3i medium (n=3) or conventional medium (n=1) were vitrified at P18 and, after warming, were cultured for a further 12 passages. These cells survived vitrification and expressed OCT4, REX1, SSEA1, ALP, SSEA1 and SSEA4.

Conclusion: These results demonstrate that putative bESC lines that express pluripotent markers can be cultured long term and retain expression of pluripotent markers after vitrification.

Keywords: Cell Signalling, ESCs, Three Inhibitors (3i)

Embryology

O-7: Improved *In Vitro* Development of Cloned Bovine Embryos Using S-Adenosylhomocysteine, A Non-Toxic Epigenetic Modifying Reagent.

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Background: Development of cloned bovine embryos.

Materials and Methods: Oocytes collection, oocyte denudation, oocyte enucleation, nuclear transfer, electrofusion, reconstructed embryo activation, culture of reconstructed and IVF embryo,

and treatment with SAH post fusion interval Treatment of reconstructed embryos with TSA for 12 hours after activation, preparation of somatic donor cells, donor cells transfection with GOF18-DPE-EGFP plasmid containing neomycin resistance genes via lipofection method, cell cycle synchronization, EGFP- POU5F1 fluorescence, immunofluorescence staining quantitative real time RT-PCR.

Results: The results of this study indicated that post-fusion treatment with SAH has a time dependent effect on DNA-methylation and histone-acetylation, developmental competence and gene expression of the cloned embryos. In addition, these results might improve quality of cloned bovine embryos to produce transgenic cattle.

Conclusion: The results of this study indicated that post-fusion treatment with SAH, a non-toxic DNMTs inhibitor, has a time dependent effect on DNA-methylation and histone-acetylation, developmental competence, and gene expression of the cloned embryos. Although this effect has been shown to be time dependent, there was not a consistent effect of a certain SAH interval on all assessed aspects of embryo development. Accordingly, while 12 hours treatment significantly increased blastocyst rate of cloned embryos compared with IVF and control SCNT, 48 hours treatment with SAH produced embryos resembling to IVF embryos in terms of DNA-methylation status, fluorescent intensity for EGFP and POU5F1 gene expression. Even though compromised results of 72 hours treatment with SAH strongly indicated that any treatment with SAH should be terminated before the critical stage of de novo methylation. To make a final conclusion on the impact of SAH treatment time interval, further studies on full term development of the resultant cloned embryos should be performed. These results might improve quality of cloned bovine embryos to produce transgenic cows because there are potentially large profits to be made from transgenic pharmaceutical productions as F-VIII, F-IX, Protein C, AT III, Fibrinogen, Albumin and Activase® (Alteplase) or t-PA factors

Keywords: SAH, SCNT Embryos, Cloned Bovine Embryos, DNMTs

O-8: Molecular Mechanisms of Membrane Fusion Involved in Fertilization

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Background: Assisted fertilization procedures are a currently widespread practice to regulate reproduction in humans and animals. The arising question is why the human being manipulating gametes to generate new individuals, if we do not understand yet the molecular mechanism of fertilization?. Successful completion of fertilization in mammals is dependent on three membrane fusion events: 1. the acrosomal exocytosis of sperm, named acrosome reaction, 2. the fusion of sperm and egg plasma membranes to form a zygote, and 3. the cortical granules exocytosis of fertilized eggs, named cortical reaction, to prevent polyspermy. The general aim of this project is to identify proteins involved in membrane fusion events during fertilization and study its possible involvement in oocyte maturation.

Materials and Methods: To analyze acrosomal exocytosis we have performed functional assays in permeabilized and intact human sperm. To analyze cortical granules exocytosis and oocyte maturation we carry out functional assays in mouse eggs activated parthenogenetically. We also perturb protein function by microinjecting blocking antibodies and interference RNA.

Results: We are characterizing the function of proteins involved in membrane fusion events during acrosome reaction, cortical reaction and oocyte maturation. We have investigated that MARCKS, a centrosome component during oocyte maturation, also plays a role in acrosomal exocytosis.

Conclusion: Acrosome reaction and cortical reaction are calcium dependent processes such as neuronal exocytosis, and the concept that the molecular mechanism of membrane fusion is similar to neurons has become generalized. However, our findings show that exocytoses participating in fertilization have distinguishing qualities. In addition, sperm is a cell in interphase, while the oocyte is in meiotic division. Studying a protein in both model cells has the advantage of discovering new functions for a given protein during cell division. Our long-term goal is contribute to the understanding of the molecular mechanisms involved in fertilization, which may affect early embryonic development and implantation.

Keywords: Fertilization, MARCKS, Acrosome Reaction, Cortical Reaction, Oocyte Maturation

O-9: The Peroxidation Indices during Vitri-fication of Bovine Ovary

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Background: Cryopreservation of ovary for preserving germ cell line is going to be an important issue. However, the physical and chemical stresses during cryopreservation are the most important factors that may influence the post freezing tissue viability and quality. Vitrification is a new approach to bypass the impacts of both stresses for gametes and embryos and it is also suggested for ovary vitrification. The aim of the present study was to evaluate an ovarian conventional vitrification protocol for some oxidative stress indices.

Materials and Methods: The bovine ovaries (n=16) were transported to the laboratory in a thermos flask (37°C). The ovaries were cut and the medulla parts were removed. The small pieces of ovarian cortex (1×1×1 mm³) were subjected to vitrification protocol. The ovary pieces were left in the vitrification solution 1 (VS1; Ethylene Glycol (20%) and Glycerol (10) in Holding medium) for 3 minutes followed by VS2 (EG (25%) and Glycerol (25%) and sucrose (0.5M)) for 1 minute. The samples were put on an aluminum foil and immersed in LN. Samples were warmed at least 48 hrs after vitrification in warming solutions (WS) with descending concentrations of sucrose; WS1: 0.5; WS2: 0.25 and WS3: 0.15M sucrose each solution for 5 min and finally samples were left in the holding medium for 10 min and subjected to the biochemical assays for carbonyl proteins, malonyl dialdehyde, and sulfhydryl groups. Fresh samples from the ovaries were also analyzed for biochemical parameters as control group.

Results: Malonyl dialdehyde (149.8 ± 25.3 and 36.9 ± 6.98 nmol/mg protein; p<0.0001) and GSH (247.2 ± 34.83 and 219.6 ± 13.44 nmol/mg protein; p=0.0007) were increased in vitrified samples compared to the fresh samples, respectively. However, carbonyl protein was reduced in the vitrified samples compared to the fresh samples (94.7 ± 10.55 and 185.7 ± 25.56 nmol/mg protein; p=0.0014).

Conclusion: The results showed that vitrification severely alter the peroxidation indices in an ovary vitrification protocol.

Keywords: Vitrification, Bovine, Ovary

O-10: Sperm Mediated Gene Transfer Using Adjuvant Preserving Fertility for Production of Transgenic Chicken Expressing Enhanced Green Florescent Protein

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Background: Low uptake of exogenous DNA by sperm and reduced number of fertilized oocyte by transfected sperm are the major obstacles for progression of sperm mediated gene transfer. Therefore, the modification of sperm mediated gene transfer procedure needs to be required. The purpose of this study was to evaluate the efficiency of FuGene 6 compare to lipofection in transfection medium for internalizing of the foreign gene in the rooster sperm cell.

Materials and Methods: Semen were washed in Beltsville extender for removal of the seminal plasma by repetitive washing. Then sperm (1×10^9 sperm/ml) was incubated with exogenous DNA (Enhanced Green Fluorescent Protein, EGFP) in Beltsville extender containing: 1. no additive, 2. lipofectamine 1000 (1:1), and 3. FuGene 6 (1:1). Transfection was measured using PCR reaction in the sperm cell and new born chickens after artificial insemination. Motility, viability, membrane integrity, acrosome integrity and their fertilizing ability using artificial insemination were considered as index of sperm quality post transfection.

Results: Although there was not significant different in rate of transfection in sperm cell between Lipofectamin (11%) and FuGene 6 (10%), they were significantly higher compare to Control group (2%). The higher significant percentage of motility, viability and fertility rate were observed in FuGene 6 (29.1 ± 1.5 , 33.5 ± 2.4 , 26.8 ± 1.8 , respectively) compare to control group (10.5 ± 1.5 , 12.8 ± 2.4 , 14.9 ± 1.8) and Lipofectamine (13.5 ± 1.5 , 16.1 ± 2.4 , 17.3 ± 1.8). Moreover, the higher significant percentage of transfection in chickens were observed in FugGen 6 (33.5%) rather than control (14%) and lipofectamine (16%) groups. For membrane integrity and acrosome integrity, no significant different was observed for all groups.

Conclusion: Our data revealed that adding FuGene 6 would result in higher rate of sperm quality after transfection which led to higher rate of fertility and production of transgenic chicken.

Keywords: Lipofectamine, FuGene 6, Rooster Sperm Transgenic Chicken

O-11: Immunoneutralization of Inhibin Alpha Subunit as A Tool for Improving Farm Animal Fertility

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Background: The objectives of a series of studies undertaken was to improve farm animal reproductive efficiency in terms of embryo production efficiency, conception rate and litter size by immunoneutralization of inhibin in conjunction with other conventional reproductive techniques.

Materials and Methods: A recombinant porcine inhibin alpha-subunit mature peptide was produced by mass scale and used as the antigen to immunize animals coupled with either standard superovulatory or oestrus synchronization protocols. Super-ovulation tests were carried out in both Holstein dairy heifers and swamp water buffaloes in single or 3 consecutive super-ovulations. Anti-inhibin or anti-follistatin antibodies were supplemented into oocyte IVM media in studies to improve *in vitro* embryo production efficiency. Oestrus synchronization was carried out in both water buffaloes and repeat breeder Holstein dairy cows. Concentrations of reproductive hormones were also monitored throughout super-ovulation or oestrus synchronization procedures.

Results: Immunization against inhibin improved blood concentrations of oestradiol during the follicular phase and progesterone during the luteal phase, stimulated expression of oestrous behavior, improved superovulatory response, and most importantly enhanced embryo quantity and also quality in both Holstein heifers and water buffalo cows. Immunization against inhibin also increased plasma concentrations of FSH and activin, and also the ratio of activin to follistatin during the follicular phase. In the *in vitro* study supplementation of anti-inhibin antibody into the oocyte IVM culture medium improved oocyte maturation rate, cleavage rate, but not blastocyst rate. However, supplementation of anti-follistatin into the IVM medium doubled the blastocyst rate as well as increasing oocyte maturation rate and cleavage. Immunization against inhibin, when combined with the oestrus synchronization protocol, also substantially increased ovulation rate and also conception rate following artificial insemination in water buffalo cows plus conception rate in repeat breeder Holstein dairy cows.

Conclusion: 1. Immunization against inhibin, through neutralizing bio-activity of inhibin, creating a hormonal effect of higher activin to follistatin or activin to inhibin ratio result in improved oocyte maturational and embryo developmental qualities and enhanced embryo quantity but also quality after super-ovulation treatment. 2. Immunization against inhibin, by bringing together the above 3 prerequisites of establishing pregnancy, i.e. higher secretion of oestraiol in follicular phase, higher progesterone secretion in luteal phase, and enhanced oocyte and embryo qualities results in improved ovulation rate and conception rates in animals after oestrus synchronization treatment.

Keywords: Immunization Against Inhibin, Embryo Production Efficiency, Ovulation and Conception Rates, Holstein Dairy Cows, Water Buffalo Cows

O-12: Studies on Sequestration of PDC-109 Protein on Cryodamage and In Vitro Fertility of Crossbred Bull Spermatozoa

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Background: Plasma membrane of the spermatozoa interacts with and is altered by abundant seminal PDC-109 present in their immediate milieu *in vivo* with damaging effect in a time- and concentration- dependent manner. Therefore we hypothesized that sequestration of detrimental protein from ejaculates would be beneficial for cryopreservation of sperm cells. To this aim we evaluated the effect of sequestration of PDC-109 either by anti-PDC-109 antibodies (Ab) or egg yolk (EY) alone or by the synergistic action of EY+Ab in minimizing cryoinjury to bull spermatozoa.

Materials and Methods: Seminal PDC-109 protein was purified and injected in rabbits to raise antibodies. Immuno-diffusion assay followed by western blot to ascertain cross-reactivity of other HBP's was carried out. Ab were quantitated and added to ejaculates, either alone or in addition to EY in Tris-Glycerol (TG) extender. Thus, ejaculates were processed in extender containing EY+TG1 (Group I), EY+TG2 (direct collection of ejaculates in EY, Group II), EY+Ab+TG (Group III) or Ab+TG (Group IV). Semen quality parameters (SQP) viz. viability, individual motility, biochemical integrity of membrane, cholesterol content of spermatozoa, acrosome integrity (Giemsa and fluorescent stain FITC-PSA), cryoinjury to spermatozoa (Chlortetracycline, CTC assay) and *in vitro* fertility parameters were evaluated.

Results: A significant ($p < 0.05$) improvement in post-thaw SQP observed at pre-freeze and post-thaw stages of cryopreservation in Group III compared with other groups indicated improvement in freezability. Study revealed highly significant ($p < 0.01$) increase in non-capacitated spermatozoa at pre-freeze as well as post-thaw stages in AB+EY treated group signifying reduced cryoinjury.

Conclusion: We report for the first time novel method of sequestration of PDC-109 protein by Ab+EY synergistically to improve freezability and minimize cryoinjury to bull spermatozoa. Further this protocol will open new opportunities for understanding and diagnosis of infertility and /or subfertility not only in bulls but in other species thereby eliminating low fertile sires, before semen is processed for cryopreservation.

Keywords: Freezability, Cryopreservation, Cholesterol, Acrosome Reaction, Cryoinjury

O-13: Phosphorylation of 4E-BP1 Promotes Translation at The Oocyte Spindle

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Background: Fully grown mammalian oocyte utilizes transcripts synthesized and stored during earlier development. In the mouse oocyte there are three forms of cap-dependent translational repressors: 4E-BP1, 4E-BP2, and 4E-BP3. The dominant form, 4E-BP1, inhibits cap-dependent translation by binding to the eIF4E translation initiation factor. Hyperphosphorylation of 4E-BP1 disrupts this inhibitory interaction and results in activation of cap-dependent translation.

Materials and Methods: We used immunofluorescence, immunoblotting, qPCR, *in situ* translation labeling, and microinjection

techniques.

Results: 4E-BP1 is highly phosphorylated after NEBD, while it is dephosphorylated after fertilization. Increased phosphorylation of 4E-BP1 (which is not detected in the cumulus cells) promotes cap-dependent translation of specific mRNAs after meiotic resumption. Our immunofluorescence analyses of the differently phosphorylated forms of 4E-BP1 in the oocytes during meiosis show even localization of 4E-BP1 and phospho-4E-BP1(T37/46) as well as spindle poles localization of phospho-4E-BP1(S65). 4E-BP1 phosphorylated on T70 co-localizes with its activator mTOR exclusively at the spindle. In addition, mTOR, and CDK1 are the main positive regulators of 4E-BP1 phosphorylation after NEBD; on the other hand, inhibition of PLK1 does not affect 4E-BP1 phosphorylation. Treatment by Rapamycin, inhibitor of mTOR, results in decreased phosphorylation of 4E-BP1 on T37/46 in the whole oocyte, while T70 phosphorylation is decreased at the spindle.

Conclusion: Our results show that 4E-BP1 phosphorylation forms promote *in situ* translation necessary to support spindle assembly and genomic stability.

Keywords: Translation, RNA, Meiosis, Oocyte, Aneuploidy

Ethics and Reproductive Health

O-14: General Governing Rules of ART Contracts Involving Third Parties

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Background: ART contracts involving third parties have been created while clinical reproductive treatments are globally widespread. Iran is pioneer in applying these treatments in middle-east due to shii'at jurisprudence prescribing them. This key role in region, has raised Iranian jurists' responsibility in developing a legal system regarding administration of ART. The most significant part of this task refers to ART contracts which should be designed as considering parties' rights and commitments. ART contracts, particularly those involving third parties, have a totally different nature in comparison with regular contracts. Intervention of human remarks and entering a third party in the ART process, has intensified the issue.

Materials and Methods: Review

Results: The authors have initiated 10 basic rules governing ART contracts involving third parties, which is listed below: 1. Sufficiency of inter-partial consent, 2. full legal capacity of contractors, 3. legitimacy of contract, 4. non-monetary subject matter, 5. free will of parties, 6. ethical interpretation of contract, 7. considering contractual motivations, 8. necessity of arbitration clause, 9. contract's irrevocably rule, and 10. personal burden of commitment

Conclusion: This article aims at analyzing these rules and comparing them with ordinary contracts governing rules in order to obtain a legal scheme of what should be concluded amongst ART parties.

Keywords: ART, Contract, Third Party, Governing Rules

O-15: Advanced Age Women and Miscarriage Rate

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Background: Study question: what is the effect of advanced aged in pregnant women using assisted reproductive technology on the rate of miscarriages? Summary answer: the advanced age effects on the rate of miscarriages. What is known already: It is well known that miscarriage risk increases with age. The proportion of women giving birth after 35 years of age has risen considerably in developing countries. Several studies have concluded that women aged ≥ 35 years have a higher frequency of spontaneous abortion, than do younger women. Maternal age especially in women using ART is associated with adverse pregnancy outcomes, but the extent of effect is not clear. The aim of this study was to evaluate the relationship between the use of assisted reproductive technology and the incidence risk of abortion in advanced aged women.

Materials and Methods: A retrospective approach was chosen to analyze the data of women who referred to Royan Institute from 24 October 2009 to 20 September 2012. In general, this study examined 4,082 women over 3 years. In this study pregnancy outcome of women less than 35 years and over 35 years were compared. Chi-square and logistic regression were used to statistical analysis.

Results: The results showed for each year increase in women's age, the odds of late abortion, missed abortion, early abortion less than 12 weeks and recurrent abortion increased by 1.06, 1.06, 1.04 and 1.12 times respectively. Limitations, reasons for caution: In this investigation there might be some source of uncertainty in the method used to calculate the outcomes. Some of outcomes might not be registered or some cases may be disconnecting their relationship with the clinic and this was out of researcher reach.

Conclusion: Advanced age of pregnant women using ART effects on miscarriage rate.

Keywords: Advanced Age, Recurrent Miscarriage, Missed Miscarriage, Early Abortion

O-16: Genetic Counseling and Necessity of Psychological Counseling Skills

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Individuals who are at risk because of their family history or concerned about the possibility of having an affected child based any reasons are seeking solution approaches. Nowadays genetic counseling offers hope to prevent from suffering some genetic diseases. Advances in genetics diagnosis testing have played effective role in this regards. Interestingly, Preimplantation Genetic Testing would be as an alternative to reduce or eliminate that affect generation in some cases, but not in all. Despite multitude progress, geneticist encounters with any ambitious in diagnosis or forecast. This ambiguity can be associated with emotional, social or financial consequences that make genetic counseling more difficult than expected. Genetic counseling is a communication process which deals with the human problems associated with the occurrence, or the risk of occurrence. A genetic counselor should be experienced in interview skills such as active listening associated with good history taking, summa-

tion, conclusion, formulation ability and empathy. Paying attention to non-verbal communication via body language such as gestures, will allow counselor to help his/her clients by providing an informed decision making procedures. On the other hand, priority and necessity of information which will be given to clients are too important. Genetic counselor must be prohibited to give information that just has emotional disturbance without useful yields. Trained genetic counselors can help not only to allay some of these emotions, but also to prevent it. Therefore, it is suggested that genetic counselors be trained by psychological counseling skills and a psychiatrist is also included in Genetic counseling team.

Keywords: Genetic Diseases, Psychological Counseling Skills, Preimplantation Genetic Testing

O-17: A Comprehensive Analysis of Maternal Mortality and Relevant Common Medical Errors: A Serious Challenge for Iranian Healthcare System

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Background: To describe factors contributed to the maternal deaths occurred in the West Azerbaijan, Iran from 2002 through 2011.

Materials and Methods: In a cross-sectional study, data on the maternal deaths obtained from the national maternal mortality surveillance system were analyzed. The three delays model was used so as to recognize contributing factors of maternal deaths due to obstetric hemorrhage and hypertensive disorders of pregnancy.

Results: There were 183 maternal deaths; therefore, the overall MMR in the province was 32.8 per 100 000 live births. The most common causes of maternal deaths were obstetric hemorrhage in 36.6% of cases and hypertensive disorders of pregnancy in 25.7%. The factors that most contributed to the deaths were all types of medical errors and sub-standard care with different proportions in management of obstetric hemorrhage and hypertensive disorders of pregnancy.

Conclusion: A sub-standard care and medical error were the major contributing factors in both obstetric hemorrhage and HDP leading to maternal mortality. Therefore, it is necessary to improve the quality of health care at all levels especially hospitals.

Keywords: Maternal Mortality, Sub-standard Care, Medical Error

O-18: Effect of Treatment Failure on Infertile Women's Quality of Life

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Background: Infertility as a global public health issue reduces quality of life (QoL), especially through negative psychological and social consequences. Due to this impact, nowadays health-systems not only focus on morbidity and mortality but also on quality of life and well-being. As increasing evidences suggest that infertility and its treatment influence quality of life, so this study was conducted to

evaluate the relationship between quality of life and the number of treatment failure in infertile women undergoing IVF treatment. Fertility Quality of Life (FertiQoL) tool was used to assess QoL. This questionnaire is composed of two modules: the Core FertiQoL and the treatment FertiQoL.

Materials and Methods: This cross sectional study was conducted in Royan Institute, Tehran, Iran. A total of 125 infertile women undergoing IVF participated in the study. The FertiQoL was administered to all participants. One-way ANOVA was used to evaluate the association between the FertiQoL and the number of treatment failure. This test was then followed by Tukey's pairwise comparisons. Statistical analyses were performed using SPSS version 20 and a p value of less than 0.05 was considered statistically significant.

Results: Patients were divided into four groups according to the number of previous failures: 0 (40.8%), 1 (25.6%), 2 (14.4%), 3 (12.8%), and 4 or more failures (6.4%). Significant difference was found among the groups on both core and treatment FertiQoL scores ($p=0.002$ and $p=0.001$ respectively). Patients with two failures scored lower than those with four or no failure on both core and treatment FertiQoL ($p<0.05$).

Conclusion: The results of this investigation show that infertile women with twice failure might suffer from lower quality of life and need to be supported by family, friends and society. Psychological interventions may improve the quality of life in these women through affecting the bio-psycho-social dimensions.

Keywords: Infertility, Quality of Life, Treatment Failure

O-19: Challenges of Donor Selection: The Experiences of Iranian Infertile Couples Undergoing Assisted Reproductive Donation Procedures

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Background: Couples seeking assisted reproductive donation procedures are faced with complex challenges throughout their treatment which can have important psychological impacts on their life. Selecting a suitable donor is one of the hardest decisions they will ever make. This study was carried out to provide an in-depth description of the experiences of couples in relation to donor selection.

Materials and Methods: In this descriptive exploratory qualitative study, 32 infertile couples who were candidate to use assisted reproductive donation procedures were purposefully selected from Montaserieh infertility center in Mashhad, Iran in 2012. Data was collected through conducting semi-structured interviews and analyzed using conventional content analysis. Member check and expert debriefing were used to enhance study rigor.

Results: The experiences of infertile couples in relation to donor selection were classified in three categories: challenging selection of type of donor, adopting selection criteria and searching for donor suitability. Most of the couples agreed with unknown donors. The most important criteria for donor selection from couples' point of view

was moral issues. Nevertheless, due to limited number of donors, in most cases couples did not search about donor before selection.

Conclusion: Limited number of donors is the most important issue in most of couples to make decision regarding donor selection, which caused them to relinquish from thinking on the type of donor and selection criteria.

Keywords: Infertility, Donor Selection, Qualitative Research, Assisted Reproductive Technologies

O-20: Commencing in Darkness: Narratives of Iranian Unconsummated Couples about Their Unmet Sexual Education Needs

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Background: The inability to have vaginal intercourse despite several attempts, defined as unconsummated marriage (UCM). It is a problematic worldwide sexual disorder. Lack of sexual knowledge has been shown affected UCM couples, so, we designed a qualitative narrative study to explain how a sample of Iranian unconsummated couples experienced sexuality when they were children and to explore what and how they learned about sexuality.

Materials and Methods: Data were collected through individually and jointed sexual history and life story interview with 20 UCM couples at Isfahan psychosexual clinic.

Results: Two main themes were emerged regarding sex educations: Formation of sexual self-concept and formal sex education. Participants discussed how Haya (conscious embarrassment) through their childhood and premarital period play a role as a barrier for openly discussion about sexual matters between them and their parents. They also narrate how magnification and catastrophizing through transmission of misinformation by their parents and peers lead to their unmet needs about sexual education, so they experienced uncomfortable feelings with their own sexuality.

Conclusion: The findings explain how the majority of the participants blamed the shortage of formal and informal sex education as the main reason for their repeated failures in sexual lives. They wish to change disciplines for sex education in their community, to an idealized multistage systemic formal education if they regressively comeback through their life stories.

Keywords: Sexual Education, Sexuality, Unconsummated Marriage, Qualitative Study, Narrative Study

O-21: Challenges of Elective Abortion in Iranian Society

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Background: Elective Abortion means termination of pregnancy by drugs or manipulation. The aim of this research is to evaluate the challenges of elective abortion in Iranian society.

Materials and Methods: This descriptive study is done on 113 pregnant women wanted to do abortion who referred to Dr Rasekh clinic that were studied through a questionnaire with six questions. Collection of information was very difficult because most of the patients who according to the documents are asked to register in our clinic initially denied their purpose to abort. Finally, after a long time of explaining about the privacy of our research as well as assuring them not to reveal their secrets, they trust and agreed answering the questions.

Results: 44% of women requestors of abortions are due to pregnancy during engagement period despite the legal marriage, 20% due to economical problems, 14% due to the multiple number of children and old age, 5% due to adultery relationship (illegitimate relationship), 4% due to genetics disorders, 4% due to fetal sex, due to struggle with her husband and divorce plan, 3% due to child apathy, and 3% due to unawareness and inaccurate consultation.

Conclusion: There is only one way to prevent illegal abortions that it is to expand the dimension of education. Contraception education is essential for every age group and promote cultural and religious precepts of the moral teachings of Islam and other divine religions. The role of the mass media as well as religious missionaries at all levels of counseling by people who have a positive impact on families and by committed physicians is important but this information must continued.

Keywords: Challenges, Elective, Abortion, Counseling, Education

O-22: The Relationship between Sexual Satisfaction and Body Image in Women

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Background: Sexual satisfaction is the fundamental role in success of family foundation. sexual satisfaction is the one of the most important components of life's satisfaction. that influence on couples' health. Although sexual function is related to sexual satisfaction but this factor cant be recognized as the only influential factor and the other factors include relational and emotional variables and individual factor such as body image can play a role on sexual satisfaction. Therefore, with regard to the impairment of past research, this study was conducted to identify the relationship between the components of body image and women's sexual satisfaction.

Materials and Methods: This correlation research was performed in 2012 on 196 women who were selected by random sequential sampling among women who attended the selected medical centers. Data was collected by sexual satisfaction scale for women and female sexual function Index (FSFI) and body esteem scale (BES)

which are highly valid and reliable. Data was analyzed by using Pearson Correlation Coefficient and linear regression by SPSS 20.

Results: There is a positive correlation between sexual function and sexual satisfaction. Subscales of sexual attractiveness and weight concern) the body esteem scale) was significantly related to sexual satisfaction and sexual function. In spite of Subscales of physical condition) the body esteem scale) was not related to sexual satisfaction and sexual function. Analysis regression showed the components of women's sexual function predicted 46% of variance and body image 20.2% of variance in sexual satisfaction.

Conclusion: Body image and sexual function predicted sexual satisfaction in women. Counselores and practionioners may consider the role and importance of body image when client present with sexual problems.

Keywords: Sexual Satisfaction, Sexual Function, Body Image

O-23: Comparison of Female Infertility Etiology in Traditional Iranian and Conventional Medicine

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Background: According to human history, "infertility" has long been of interest to scientists, which has led to the discovery of its causes. Nevertheless 15-25% of infertility cases are still termed as idiopathic. In Iranian traditional medicine school, special attention has been paid to this issue, so part of the great written documents of this school is dedicated to the study of the causes and treatment of infertility.

Materials and Methods: Within a library method, a search was performed on "Oqr" or infertility issues through the three main reference books of Iranian traditional medicine: the "Canon" of Ibn Sina, "Zakhireh Kharazmshahy" by Hakim Jorjani and "Exire Aazam" by Hakim Mohammad Aazamkhan. The results were classified and arranged in tables which compare the findings in traditional and current medicine.

Results: When the factors considered in modern medicine are compared to those mentioned by Jorjani, it becomes clear that almost all the causes which are cited in modern medicine have been previously discussed by Jorjani. Regarding Ibn Sina, his viewpoints show his great and correct knowledge of infertility and many of its causes. Interestingly, the comparison between the views of Ibn Sina (370- 429 AH) and Jorjani (435-531 AH) on infertility shows the extent of scientific advances in traditional medicine during this time. Jorjani further extended the knowledge and classified infertility causes into four groups. The scientific development of traditional medicine continued and in the thirteenth century AH, Hakim Aazam Khan found more factors and classified the infertility etiology in seven major categories.

Conclusion: By comparing the causes of infertility in women in modern and Iranian traditional medicine and integrating them, a new window can be opened to diagnosis and holistic approach to reproductive issues.

Keywords: Female Infertility, Traditional Iranian Medicine, Conventional Medicine

O-24: Healthy Menopausal Women's Related Factors of Sleep Quality in West of Tehran

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Background: Sleep is an essential aspect of life and Insomnia is associated with negative health consequences including fatigue, impaired daytime function, reduced quality of life, and increased visits to healthcare providers. Women tend to report sleep disturbances more often than men in the same age. Also it is one of the important symptoms observed during menopause with high rates and need to assess its related factors for better prevention and management. The objective of this study is to assess related factors of sleep disturbance in healthy 50-60 years old Menopausal.

Materials and Methods: In this cross sectional study, by randomized sampling, 700 healthy volunteer Menopausal women, with age 50- 60 years, whom had been visited in Health Clinics of West of Tehran (Year 2012) had been interviewed. Participants were at least one year after entering menopause period. The study questioner included two main parts of personal characteristics and the Pittsburgh Sleep Quality Index (PSQI). Data were analyzed by using SPSS 14 software. All ethical points were considered and approved by research ethics committee of Tehran University of Medical Sciences.

Results: Average of age was 52.9 ± 3.3 , and 62.5% had sleep problems. Average of PSQI score was 7.84 ± 4.4 . There were significant correlation between sleep disturbance and occupational status, educational status, husband's occupational status, and economical status ($p=0.002$). There were no significant correlation between sleep disturbance and other personal characteristics, and consumption of tea, coffee, or cola.

Conclusion: Frequency of sleep disturbance in previous study of research group with lower samples, were 70% and in this study was 62.5%. It is suggested to provide suitable guiding and counseling regarding to related factors for maintaining the quality of life, especially during Menopause time. Acknowledgment: This is the first phase of study, which received grants from Research Department of Tehran University of Medical Sciences. (2010-2012).

Keywords: Menopause, Quality of Sleep, Pittsburgh Sleep Quality Index (PSQI)

Female Infertility

O-25: The Risk of Major Birth Defects in-ICSI and Normal Infants

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Background: In the past 22 years, intracytoplasmic sperm injection (ICSI) on human oocytes become a successful method of treatment for most categories of infertility. But there are real concerns that possible malformations among ICSI infants are still not fully recognized. Despite the success of ICSI, the risk of major birth defects due to various parental factors or treatment may increase. We report our experience to determine whether use of ICSI is associated with an increase in major birth defects or adverse pregnancy outcomes.

Materials and Methods: Historical cohort study of Major Birth defects was performed in 789 births in Royan Institute. The data for this analysis were derived from Tehran's ICSI linked data file by simple sampling method. In our study, the risk of birth defects was compared in 263 ICSI infants and 526 naturally conceived (NC) infants. We also performed multiple logistic regression analyses to calculate the odds ratio (OR) and 95% confidence intervals for the independent association of ICSI on each outcome.

Results: We found 50 infants with major birth defects: these included 24 NC infants (4.6%) and 26 ICSI infants (9.9%). In comparison with NC infants, ICSI infants had a significant 2.29-fold increased risk of major birth defects. After adjustment for maternal age, infant's sex stillbirth, abortion and type of delivery, we found a relatively difference in risk ($OR=1.51$). In this study, the majority (93.7%) of all infants were normal but 6.3% of infants had at least one major birth defect.

Conclusion: Although available data are limited, there appears to be no indication that birth defects increased in pregnancies resulting from ICSI. In this study, we reported major birth defects in ICSI infants more than naturally conceived infants. We also found evidence of a difference in risk of major birth defects between normal and ICSI. Musculoskeletal and urogenital malformations were the most reported major birth defects in ICSI infants according to organs and systems classification

Keywords: ICSI, Reproductive Technique, Birth Defects

O-26: Importance of IL-18 in Serum and Follicle Fluid in The Context of Fertility Treatment

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Background: Some authors detected significantly higher IL-18 levels in serum, peritoneal, and pleural fluids of patients with severe OHSS as compared with control groups and suggest a role of IL-18 as a marker of OHSS. Lower levels of IL-18 have been found to characterize unexplained infertility. In this study, we analyzed the importance of IL-18 levels in serum and follicle fluid in response to ovarian stimulation with gonadotropins, in correlation to pregnancy rate and also to clinical parameters.

Materials and Methods: From a total sample of 90 patients (2011-2013), we analyzed the level of IL-18 in serum and FF on the day of FP. IL-18 mean and cut-off levels were evaluated in serum and FF on the day of FP by the ELISA method: a. in response to ovarian stimulation with gonadotropins, between patients with a poor and a good response. b. To the resulting pregnancy rate and c. by comparing BMI

Results: IL-18 levels in serum were significantly higher than in FF ($p < 0.001$) revealing a positive and significant correlation ($r = 0.84$, $p < 0.001$). IL-18 levels in serum and in FF of patients with a BMI lower than 21 were significantly lower than in patients with a BMI higher than 22 ($p = 0.015$). Patients with a good response ($E2 \geq 2500$ pg/ml, number of follicles ≥ 7 on the day of hCG injection) showed significantly higher IL-18 mean levels on the day of FP than patients with a poor response ($E2 \leq 2500$ pg/ml, follicles ≤ 6 , $p < 0.001$). We found a cut-off level of 158.6 (ng/ml) in serum with a sensitivity of 61% and a specificity of 64.5%. The pregnancy rates were 39% below and 32% above this cut-off level.

Conclusion: Our data suggest that IL-18 exerts an influence on ovarian function and thus on the result in hyperstimulated IVF/ICSI patients. Cut-off level for IL-18 is clinically relevant.

Keywords: IL-18, Cut-off Level, Poor Response, Pregnancy, BMI

Genetics

O-27: Genome Instabilities in Preimplantation Development Leading to Genetic Variation between Tissues of Normal Human Fetuses

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Background: Origin of midlife copy number variations (CNVs) between tissues in non-genetic diseases is unknown. Such genomic differences caused by post-zygotic events. They might either happen during the life or due to prevalent mosaicism in preimplantation stage. We aim to explore fetal mosaicism and its origins.

Materials and Methods: Two apparently normal fetuses

were achieved following the therapeutic abortion due to maternal indications on fourth month of pregnancy. DNAs from 22 tissues of each fetus were studied by array CGH using slides contain 195000 probe. Copy number calling was performed using Circular Binary Segmentation (CBS) method. Reciprocal CNVs as high confidence CNVs was validated by qPCR. Functional analysis was performed by gene ontology (GO).

Results: About 60 CNVs was observed in each fetus. The frequency of reciprocal CNVs varied from 2 to 18. Analysis of the CNVs by array CGH and qPCR showed that quantity of their change were not mostly integer multiples. Some of CNVs were shared between both fetuses, some were found in the same tissues, whereas some in different tissues. GO showed that altered genes are mostly involved in embryonic development pathways. Tissues clustering according to CNVs revealed that those from the same embryonic origin in some cases are close together in a cluster; however, there were large disagreements with clustering of embryonic layers derivatives.

Conclusion: According to distribution pattern of frequent CNVs, their origin should be early development, some preimplantation and some postimplantation. CNVs with low frequency seem to occur in later stages. Each organ inherits CNVs with a unique pattern regarding to extensive cell mixing/migration in embryonic development. Shared variations seem to be hotspots for CNV events, those occur in the same tissues might be functional. Regarding preimplantation origin of some CNVs, PGD methods with ability of mosaicism and CNVs detection could be helpful to transfer the healthier embryos.

Keywords: Copy Number Variation, Fetus, Origin

O-28: Endometriosis Is Influenced by The Promoter Haplotype-Based Expression of Macrophage Migration Inhibitory Factor (MIF)

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Background: Macrophage migration inhibitory factor (MIF) is a key pro-inflammatory cytokine that is secreted by accumulated active macrophages in ectopic tissue of endometriosis. MIF is involved in pathophysiological events of endometriosis, such as angiogenesis and cell proliferation. MIF that stimulates the synthesis of PGE₂, leads to over-expression of local estradiol synthesis in endometriosis.

Materials and Methods: Genomic DNA of 70 patients with endometriosis, who had undergone laparoscopy during 2012-13, and 70 fertile women were amplified via PCR. Restriction fragment length polymorphism which was applied to determine -173G/C polymorphism. ORF polymorphisms and -794(CATT)5-8 were detected by sequencing. Q-PCR was performed for mRNA expression level of MIF in 14 ectopic tissues. Statistical analysis was done by Chi-square test and one way ANOVA.

Results: The genotype analysis of ORF polymorphisms revealed three SNPs: +254, rs2096525 ($p = 0.84$), +656, rs2070766 ($p = 0.88$),

+626, rs33958703 (p=0.04). Homozygosity of -794(CATT)5, as normal type, was only observed in control group. Promoter haplotype (-173G/C and -794(CATT)5-8) was different in control and patient groups. Expression level of MIF in ectopic tissues with -794(CATT)6,7/-173GC was significantly more than the other haplotypes (p=0.00) and in -794(CATT)6,6/-173GC group was significantly higher than -794(CATT)5,5/-173G (p=0.02). We found maximum prevalence of endometriosis at those being 26-30 years. Obese women with BMI \geq 30 have shown lower risk of endometriosis (p=0.00).

Conclusion: We report for the first time, both increased number of CATT repeat in -794 and presence of -173 C in ectopic tissue of endometriosis stimulated MIF promoter activity. It seems haplotype of -794(CATT)5-8/-173G/C and +626 rs33958703 SNP were significantly correlated with susceptibility of endometriosis that might play an important role in pathophysiology of endometriosis.

Keywords: Endometriosis, Haplotype, Macrophage Migration Inhibitory Factor, Polymorphism

O-29: Differences in The Transcriptional Profiles of Human Cumulus Cells Isolated From MI and MII Oocytes of Patients with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder in women. The abnormalities of endocrine and intra-ovarian paracrine interactions may change the microenvironment for oocyte development during the folliculogenesis process and reduce the developmental competence of oocytes in PCOS patients who are suffering from anovulatory infertility and pregnancy loss. In this microenvironment, the cross talk between an oocyte and the surrounding cumulus cells (CCs) is critical for achieving oocyte competence.

The aim of our study was to investigate the gene expression profiles of CCs obtained from PCOS patients undergoing IVF cycles in terms of oocyte maturation by using human Genome U133 Plus 2.0 microarrays.

Materials and Methods: A total of 59 genes were differentially expressed in two CC groups. Most of these genes were identified to be involved in one or more of the following pathways: receptor interactions, calcium signaling, metabolism and biosynthesis, focal adhesion, melanogenesis, leukocyte transendothelial migration, Wnt signaling, and type 2 diabetes mellitus. According to the different expression levels in the microarrays and their putative functions, six differentially expressed genes (LHCGR, ANGPTL1, TNIK, GRIN2A, SFRP4, and SOCS3) were selected and analyzed by quantitative RT-PCR (qRT-PCR).

Results: The qRT-PCR results were consistent with the microarray data. Moreover, the molecular signatures (LHCGR, TNIK, and SOCS3) were associated with developmental potential from embryo to blastocyst stage and were proposed as biomarkers of embryo viability in PCOS patients.

Conclusion: Our results may be clinically important as they offer a new potential strategy for competent oocyte/embryo selection in PCOS patients.

Keywords: cDNA Microarray, Cumulus Cell, Embryo Development,

Oocyte Maturation, PCOS

O-30: Comparing Expression Patterns of Endometrial Genes in Implantation Failures and Recurrent Miscarriages with Fertile Couples Following ICSI/IVF Using in Silico Analysis

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Background: To screen and diagnose patients with recurrent abortions and implantation failure after IVF/ICSI, differentially expressed genes of endometrium through DNA microarrays were monitored.

Materials and Methods: Microarray expression profile of GSE26787 dataset from GEO database was used to analyze gene expression profiles of 15 endometrial biopsy samples- five from control fertile (CF) couples, five from implantation failure (IF) and five from recurrent spontaneous miscarriage (RM), prepared by using Affymetrix Human Genome U133 Plus 2.0 system. Raw data was normalized by RMA (Robust Multichip Average) algorithm and cluster analysis was applied to compare gene expression patterns between IF and RA with CF (2 samples Bayes Test; p value < 0.05). Genes exhibiting at least 2-fold changes were selected for further analysis and also analyzed to generate a putative interaction map including proteome map based on biological processes and molecular functions of proteins.

Results: The down-regulated genes in IF versus FC cases were PAEP, CXCL14, GPX3, TSPAN8 and DNER; while in RM versus IF were PDS5B, HLA-DQA1, LOC100505967, NTRK3, and PBK. Up-regulated genes in IF versus FC included LOC100505912, XDH, MMP26, LOC100505967, and CTNNA2 and in RM versus IF the up-regulated genes were GABRA2, TCN1, SLC37A2, C2CD4A, and MFSD4.

Conclusion: The different expression of endometrial genes showed the extent of changes within gene expression of endometrium prior to conception that plays an important role in infertile couples especially in patients who have IF or RM. We will further analyze the findings to generate a putative interaction map including proteome map based on biological processes and molecular functions of proteins.

Keywords: Implantation Failure, Recurrent Spontaneous Abortion, Gene Expression Patterns, Interaction Network

O-31: Epigenetic Aberration of HOXA10 Gene in Human Endometrium throughout The Menstrual Cycle in Endometriosis

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Background: Epigenetic aberration such as DNA methylation and histone modifications appear to be involved in various diseases such as Endometriosis. Here, we investigated the epigenetic regulation of HOXA10 promoter, as a crucial gene, responsible for uterine organogenesis, functional endometrial differentiation and endometrial receptivity, and its correlation with mRNA expression of this gene in eutopic and normal endometrium, during menstrual cycle.

Materials and Methods: Epigenetic analysis of eutopic and normal endometrium which were assayed by Chromatin Immunoprecipitation (ChIP), by using anti- H3K9ac, MeCP2, H3K9Me2, H3K27Me3, H3K4Me3 antibodies and precipitated chromatin DNA was evaluated quantitatively by real-time PCR technique. To this end, eutopic endometrium samples were collected using laparoscopy from 20 women with documented endometriosis, and endometrial tissues were collected from 18 healthy fertile women as well who underwent laparoscopy for tubal ligation surgery as a control group. Ethical approval and informed patient consent were gained for the use of tissue samples.

Results: Data showed a harmonious pattern between mRNA expression of HOXA10 and epigenetic regulation of its promoter region. In the way that in secretory phase, the epigenetic marks of H3K9ac and H3K4Me3, known to be associated with gene activation, were lower in eutopic endometrium in comparison with control group and also H3K9Me2, H3K27Me3 and MeCP2 marks, known to be associated with gene repression which were higher in eutopic endometrium in comparison with control group.

Conclusion: Our findings suggest that epigenetic be greatly responsible for aberrant expression of HOXA10 gene in endometrium of patient with endometriosis.

Keywords: HOXA10 Gene, Endometriosis, Menstrual Cycle, Epigenetic

secondary outcomes were duration of procedure and total volume of required saline for adequate visualization of endometrial cavity. Pain was quantified with 10-point visual analog scale.

Results: One hundred and forty eight subjects were randomly assigned to intracervical balloon insertion and 152 to intrauterine placement. There were no significant differences in inflation and deflation pain and total procedure time between two groups. The total volume of required saline for adequate distention of cavity was significantly lower in cervical group than intrauterine group. The nulliparous women experienced significantly more pain after initial inflation of balloon compared to multiparous women ($p= 0.04$). Pain scores were not associated with patient's age, volume of saline infused and presence of pathology. But pain scores were significantly correlated with total procedure time.

Conclusion: Intracervical catheter placement does not reduce pain during or after SHG. But intracervical balloon insertion requires less significant volume of saline compared with intrauterine placement which reduces the risk of intrauterine infection and spread of malignant endometrial cells into the peritoneal cavity at the time of procedure

Keywords: Sonohysterography, Pain, Catheter Placement

Reproductive Imaging

O-32: Pain Experienced during Sonohysterography: A Randomized Comparison of Cervical to Uterine Catheter Placement

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Background: The aim of this study is to evaluate whether the location of the balloon placement into either the cervical canal or uterine cavity during sonohysterography can affect the intensity of pain.

Materials and Methods: A total of 300 infertile women undergoing sonohysterography were randomized to intracervical or intrauterine balloon placement. The examination was scheduled in the early follicular phase of menstrual cycle, before day 10. The primary outcome measures included the degrees of perceived pain after inflation and then after deflation of the balloon catheter. The

Andrology

P-1: Study The Anti-inflammatory Effect of 1- (1- (4-Chlorobenzoyl) -6 -methoxy-2-methy l-1H-indol -3-yl) butan -2 -one on Leukocytospermia

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Background: Leukocytospermia is found in both fertile and infertile men with and without evidence of infections of the genital tract. However, despite extensive research done in this subject, the clinical implications of this condition remain unclear. Leukocytes are also commonly found in semen samples, and a level above $1 \times 10^6/\text{mL}$ is considered pathologic according to WHO criteria. Non-steroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents for the treatment of the inflammation related to large variety of pathologies. NSAIDs inhibit the cyclooxygenase (COX) activity resulting in decreased synthesis of prostaglandin, leukotriene and thromboxane precursors. Several reports indicate that NSAIDs can prevent the development various human tumors, including colon, breast, lung, gastric, and esophageal neoplasias.

Materials and Methods: Semen samples were obtained from Leukocytospermic infertile men. This study includes 37 sub-fertile male partners between July 2013 to April 2014, from couples who had been consulted the infertility clinic of the Babil hospital of maternity (Hilla city/IRAQ). The Semen specimens were divided into four fractions. First part was control, 0.5ml of liquefied semen mixed with 0.5ml fertiCult medium and incubated at 37° C for 30 minutes. Second part (antibiotic 1), 0.5 ml liquefied semen was mixed with 0.5ml fertiCult medium supplemented with 0.02 mg/ml antibiotic and incubated at 37°C for 30 minutes. Third part (antibiotic 2), 0.5ml liquefied semen was mixed with 0.5ml fertiCult medium supplemented with 0.04 mg/ml antibiotic and incubated at 37°C for 30 minutes. Fourth part (antibiotic 3), 0.5ml liquefied semen was mixed with 0.5ml fertiCult medium supplemented with 0.06 mg/ml antibiotic and incubate at 37°C for 30 minutes. After semen specimen treated in all fractions, they were examined and assessed for macroscopical and microscopical changes.

Results: The treatment of low-level leukocytospermia with present antibiotic show significantly enhancement in the semen parameters among the treated patients. Low-level leukocytospermia resolved in good percentage of the treatment groups.

Conclusion: The results of this study indicated that adding present antibiotic to the culture medium for *in vitro* sperm activation leads to an improvement in certain sperm function parameters.

Keywords: Leukocytospermia, Indomethacin, Derivative, Ester

P-2: Zaralenone-Induced Damages in Testicular Tissue Correlates with Mitochondria Damage, Er- α Receptors Expression and Leydig Cells Aromatization

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Background: Zearalenone (ZEA) as a myco-oestrogen competes with the natural 17- β estradiol to bind with the specific binding sites of the Ers. Therefore, there are increasing attentions on its oestrogen mimic properties. In this line, the Ers are directly involved in intra-testicular endocrine activities by controlling the Leydig Cells aromatization. Therefore, present study was designed in order to analyze the probable effect of ZEA on Er- α expression in Leydig Cells as well as ZEA-induced changes in Leydig cells steroidogenic foci. Moreover, the ZEA-decreased endocrine status induced cellular apoptosis was evaluated.

Materials and Methods: Thirty mature male rats were divided into four groups including; control-sham (2 mL, normal slain, ip), low dose ZEA-treated (1mg/kg, ip), medium dose ZEA-treated (2mg/kg, ip), high dose ZEA-treated (4mg/kg, ip). All animals received chemicals for 21 continuous days. The Er- α mRNA and protein expressions were evaluated by using semi-quantitative RT-PCR and immunohistochemical analyses, respectively. The mitochondria content of the cells were evaluated by using histochemical staining Acid Fuchsin staining. The Leydig cells steroid foci and the germinal cells apoptosis were assessed by using the epi-fluorescent and Annexin-V staining techniques, respectively.

Results: Observations revealed that, ZEA resulted in significant reduction at mRNA and protein levels of Er- α in a dose dependent manner. The germinal cells of the ZEA-induced testicles were exhibited significantly lower mitochondria contents. Accordingly, the high dose ZEA-received animals showed the lowest mitochondria content. Moreover, the ZEA, dose dependently, reduced Leydig cells steroidogenic activity and intensively elevated cellular apoptosis.

Conclusion: Our data showed that, ZEA with longer nuclear retention to Ers affects the Er- α expression, which in turn influences the Leydig Cells aromatization. Ultimately, the down-regulated endocrine status promotes the cellular apoptosis.

Keywords: Zearalenone, Er- α , Steroidogenic Activity, Leydig Cells, Apoptosis, Mitochondria

P-3: Complete Reproductive Hormonal Study in Nonobstructive Azoospermic (NOA) Patients for Determining Reliable Markers of The Presence of Spermatozoa in Their Biopsies

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Background: Various hormone tests of the presence of spermatozoa on testicular sperm extraction (TESE) in azoospermic men have been investigated. Because of its clinical importance, we conducted this study to evaluate complete reproductive serum hormone levels in nonobstructive azoospermic (NOA) patients for determining markers which can predict the spermatogenesis.

Materials and Methods: Seventy one NOA infertile men who were referred to the Infertility and Andrology Clinic from February 2012 till March 2013 were participated in this Cross-sectional study. Blood samples were obtained and serum level of Inhibin B (Inh-B), Anti-Mullerian hormone (AMH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone and estradiol were measured (double-checked). The TESE was performed under general anesthesia and tissue samples were sent for sperm retrieval.

Results: In 39 patients (55%) spermatozoa was detected in their biopsies (successful TESE) and in 32 patients (45%) it was not found

(failed TESE). Serum LH level was significantly less in patients with successful TESE compared to the failed TESE group. (5.17 ± 2.62 mIU/ml vs. 9.28 ± 7.74 mIU/ml; p value: 0.007). Also, serum levels of AMH and FSH were significantly less in successful TESE group considering $\alpha=0.1$ and confidence interval = 90%. (5.45 ± 3.45 ng/ml vs. 9.57 ± 8.89 ng/ml; P value: 0.073 for AMH and 10.90 ± 14.55 mIU/ml vs. 17.56 ± 17.54 mIU/ml; p value: 0.085 for FSH). Other serum hormone levels were not significantly different between the two groups.

Conclusion: Triple serum hormone testing including LH, FSH and AMH can be recommended as a predictive hormonal study of the presence of testicular spermatozoa in nonobstructive azoospermic patients, although more studies with larger sample sizes are needed to confirm our results

Keywords: Nonobstructive Azoospermic (NOA) Azoospermic Patients

P-4: Grape Seed Extract Alleviate Male Reproductive Toxicity Caused by Busulfan in Rat Model

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Background: The alkylating agent busulfan adversely affects spermatogenesis in mammals and impairment of spermatogenesis has been identified as an inevitable side effect of cancer treatment drugs then the aim of this study was to investigate the possible protective effects of Grape seed extract with potent antioxidant activity, on busulfan-treated adult male mice.

Materials and Methods: In this study 40 adult male rats were randomly divided into 4 groups (n=10 each) consisting of a control and 3 experimental groups. The animals in the control group received dimethyl sulfoxide (DMSO and distilled Water), a solvent, the second group was gavaged with busulfan solution, and 2 test groups received Busulfan and grape seed extract daily orally by gavage. All rats in different groups were sacrificed after 45 days and histological analysis of testis and the cauda epididymal sperm analysis for sperm motility, DNA damage concentration in the cauda epididymis, viability and sperm DNA damage was carried out (p<0.05).

Results: The histological results obtained from this study revealed that busulfan induced significant histopathological changes in the testis such as decrease in seminiferous tubules diameter (STD), the percentage of seminiferous tubules with positive tubular differentiation (TDI), repopulation (RI) and spermiogenesis (SPI) indexes (p<0.05) than those animals in Grape seed extract -treated groups and the total number of Leydig cells were increased in Grape seed extract -treated groups. The cauda epididymal sperm analysis showed that busulfan caused significant decrease in sperm count, motility, and viability, while abnormal sperms increased as compared to control. These changes were associated with significant increase in DNA damage and chromatin abnormality. Notably administration Grape seed extract caused a considerable recovery in above-mentioned parameters (p<0.05).

Conclusion: The results suggest that Grape seed extract as an antioxidant have a possible protective effect against busulfan-induced testicular damages.

Keywords: Grape Seed Extract, Busulfan, Testis, Sperm, Rat

P-5: Effect of Total and Severe Teratozoospermia on The Outcome of ICSI

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Background: We propose in this work to assess the effect of total and severe teratozoospermia on the outcome of intra cytoplasmic sperm injection (ICSI) essentially the biological side and try to highlight the factor in the success or failure of the attempt in cases of extreme or total teratozoospermia.

Materials and Methods: This is a comparative retrospective study including 140 couples who underwent medically assisted reproduction by the technique of ICSI identified in the unity of "assisted reproduction". This study includes two groups: one group comprising 100 couples in which the indication of ICSI is a total or extreme teratozoospermia (abnormal forms $\geq 97\%$) (Group I) and a group with 40 other couples for abnormal semen analysis (Group II).

Results: The fertilization rates respectively in the teratozoospermia group and control group were 68% and 65%, segmentation rate were 96.9% and 97%, and top quality embryo were 70% and 81%. Those rates were similar for the group total and extreme teratozoospermia and the control group (DNS). The rate of maturation showed a significant difference with a rate of 70% in the study group and a rate of 86% in the control group (p= 0.01). In the group teratozoospermia, we found a significant difference in fertilization rate when an abnormality concerns the head or another part of the spermatozoid (p= 0.04). Concerning head abnormalities, we found also a significant difference in fertilization rate between acrosomal and non acrosomal abnormalities.

Conclusion: Head and acrosomal seem to have a side effect on the biological results of ICSI in case of teratozoospermia. It does not seem to be a poor prognostic factor in the success of the attempt of ICSI.

Keywords: Teratozoospermia, Outcome of ICSI, Acrosomal

P-6: The Impact of Cigarette Smoking on Semen Quality in Infertile Couples

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Background: Approximately, one third of world's population older than 15 years of age smokes. Cigarette smoke contains toxic chemical, mutagenic and carcinogenic compounds. Some of these substances were found in seminal plasma. Over the past two decades, there has been an increasing body of evidence that cigarette smoking may impair human semen quality. The results of these studies are conflicting, particularly with regard to the sperm concentration and morphology.

Materials and Methods: This prospective cross-sectional study was conducted from 2009 to 2013 in ART unit of the Tehran General Women Hospital, Iran. The volunteers were 1275 idiopathic infertile men, attending the andrology laboratory in context of infertile

ity investigation. The current smoker was defined as ≥ 1 cigarettes / day in the last six months. The volunteers were undergoing semen analysis. Each subject was answered to a questionnaire about his smoking habits, lifestyle. Appropriate semen and smoking data were available for 1263 men, including 315 (24.9%) smokers and 948 (75.05%) nonsmokers. Standard semen analysis was performed according to WHO criteria. Chi-square and Student t tests were used for statistical analysis.

Results: Statistical analysis demonstrated significantly lower semen quality in smokers compared with nonsmokers. Cigarette smoking was associated with a significant decrease in the mean sperm concentration (-10.4%) ($p < 0.0001$), total number of motile sperm (-12.7%) ($p < 0.0001$). The percentage of normal forms was significantly reduced in smokers (-6.17%) ($p < 0.0001$). The semen volume was not different between two groups.

Conclusion: Although the exact impact of smoking on male fertility remains controversial, in present study, we observed the negative effects of current cigarette smoking on sperm parameters quality in idiopathic infertile men. Therefore, it would be sensible to advise men to complete smoking cessation before any parental project

Keywords: Infertility, Semen Analysis, Cigarette Smoking, Asthenospermia

P-7: Sperm DNA Assessment as A Tool for Partial Resolver of The Unexplained Infertility Problem

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Background: To establish importance of sperm DNA assessment in resolving the unexplained infertility problem.

Materials and Methods: This article is based on the careful observation of texts and papers from 2000-2014 about the DNA damage assessment as a significant parameter of sperm function test.

Results: Acidic Aniline blue, Sperm Chromatin Dispersion test (SCD), Malondialdehyde concentration, 8-OH dG and total antioxidant capacity (TAC) are tools for studying of sperm packaging density, Sperm Nuclear Matrix Assay and oxidative stress respectively and we can use each of them to clear the dark edges of unexplained infertility in clinical practice.

Conclusion: Increased amounts of DNA Fragmentation Index (DFI) and by products of lipid peroxidation and DNA denaturation have reserve proportional to the rate of fertilization and pregnancy rates in the era of spontaneous and assisted pregnancy. The exact prediction of each of this matters requires exact evaluation of the background pathology.

Keywords: Unexplained Infertility, Sperm Chromatin Dispersion Test (SCD), Reactive Oxygen Species (ROS), Total Antioxidant Capacity (TAC), DNA Fragmentation Index (DFI)

P-8: Protective Effect of Royal Jelly versus Vitamin C against Anemia-Induced Damages in Testicular Tissue

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Background: Phenylhydrazine (PHZ) is used to induce experimental anemia in animal models. On the other hand royal jelly is known as antioxidant compound. Therefore, here in present study we aimed to evaluate the protective effect of royal jelly on PHZ-induced damages in testicular tissue and compare its impact with vitamin C.

Materials and Methods: Eighteen mature male mice were randomly divided into 3 groups. In order to induce experimental anemia, PHZ was administered at dose of 8 mg/kg, ip and then it was continued as 6 mg/kg, every 48 hours, ip in all groups. The test groups subdivided into non-treated PHZ-received, royal jelly-received (100 mg/kg, orally) and vitamin C-received (250 mg/kg, ip). After 35 days, the Leydig Cells steroidogenic Activity was evaluated by using special fluorescent staining for intra-cytoplasmic steroid foci and analyzing serum level of testosterone. The spermiogenesis index and germinal epithelium RNA damage were assessed. Finally, tissue levels of total antioxidant capacity (TAC) and total thiol molecules (TTM) were investigated.

Results: Observations demonstrated that PHZ resulted in significant ($p < 0.05$) reduction in Leydig cells steroidogenic Foci ratio. Meanwhile, royal jelly enhanced the intra-cytoplasmic steroid accumulation and elevated serum level of testosterone versus non-treated animals. There were no significant differences between vitamin C and royal jelly. Moreover, royal jelly-treated animals exhibited remarkably lower percentage of tubules with damages RNA as well as increased tissue TAC and TTM ratio.

Conclusion: Our data showed that royal jelly by enhancing the antioxidant status of the testicular tissue inhibited the PHZ-induced RNA damage and up-regulated the testicular endocrine activity.

Keywords: Phenylhydrazine, Royal Jelly, Vitamin C, Sperm, RNA Damage

P-9: Investigate The Effects of Polyphenol Gossypol as A Male Antifertility Agents, How to Make Male Contraceptive?

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Background: Gossypol is a polyphenol isolated from the cotton plant (*Gossypium* sp.). The substance, shows promise for use as a male contraceptive, is a derivative of cottonseed oil. Various species of animals have been tested and some are more sensitive than others, and in men it causes spermatogenesis arrest at relatively low doses.

Materials and Methods: This routine data base study re-examines these data and points out the gossypol, as a male antifertility agents, how to make male contraceptive? In 2009 to 2011 we performed a study with polyphenol gossypol involving 42 participants, and in 2011 to 2013 we conducted another study of 28 participants. The first study was aimed at confirming gossypol's antifertility efficacy and determining the existence of side effects. It acts as a natural defensive agent against predators, provoking infertility.

Results: Studies showed 87 percent of men taking a daily gossypol pill (20 mg/kg body weight/day) had reliable contraception and no

complaints about change in libido, and 99.9 percent of the subjects had sperm concentrations reduced to c 4 million/mL. Other studies revealed that sperm cells themselves are more visibly damaged than Sertoli or Leydig cells. Spermatids are affected first, with damage progressing back to less mature sperm-cell stages if the dose is prolonged or excessive.

Conclusion: Studies on gossypol have paved the road for the search for male contraceptive agents from phenolic compounds. The synthesis of gossypol analogues and derivatives may offer good prospects for developing a series of male antifertility agents. However, the studies revealed two serious flaws: disruption of potassium uptake and incomplete reversibility. Gossypol should be prescribed preferably to men who have completed their families or for those who would accept permanent infertility after a few years of use.

Keywords: Gossypol, Male, Contraceptive, Antifertility Agents

P-10: Aqueous Extract of Rosa Damascena Protects Mice against Formaldehyde-Induced Testicular Damages

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Background: Formaldehyde is widely used in industry and in medicine as tissue fixative and disinfectant. It contains reactive molecules which have been known for its cytotoxic effects. In the present study, the probable protective properties of Rosa damascena aquatic extract, against the harmful effects of formaldehyde was evaluated by consideration of sperm count, motility, viability, morphology and serum testosterone level in male mice.

Materials and Methods: Twenty-eight healthy NMRI male mice were randomly divided into four groups (n = 7). Group one was negative control which received distilled water while groups two, three and four were received intra-peritoneally 10 mg/kg diluted formaldehyde, 40 mg/kg extract, 40 mg/kg extract+ formaldehyde, respectively. The trial was continued for 35 days.

Results: The result showed that formaldehyde could lead to significant adverse effects on motility, viability, concentration and morphology of sperm compared to group I (p<0.01) and decreased serum level of testosterone compared to group I (p<0.05). In group III, the extract caused to significant increase in motility, viability, count of sperm and increased serum testosterone compared to control and second groups (p<0.01). In group IV, it was determined that the produced damages, caused by formaldehyde, were improved after extract injection compared to group II (p<0.01).

Conclusion: It is concluded that serious damages are occurred via formaldehyde injection in male reproductive system and the aqueous extract of Rosa damascena provides protective effects against these damages.

Keywords: Formaldehyde, Spermatogenesis, Rosa Damascena, Testis, Testosterone

P-11: Evaluation of Heat Shock Protein A2 in Male Rats before and after Varicocele Induction

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Background: Varicocele is a major cause of male infertility, as it may impair spermatogenesis through several distinct pathophysiological mechanisms including hypoxia, renal-adrenal reflux, hormonal dysfunction, autoimmunity, oxidative stress and hyperthermia. HSP70s are members of the heat shock protein (HSP) family, which are considered intracellular chaperones. Their expressions can increase when cells are exposed to metabolic and/or heat stress. Therefore, the aim of this study was to evaluate the effect of varicocele induction on the heat shock protein A2 (HSPA2) expression in rat's model.

Materials and Methods: Twenty Wistar male rats divided into two groups including; left varicocele and control group. Two month after the operation, all animals were killed and both testicles were harvested, weighed and sperm cells were collected from caudal left epididymis. Evaluation of HSPA2 expression was assessed by Western blot technique.

Results: Using specific antibodies and western blot analyses, we demonstrated that the expression of HSPA2 in left testis was significantly higher than right testis of varicocele-induced rats and also this parameter was insignificantly higher in testis of varicocele-induced rats compared to control (p>0.05). In addition, expression of HSPA2 was significantly higher in left caudal epididymis sperms compared to right caudal epididymis sperms (p<0.05).

Conclusion: The result of this study demonstrated that normal level of HSPA2 is essential for sperm maturation and function. With induction of varicocele in rat's model, level of HSPA2 has increased that, it is as indicator of activation a compensatory mechanism against induced heat condition by varicocele.

Keywords: Varicocele, Epididymis, Testis, HSPA2

P-12: Effect of Centrifugation on Levels of Stress Oxidative of Human Spermatozoa

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Background: Cryopreservation has adverse effect on human spermatozoa via exposure to physical and chemical stress that may lead to alter in reactive oxygen species (ROS) levels. During sperm preparation or cryopreservation, centrifugation as a physical stress may effect on sperm quality probably with stress oxidative production and membrane damage. Therefore the aim of this study was to evaluate the effects of centrifugation on stress oxidative level before and after cryopreservation.

Materials and Methods: This study was performed on 23

semen samples that were considered normal according to the world health organization (WHO) criteria. Fresh and frozen-thawed semen sample were divided into two equal portions. One portion was directly used for assessment of stress oxidative and named unwashed, while the other fraction was washed and labeled as washed group. Percentage of ROS-positive sperm were evaluated by H2DCFDA (2', 7'-dichlorodihydrofluorescein diacetate) probe.

Results: ROS-positive sperm were significantly higher in the washed groups compared to the unwashed groups before freezing (71.51 ± 3.68 vs. 61.66 ± 4.40 , $p=0.00$) and following freezing (58.01 ± 4.65 , vs. 45.99 ± 4.40 , $p=0.00$).

Conclusion: The results of this study revealed that centrifugation increase the percentage of ROS-positive sperm. These increase probably due to centrifugations force, removing antioxidants from semen plasma during the washing process or alter in membrane structure sperm.

Keywords: Centrifugation, Cryopreservation, Stress oxidative

P-13: Comparison of Sperm Quality, Oxidative Stress, DNA Fragmentation, Protamine Deficiency, and DNA Methylation in Varicocele and Fertile Individuals

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Background: There are many approaches that gene expression is controlled in eukaryotes. DNA methylation is one of several epigenetic mechanisms that cells use to control gene expression and lock genes in the "off" position. In addition, sperm DNA damage can correlate with DNA methylation defect. There is evidence that sperm of infertile men contain more DNA damage than fertile men and that may have a negative effect on fertility potential of these patients. Therefore, we aimed to evaluate and compare effective factors on fertility such as oxidative stress, DNA fragmentation, protamine deficiency, DNA methylation between fertile and varicocele individuals.

Materials and Methods: Semen samples were collected from 44 varicocele individuals with grad II and III and 15 fertile individuals who referred to Isfahan Fertility and Infertility Center. A portion of semen sample was used for routine semen analysis according to WHO criteria and the remainder were evaluated to DNA fragmentation by using TUNEL assay, oxidative stress by using DCFH-DA staining and protamine deficiency by using CMA3 staining. As well as global DNA methylation was evaluated by using flow cytometer.

Results: Quality of semen samples were significantly lower in the varicocele compared to fertile individuals. The percentage of DCF- positive sperm and TUNEL-positive and CMA3 positive were significantly higher in varicocele compared to fertile individuals however intensity of DCF in sperm were significantly lower in varicocele individuals compared to fertile individuals. Percentages of global DNA methylation significantly were higher in fertile compared to varicocele individuals.

Conclusion: This result clearly showed oxidative stress, DNA fragmentation, and protamine deficiency can effect on DNA methylation alternations. These deficiencies is higher in varicocele individuals compared to fertile that it is possibly due to spermiogenesis disorders in these individuals.

Keywords: Global DNA Methylation, DNA Fragmentation, Protamine Deficiency, Stress Oxidative, Varicocele

P-14: The Effects of Depression on Reduced Sperm Motility in Male Rats

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Background: Infertility is a problem for 15 to 30% couples. Male infertility may be a result of insufficient number of sperms and poor motility. Spermatozoon consists of a head, midpiece and tail. The long tail consist micro-tubules that from part of the propulsion system of the spermatozoon. Motility of sperm is the result of relation between ATP, micro-tubules and dynein. Depression is one of the most prevalent and disabling disorders, in the world. Depression can effect in fertilization by decreasing motility of sperm. Study there is any sperm motility dysfunction in stress induced depression.

Materials and Methods: 40 male sprague dawley rats entered the study. Half of them had unpredictable chronic mild stress (UCMS) for 21 days in order to get depressed. Forced swim test (FST) showed the immobility time (IB), the symptom of depression. One week after behavioral test, rats were prepared for transcardial perfusion. Then after paraffin, fixed brain was taken off and hippocampus was prepared for nissl staining. Fresh epididym were taken off and got homogenized and the supernatant was reserved for Real Time PCR evaluation. This evaluation how the level of dynein expersion. All above procedures were performed for control group too, instead of inducing UCMS.

Results: Our results showed low weigh gain and increased IB in UCMS groups ($p<0.05$). There was a higher concentration of serum IL6 in depressed groups in comparison to control one ($p<0.05$). Nissle staining showed a prominent neural degeneration in UCMS group hippocampus ($p<0.05$). At last RT PCR showed a lower permission of dynein gene in epididym ($p<0.05$).

Conclusion: Our studies showed different manifestations of depression after UCMS. It showed that UCMS can lead to mental depression. RT-PCR studies showed low expersion of dynein ($p<0.05$), which is equal to impairment of sperm motility. In this study we showed that depression can reduce sperm motility by low expression of dynein that existed in tail of sperm.

Keywords: Depression, Dynein, Sperm Motility

P-15: Effect of Testosterone on Antioxidant Biomarkers in Mice with Spinal Cord Injury

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Background: Spinal cord injury (SCI) is a traumatic or non-traumatic injury that can lead to loss of functions such as feeling or mobility along with permanent and debilitating influences on the patient's quality of life. Patients with SCI have weakened sexual function and a distinctive sperm profile described by normal to high sperm concentrations and unusually low sperm mobility. The reason for this condition is unknown but might be related to abnormalities in the seminal plasma. These abnormalities might be attributed to the existence of constant inflammatory process, producing cytotoxic agents in semen like reactive oxygen species (ROS).

Materials and Methods: In this study we tested the effects of exogenous testosterone on oxidative biomarkers in mice (14 groups) after SCI. Oxidative stress was evaluated by superoxide dismutase and glutathione peroxidase activity, protein carbonyl content, malondialdehyde (MDA) and total antioxidant capacity (TAC).

Results: After injury, administration of exogenous testosterone, resulted in decreasing the MDA levels, super oxide dismutase and glutathione peroxidase activity but not about protein carbonyl. The content of TAC remained unchanged except for shams and SCI groups without testosterone treatment, which showed remarkable increase. TAC was determined after administration of testosterone in SCI-operated groups. These results showed that administration of exogenous testosterone did not have positive effect on plasmatic TAC. Moreover, the serum testosterone was markedly decreased as the consequence of exogenous testosterone.

Conclusion: Therefore, administration of exogenous testosterone cannot offset sexual hormone tribulation along with anti-oxidative protective effects. These results, suggest that SCI can cause sterility via disturbance in plasmatic testosterone balance, which administration of exogenous testosterone was unable to restore the regular level of endogenous testosterone.

Keywords: Spinal Cord Injury, Infertility, Testosterone, Oxidative Stress, Reactive Oxygen Species

P-16: Effects of Urtica Dioica Extract on Histomorphometric Structure of Rat Testis Induced by High Dose Testosterone

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Background: Hormonal disorders such as hyperactivity of testosterone are one of the causes of infertility. In the present study, effects of Urtica dioica root extract on histomorphometric changes of rat testes induced by high dose testosterone was investigated.

Materials and Methods: For this purpose, 25 male mature wistar rats were divided in 5 groups, group 1 was control, group 2 received testosterone with dosage of 10 mg/kg subcutaneously, group 3 received solvent of testosterone (almond oil) with 10 mg/kg dosage subcutaneously, group 4 received Urtica dioica root extract in dosage of 50 mg/kg orally and group 5 received testosterone (10 mg/kg) along with Urtica dioica root extract (50 mg/kg) for 6 weeks daily. The rats were euthanized and testes were removed and the macroscopic parameters (weight and volume) measured, then, they were fixed in buffer formalin 10%. The 5-6 μ sections were made and stained with HandE. The micrometric measurement were done using Dino digital Lens and Dino software capture 1.

Results: The ratio of testis weight to body weight showed a

significant reduction in the testosterone group compared to Urtica dioica and testosterone+Urtica dioica groups ($p < 0.05$). Spermatogenesis evaluation indicated that testosterone group caused a significant increasing in RI compared to control ($p < 0.05$), Urtica dioica and almond oil groups ($p < 0.01$). TDI and SI increased in testosterone group compared to Urtica dioica group significantly ($p < 0.01$). The size of Leydig cells and thickness of germinal epithelium in testosterone+Urtica dioica group decreased compared to control group significantly ($p < 0.05$). The seminiferous diameter was not changed in different groups.

Conclusion: Urtica dioica has a significant effect on reducing testis weight and TDI2 and SI3 following testosterone inducing in high dosage.

Keywords: Urtica Dioica, Histomorphometric, Rat Testis, Testosterone

P-17: Body Mass Index and Sperm Counts Associated with Varicocele in Infertile Men

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Background: Varicocele is a progressive disease and etiology of varicocele is not well known. Some studies have been reported that prevalence of varicocele decreases with increasing BMI. The aim of this study was to determine interrelation of varicocele with height, body mass index (BMI) and sperm counts in infertile men.

Materials and Methods: We retrospectively evaluated the data of all patients who consulted for infertility at Fatemezhra Infertility and Reproductive Health Research Center for six months. We filled out a form providing the age of men, marital age, height, weight, BMI, semen analysis, and presence or absence of varicocele.

Results: The data of 241 men, aged 21-64 years, were included in the study. There were 94 (38.7%) men with varicocele and 149 (61.3%) men without varicocele. The mean age and BMI of the participants was 31.5 ± 6.3 and 27.7 ± 4.8 kg/m², respectively. There were no significant differences in age, height, weight, and BMI among the men infertile with and without varicocele. Percentage of varicocele was the highest in oligospermia males and significantly higher than the men with normal sperm count ($p = 0.035$).

Conclusion: The findings of the present study indicated that varicocele have some effects on sperm count as well as on pregnancy. The men with a greater BMI have not advantages in relieving the varicocele. Therefore, further study is proposed to elucidate association between BMI and varicocele.

Keywords: Infertility, Male Infertile, Varicocele, BMI, Sperm Count

P-18: The Effect of Cell Phone Waves and Severity Noise on Sperm Motility and Sexual Hormones in Male Rats

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Background: There is a great concern for the possible adverse effects of cell phones and noise waves and it looks these wave effects on the individuals' health. Thus, the present study was conducted to examine the effect of cell phone waves, severity noise, and simultaneous effect of cell phone waves and severity noise on sperm motility and sexual hormones in adult male rats.

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 wave group, exposure to noise group, and simultaneous exposure to cell phone simulated wave and sound group. The means of sperm motility in all groups were determined with criteria of WHO and sexual hormones concentration of blood plasma in all groups were determined by RIA. The results were analyzed by one-way ANOVA statistical technique followed by Tukey test using SPSS (version 16) software.

Results: Sperm motility in exposure to cell phone simulated waves and simultaneous exposure to cell phone simulated wave and noise groups decreased significantly compared with control group ($p<0.05$). The testosterone hormone concentration of blood plasma in all exposure groups decreased significantly compared with control group ($p<0.05$).

Conclusion: Exposure to cell phone waves and simultaneous exposure to cell phone simulated waves and sharp noise can cause a significant decrease in sperm motility and, also, can cause a significant decrease in testosterone hormone concentration in adult male rats resulting in abnormality fertility.

Keywords: Cell Phone, Sperm Motility, Sexual Hormones, Male Rat

P-19: The Protective Effect of Vitamin E against Damage Caused by Formaldehyde on The Sex Hormones Level and Sperm Parameters of Adult Male Rats

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Background: Formaldehyde (FA) is a suspected reproductive toxicant, which may cause significant adverse effects on human reproductive system. Given to its widespread use in occupational setting, and more recently, in several industries related to construction and household products, everyone may be exposed to FA. This study was designed to determine the effect of FA and to investigate possible protective role of vitamin E (VE) on male rat reproductive system.

Materials and Methods: 32 adult male wistar rats were randomly divided into 4 groups: (1) control rats, (2) Rats treated with vehicle (corn-oil), (3) rats treated with FA (FAt), and (4) rats treated with FA plus VE plus vehicle (FAt + V E) groups. FAt and FAt + VE groups were exposed to FA by intraperitoneal injection (10 mg/kg/day) for 2 weeks. Furthermore, FAt + VE group were orally administered VE (30mg/kg/day) simultaneously. After the treatment, serum luteinizing hormone (LH), Follicle-stimulating hormone (FSH) and Testosterone were examined. More over the count, morphology and motility of sperm, were observed.

Results: The quantity and quality of sperm was significantly decreased in FAt group compared with those in the control and vehicle-treated group. VE treatment significantly improved these parameters in FAt+ VE group. Moreover, in comparison with control, the serum testosterone level mildly decreased and the serum FSH and LH level mildly increased in the formaldehyde exposure groups, but no statistically significant difference was observed. VE treatment restored these hormone level changes at a level similar to the control and vehicle- treated group in FAt + VE group.

Conclusion: Our results indicate that Vitamin E appeared to ameliorate the adverse effects of FA on reproductive system of the adult male rat.

Keywords: Formaldehyde, Vitamin E, Rats, Reproductive Toxicity

P-20: Long-Term Effects of Dexamethasone on Rate of Epididymal Sperm and Fertility in Male Mice

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Background: Today, one of the world's most complex issues of fertility - infertility is becoming a concern. Under such conditions, evaluation mechanism and factors affecting fertility to control problem appears to be necessary and reasonable. Among these side effects of drugs should not be neglected. This study was designed to investigate the possible effects of Dexamethasone on rate of epididymal sperm and fertility in male mice.

Materials and Methods: Fifty matured male mice were divided into five groups including control, placebo and three treatment groups. Control group was no injected, placebo group received normal saline only and treatments were Dexamethasone (0.1, 0.5 and 1 mg/kg) which were injected in peritoneum every other day for a period of twenty days. After 20 days, two mice from each group were selected to measure fertility and each male with two females were kept for 15 days. After 15 days, the female mice were anesthetized and the number of embryos in the uterine horn was counted. However, epididymal sperm was counted in other mice by preparation epididymal suspension. The obtained data were analyzed using Duncan's multiple ranges test by SPSS program.

Results: Study sections of epididymal and sperm counts under the microscope showed that all doses of Dexamethasone significantly reduces the amount of sperm in these sections compared to control group ($p<0.05$). Also the fertility rate in all doses of Dexamethasone significantly decreased ($p<0.05$).

Conclusion: According to the evidence it can be said that since Dexamethasone is a synthetic Glucocorticoid and causes the immune system to be suppressed, cellular immunity is also reduced, so that, the cells become more sensitive to environmental factors and stress. Moreover, the level of apoptosis will be increased and causes impaired testicular function. Therefore, Dexamethasone has a negative effect on male reproduction.

Keywords: Dexamethasone, Epididymal Sperm, Fertility, Mice

P-21: Study of The Effect of Oral Zinc Supplementation on Superoxide Radical Scavengers in Spermatozoa of Patients with Asthenospermia

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Background: Oxidative stress and decreased antioxidant levels have been projected as a potential factors involved in the pathophysiology of diverse male infertility types, including asthenospermia. The generation of reactive oxygen species (ROS) in the male reproductive tract has become an actual concern because of their probable noxious effects, at high levels, on physical properties of sperm quality. ROS are extremely reactive oxidizing agents, which a member of the class of free radicals. The present study was conducted to study the effect of zinc supplementation on the quantitative and qualitative characteristics of semen along with oxido-sensitive index level in the seminal plasma of asthenospermic patients.

Materials and Methods: Semen samples were obtained from 60 fertile (age 31.6 ± 3.3 year) and 60 subfertile (age 32.5 ± 3.23 year) men with asthenozoospermia between July 2012 to July 2013, from couples who had consulted the infertility clinic of Babil hospital of maternity (Hilla city/ IRAQ). The sub-fertile group treated with zinc sulfate, every participant took two capsules of zinc sulfate per day for three months (each one 220 mg). Semen samples were obtained (before and after zinc sulfate supplementation). After liquefaction seminal fluid at room temperature, routine semen analyses were performed. Oxido-sensitive index level, catalase like activity and various sperm parameters were compared among fertile controls and infertile patients (before and after treatment with zinc sulfate).

Results: Compared with healthy controls, the value of the oxido-sensitive index was found to decrease significantly in seminal plasma and spermatozoa of patients with asthenozoospermia. Catalase-like activity is increased significantly in spermatozoa and plasma of patients with asthenospermia compared with that of healthy controls.

Conclusion: Zinc supplementation restores oxido-sensitive index and catalase-like activity in spermatozoa and seminal plasma of asthenozoospermic subjects to normal ranges. Volume of semen, progressive sperm motility percentage and total normal sperm count were increased after zinc sulfate supplementation.

Keywords: Zinc Supplementation, Oxidative Stress, Superoxide Radical Scavengers, Asthenozoospermia, Oxido-Sensitive Index

P-22: Protective Effect of Grapes Seed Hydroalcoholic Extract against Fluoxetine-Induced Fertility Toxicity in Male Mice

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Background: Fluoxetine, a selective serotonin reuptake inhibitor, has been prescribed for the treatment of depression and has shown also efficacy in the treatment of bulimia nervosa and obsessive-compulsive disorder. Grape seed extract is a natural extract from the seeds of grapes. It contains the most beneficial groups of plant flavonoids, proanthocyanidins oligomers. These flavonoids are potent antioxidants and exert many health-promoting effects. The present investigation was directed to study the possible chemoprotective activity of orally administered grape seed extract (GSE)

against Fluoxetine-induced fertility toxicity in male mice.

Materials and Methods: In this study, 24 mature male mice were used. The animals divided into four groups as, control, FLX (20 mg/kg), FLX (20 mg/kg) + GSE (100 mg/kg) and GSE (100 mg/kg) orally for 42 days. The testis tissue were obtained on day 42 in all groups. Leydig cell and Mononuclear leukocytes number (MNLN) and weight of body mice and testis tissue were measured.

Results: The results revealed that the male mice exposed to fluoxetine had significantly ($p < 0.05$) reduced in (LCN) and significantly ($p < 0.05$) increased in (MNLN). Also weight of body and testis decreased significantly ($p < 0.05$).

Conclusion: Fluoxetine can cause infertility in male mice, by the destruction of testicular tissue, Grape seed extract combined with fluoxetine can reduce the negative effects of fluoxetine.

Keywords: Fluoxetine, GSE, Mice, Leydig Cell

P-23: The Protective Effect of Ziziphora Tenuior Hydro Alcoholic Extract on Spermatogenesis in Mice

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Background: Spermatogenesis is the major factor of male fertility which is pendent on hormones specially testosterone. Testosterone signalling leads to activation of some kinases which are required for progression and maintaining of spermatogenesis. On the other hand, oxidative stress, as the most prevalent factor, influences on fertility. Also, it was shown that chronic oxidative stress in rat testis is irreversible. Ziziphora tenuior extract has anti-oxidant, anti-inflammation and anti-tumorigenic properties. Therefore, in the present study the protective effect of Ziziphora tenuior extract on sperm quality and serum testosterone level were evaluated.

Materials and Methods: Thirty NMRI male mice were categorized to five random groups (n=6) including negative and positive controls which received IP injection of distilled water and formaldehyde, as an oxidative stress inducer factor, respectively. The other experimental groups were received IP injection of formaldehyde plus 50, 100 and 150 mg/kg of Ziziphora tenuior hydro-alcoholic extract, respectively. This trial were continued for 35 days. Then sperm evaluation parameters (count, viability and motility) and serum testosterone level were assessed.

Results: Ziziphora tenuior extract with dose of 50 and 100 mg/kg increased testosterone level in comparison with group which had received formaldehyde. Also, sperm count, motility and viability were improved. Although the extract with the dose of 150 mg/kg, caused to more testosterone secretion and better results than negative control group, its effects were lower than the other experimental groups, so this dose may result to some adverse effects.

Conclusion: Ziziphora tenuior extract with lower doses can provide protective effects against oxididative stress of testis and results to more testosterone secretion and improved spermatogenesis but with higher dose although it is effective but it is not so safe.

Keywords: Ziziphora Tenuior, Spermatogenesis, Testosterone, Testis, Formaldehyde

P-24: Beta Defensin 126 and Its Role in Intra-Uterine Insemination Outcome

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Background: Human β -defensin 126 is a small cationic glycoprotein that coats the plasma membrane of sperm during epididymal transit. It provides protection for sperms from infection-causing microbes and against the female immune system. Defensin remains on the sperm until sperm become capacitated in the female reproductive tract. DEFB126 gene is located on the subtelomeric end of 20p13 in human. DEFB126 variations were found to affect the functionality of sperm cells and thus may have an important role in the success or failure of intra-uterine insemination (IUI). Therefore, this study was designed to verify the correlation of this gene mutation with the success rate of IUI.

Materials and Methods: Two cytosine nucleotide deletion of DEFB126 gene in the blood of 76 Iranian men with unexplained infertility whose wives had undergone IUI were assessed. Standard PCR and Single-strand conformation polymorphism (SSCP), Tetra PCR and Sequencing were used to confirm the results. ELISA and Immunochemistry were performed for the assessment of protein expression on sperm cells.

Results: Data revealed that 24.4 percent of men, whose wife showed a negative result for IUI, were homozygote for this mutation ($p \leq 0.05$) and couples with positive IUI result showed no mutation in this gene. Also, DEFB126 protein was not observed in men who were homozygote for this mutation.

Conclusion: The present study suggested that this common alteration in DEFB126 could be considered as a critical factor in the success rate of IUI operation. It is concluded that men with a homozygote mutation for DEFB126 are less fertile compared to those with a wild type DEFB126 for this mutation.

Keywords: β -defensin 126, IUI, Glycocalyx, Spermatogenesis

P-25: Effect of Water with Different Temperature with or without Forced Swimming on Sperm Parameters in Adult Mouse

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Background: Sports, hypothermia and hyperthermia as some stressors inhibit male reproductive functions and are associated with subfertility or infertility. We investigated whether water temperature and chronic swimming are effective on mouse sperm parameters.

Materials and Methods: Adult male mouse (N=35) were randomly divided into 7 groups: 1-controls 2-cold water (10°C) with

swimming 3- cold water (10°C) without swimming 4- lukewarm water (23°C) with swimming 5- lukewarm water (23°C) without swimming 6- warm water (36°C) with swimming 7- warm water (36°C) without swimming. All animals in swimming groups were submitted to forced swimming in a pool for 5 minutes daily for 5 days/week during five weeks. However the groups of 3, 5 and 7 were just standing on a raised platform inside the water in pool without swimming for the same time and duration. Sperm analysis was performed after 35 days from tail part of epididymis. Analyses included sperm count and morphology of head, mid piece and tail, were assayed using a hemocytometer slide and Papanicolaou staining, respectively. Data were statistically analyzed with ANOVA and post Hoc Tuckey test.

Results: Cold water (10°C) with or without swimming had no effect on sperm counts. However, the percent of abnormal tails increased significantly when compared with the controls, ($p < 0.05$). Warm water (36°C) statistically reduced sperm counts and increased abnormal morphology in mid piece and tail in both groups of with or without swimming ($p < 0.05$) when compared with controls. Warm water without swimming increased the percent of abnormal head too. Lukewarm water without swimming had no effect on both sperm counts and morphology; however, lukewarm water with swimming increased sperm abnormal tail morphology ($p < 0.05$).

Conclusion: This study indicates lukewarm water itself and without swimming doesn't have adverse effect on sperm parameters; however, swimming for chronic period in different degrees of water may disrupt mouse sperm parameters and fertility.

Keywords: Cold, Lukewarm, Warm Water, Sperm Parameters, Swimming

P-26: Effect of Genistein on Morphine-Induced Injury on Sperm Parameters

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Background: An estimated six percent of adult males are thought to be infertile. The opioid peptides, in particular enkephalins, are implicated in pain perception, in response to stress, and in reinforcement. Many studies have been performed on morphine and its deleterious effects on different parts of the body and reproductive system of animals and human. Soybean, widely used in animal and human nutrition is rich in phytoestrogens and especially in genistein. The aim of present study was to determine Effect of genistein on morphine-induced injury on sperm parameters.

Materials and Methods: In this experimental study 40 male rats with 28 weeks of age and limited weight of 220 to 250 grams were selected. They were divided into five groups of 8, untreated control group; morphine-treated group (2.5 $\mu\text{g}/\text{kg}/\text{day}$); genistein -treated groups (1, 2, 4 mg/kg/day); and morphine and genistein treated group interperitoneal administration for successive 28 days. After 24 hours animal were killed Sperm count and viability were measured through WHO protocols. Then data were analyzed by computerized statistical program (one-way ANOVA). In all cases $p < 0.05$ was considered as the minimum level of significant differences among groups.

Results: The results indicated that increasing the dose of genistein significantly increased reproductive indices such as sperm count, motility and testis weight in most of the groups ($p \leq 0.05$). In contrast the showed data in morphine intoxicated group showed significant and decrease in sperm count, motility and testis weight.

Conclusion: These findings imply that genistein can significantly improve the spermatogenesis process in rats and suggest its po-

tential healing properties to reduce the effects of morphine in humans.
Keywords: Genistein, Morphine, Reproductive Parameters, Male Rat

P-27: The Effect of Varicocele on The Serum Testosterone Level in Infertile Men with Grade (II - III) Varicocele

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Background: To determine whether men with varicoceles have lower testosterone levels than those without and to ascertain if testosterone levels increase after varicocelectomy and or to determine whether the varicocele grade is related to the degree of improvement in serum testosterone levels after varicocelectomy and also older age is associated with improvements in testosterone after varicocelectomy.

Materials and Methods: We measured preoperative testosterone levels in 70 men with palpable varicoceles (grade II and III) and in 79 fertile men who served as a comparison group and patients were categorized into two age groups (Less than 35 years, More than 35 years). The testosterone levels between groups were compared by age. 70 had data on both pre- and post-operative testosterone levels, which were compared to assess postoperative changes.

Results: A total of 79 patients had undergone varicocelectomy. The mean of serum testosterone levels before surgery in infertile men with varicocele and fertile men were respectively 590 (230) versus 583(237) ng/dl (p=0.70). No statistically significant changes were noted in serum testosterone levels for any of the groups. Mean serum testosterone levels were significantly increased in infertile men with varicocele compared with preoperative levels. The serum testosterone levels increased after repair from 590 (230) to 663 (242) ng/dl (p=0.009). This difference persisted when analyzed by age. These groups of patients were categorized into two age groups. Group I, less than 35 years (<=35), and group II, more than 35 years (>35). Also the testis volume of patients has been examined which were divided into two groups included the men with testis volume less than 16ml (<16) and more than 16ml (>=16).

Conclusion: Varicocelectomy resulted in significant increase in the serum testosterone level and improved testicular leydig cell function.

Keywords: Androgen, Testosterone, Varicocele, Varicocelectomy, Infertility

P-28: Effects of Resveratrol on Nicotine Toxicity of The Spermatogenesis of Rats

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Background: Nicotine is a major toxic component of cigarette smoke and it is a major risk factor in the development of functional disorder of several organ systems. Resveratrol (trans-3,5,4'-trihydroxystilbene; RES) is a naturally occurring polyphenol synthesized by a variety of plant species in response to injury, UV irradiation and fungal attack. It is present in grapes, berries, peanuts. The aim of present study was to determine resveratrol effect on Nicotine Toxicity of the Spermatogenesis of Rats.

Materials and Methods: Fifty mature rats of Wistar race were divided into 5 groups of ten as experimental and control groups. Resveratrol and nicotine were given to 50 rats for a period of 28 days. The rats were weighted and after anesthesia, their epididym was taken out and tissue dissections were obtained. Data was analyzed by one-way ANOVA and p<0.05 was considered significant.

Results: The showed data in nicotine intoxicated group showed significant and decrease in sperm motility. In contrast, resveratrol significantly improved sperm motility in groups the nicotine.

Conclusion: The findings refers that resveratrol can significantly improve the spermatogenesis process in rats and is suggested that suggest its potential properties to reduce the side effects of nicotine in humans.

Keywords: Resveratrol, Nicotine, Spermatogenesis, Rat

P-29: Study of The Environmental Effects of EDCs, Perfluorooctanoic Acid and Diethylstilbestrol on Human Reproductive Parameters and Fertility Outcomes

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Background: There is an important evidence that prolonged exposure to several endocrine disrupting chemicals (EDCs). These are persistent materials with unique environmental properties such as intake of contaminated food, water and air, irrespective of proximity to industries, which are suspected to have endocrine disrupting activities. Perfluorooctanoic acid (PFOA) and diethylstilbestrol (EDCs) are risk factors for reduced fertility in men.

Materials and Methods: Studies were found by searching the PubMed database for articles published up to 2013. The PubMed database on associations between PFOA and DES exposure men reproductive health are summarized and classified as fertility outcomes, transgenerational exposure and effects.

Results: Many studies on EDCs such as PFOAs and DES are not always consistent in part due to limitations imposed by practical constraints. These results are discussed in the context of EDCs properties of PFOAs and DES substances that have been characterized in human studies. In order to make progress in this filed, we recommended taking advantage of monitoring and biobanks, including

the development of appropriate biomarkers. Further human studies are warranted with particular focus on impaired fertility. They affect reproductive hormone levels, testicular volume, and semen quality. Each man provided a semen sample and a blood sample. Semen samples were analyzed for sperm concentration, total sperm count, motility, and morphology, and blood samples were used to measure reproductive hormones.

Conclusion: The results suggest that exposure to PFOA and DES may affect human male semen quality and reproductive hormone levels, in other hand a detailed appraisal compounds specifically related to adverse reproductive outcomes is significant for prevention.

Keywords: Endocrine Disrupting Chemicals, Fertility, Semen Quality, PFOA, EDCs

P-30: The Effect of The T26248G Polymorphism on Putative Methyltransferase Nsun7 Protein Function and Its Role in Male Infertility

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Background: Male infertility has many causes, including genetic infertility. The NOP2/Sun domain family, member7 (Nsun7) gene, which encodes putative methyltransferase Nsun7, has a role in sperm motility. The aim of the present study was to investigate the effect of the T26248G polymorphism on Nsun7 protein function and its role in male infertility.

Materials and Methods: Semen samples were collected from the IVF centre and exon7 was amplified using forward and reverse primers. Bands on samples from oligoasthenospermic men that exhibited different patterns of movement on single-strand conformation polymorphism (SSCP) gels compared with normal samples were identified and subjected to sequencing. Results were investigated with informatics tools.

Results: Direct sequencing of PCR products, along with their analysis, confirmed T26248G-transversion mutation in oligoasthenospermic men. Comparison of normal and mutant protein structures of Nsun7 indicated that the amino acid serine was converted to alanine, the structure of the helix, coil and strand was changed, and the protein folding and ligand binding sites were changed, indicating impairment of protein function.

Conclusion: The T26248G is a sense mutation, which is apparently correlated with changes in the physicochemical properties of the Nsun7 proteins (e.g. molecular weight, aliphatic index, instability, hydrophobic index, protein folding) and sperm motility in infertile men. Furthermore, changes in binding energy compared with normal result in changes to ligand-protein interactions and impair methyltransferase activity. Molecular docking studies with residues and ligand in the binding cavity of the Nsun7 protein showed that protein from normospermic men has a better re-rank score, docking score, hydrogen bonding energy and ligand-protein interaction energy and, as such, has a more favorable interaction compared with proteins from infertile men. Because Nsun7 has a role in mitochondrial rRNA processing in post-meiotic spermatozoa, protein dysfunction leads to a general lack of translation of mRNA and proteins needed for sperm motility, causing sperm motility deficits and male infertility.

Keywords: Male Infertility, Genetic Infertility, Folding Protein, Nsun7 Gene

P-31: The Alteration of Spermatogenesis Has A Correlation with Sertoli Cell Mitochondrial Abnormal Morphology in Cytotoxicity of Testicular Tissue Mediated with Monosodium Glutamate

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Background: Monosodium glutamate (MSG), L-form of glutamic acid, is a food additive which acts as preservative or enhancer of palatability. MSG has toxic effects on human and animal's tissues. Some neurotoxic alterations such as brain damage and endocrine disorders have been seen after administration of MSG. Male infertility, obesity and hypogonadism has been reported following administration of MSG. Testicular hemorrhage and alteration of sperm production and morphology are the most reported changes in cases of male infertility after administration of MSG. According to the role of Sertoli cells in normal spermatogenesis, the aim of this study was to find a correlation between microscopic changes of spermatogenesis and the ultrastructure of Sertoli cells following treatment with MSG.

Materials and Methods: Monosodium glutamate was dissolved in distilled water and administered (4 ml/Kg body weight, IP) for 28 days. For electron microscopy, each testis was separated from its adjacent epididymis and cut into small pieces and fixed in 3% glutaraldehyde. The ultrathin sections were mounted on copper grids and stained with uranyl acetate and lead citrate and observed with a Philips C-100 Bio transmission electron microscope.

Results: The ultrastructural study of Sertoli cells showed that, these cells had remarkable alterations in their fine structure. The oval-shape nuclei became irregular and some abnormalities were seen in cytoplasmic organelles including mitochondria and endoplasmic reticulum. The most remarkable changes in cellular organelles were observed in mitochondria. Normally formed mitochondria of the vesicular type were transformed to giant organelles with irregular cristae. These organelles reduced in number and were arranged in screwed and/or doughnut-shaped pattern.

Conclusion: The findings of this study indicate that, long term administration of MSG can adversely damage the ultrastructure of Sertoli cells. These changes have a direct relationship with alteration of spermatogenesis and subsequently lead to abnormal development of spermatozoa.

Keywords: Mitochondria, Monosodium Glutamate, Sertoli Cells, Spermatogenesis, Testis

P-32: Histological Study of Cauda Epididymis in Adult Rats Treated with Methyphenidate

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Background: Methylphenidate commonly known as Ritalin is one of the most common medications. The administration of Ritalin leads to increase of the activity of central nervous system. Because of this property, Ritalin may be used for maintaining alertness and improving of attention which may lead to increase of the risk of substance abuse in some cases. There is a little data about the effects of long term treatment with Ritalin on body organs involved in fertility ability. This study was designed to investigate the time dependent effects of MPH on the activity of male reproductive system.

Materials and Methods: Methylphenidate was administered to adult rats (10 mg/kg/day) in two experimental groups for period of two and eight weeks. Histological study was performed on the cauda epididymis for evaluation of microscopic changes.

Results: The administration of methylphenidate for eight weeks caused some alteration in epididymis tissue such as transformation of nucleus, reduction of cilia and decrement of height of epithelial cells of ducts. Severe reduction of epididymal sperm population and increment of connective tissue volume also was seen. The above mentioned changes were seen in other treatment group but in lower intensity.

Conclusion: The findings of this study indicate that, long term administration of methylphenidate can adversely damage the male fertility due to impairment of structure and function of male reproductive system.

Keywords: Cauda Epididymis, Histology, Methylphenidate, Rat

P-33: Epididymal Sperm Analysis after Time Dependant Administration of Methylphenidate in Adult Male Rats

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Background: Methylphenidate is one of the most common medications used for treatment of attention-deficit/hyperactivity disorder. It may be used for maintaining alertness and improving of attention which may lead to increase of the risk of substance abuse in some cases. The occurrence of alteration in male reproductive system such as hormonal secretion changes and alteration of testicular spermatogenesis has been reported. The aim of this study was to evaluate the effect of methylphenidate related spermatogenic alteration on sperm production.

Materials and Methods: Methylphenidate was administered (10 mg/kg/day) for period of 14 and 56 consecutive days. For analyses of sperm, the cauda epididymis was separated from testis and cut into small pieces in one milliliter of Ham's F10 culture medium. The epididymal sperm count was evaluated by hemocytometer with light microscope. Sperm motility was assessed with phase contrast microscope at 400× magnification. In an average ten microscopic fields were observed and the mean of counted sperms was considered as sperm motility for each rat. To estimate the percentage viability, a volume of 20 µl of sperm suspension was mixed with an equal volume of 0.05 percent eosin-Y. Two hundred sperms were considered for calculating the indices for the experimental groups.

Results: All indices of sperm analysis were reduced significant after 56 days administration of methylphenidate. The administration of this drug for 14 days was not altered sperm analysis indices significantly.

Conclusion: The results of this study showed that the reduction of sperm analysis indices following long term use of methylphenidate has a

direct relationship with microscopic changes of spermatogenesis.

Keywords: Epididymis, Methylphenidate, Rat, Spermatozoa

P-34: Expression of Toll-like Receptor 2-3 Genes in Sertoli Cells of Patients with Azoospermia

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Background: Toll-like receptors (TLRs) constitute a major part of innate immunity, which can distinguish pathogen associated molecular pattern. Sertoli cells create a special immunological niche that protects somniferous tubules from auto antigens and pathogens. These cells are the only somatic cells in somniferous that protect testis cells against pathogens. The purpose of this study was to evaluate the expression of TLR 2 and TLR3 in sertoli cells patient Azoospermia.

Materials and Methods: Biopsies were obtained from 10 men who underwent TESE. All men taking part in this study had no history of infection and congenital disorders. Tissue samples were transferred to lectin coated Petri dishes after enzymatic dissociation and isolation. After few passages, all the cells were harvested and sorted by flowcytometry. Then, TLR gene expressions were determined by RT-PCR.

Results: Isolation, purification and culture of the human sertoli cells were performed successfully. Also, it was shown that TLR 2 and TLR3 genes are expressed in these cells. In addition, it seems that TLR3 is expressed more than TLR2.

Conclusion: This results showed that the expression of TLR genes in human sertoli cells specially TLR 2-3, could play an important role in developing immunity against pathogens as well as allo and auto-antigens in somniferous tubules.

Keywords: Sertoli Cell, TLRs, Lectin, Cell Culture, Sorting Flowcytometry

P-35: Celecoxib and Silymarin Ameliorated The Varicocele-Induced Inflammation and Oxidative Stress; Evidence for CoxII, iNOS and Enzymatic Antioxidant Status

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Background: Varicocele (VCL) exerts its impact via enhancing inflammation and down-regulating testicular endocrine and antioxidant statuses. Celecoxib (CCB) is a non-steroidal anti-inflammatory drug (NSAID), which its effects based on the inhibition of cyclooxygenase (COX) enzymes. On the hand, silymarin (SMN) is known for its remarkable anti-oxidative impact. Therefore, present study was designed in order to evaluate the protective effects of CCB and SMN on VCL-induced biochemical derangements in testicular tissue.

Materials and Methods: VCL was induced in 24 mature male rats and then animals were randomly divided into four groups including; non-treated VCL-induced, CCB-treated (10 mg/kg, orally), SMN-treated (100 mg/kg, orally), SMN+CCB-treated groups. Following 60 days after VCL induction, the mRNA levels of CoxII and iNOS were assessed by using semiquantitative real time-PCR. Moreover, histological alterations and germinal cells RNA damage were analyzed by using light and epifluorescent microscopy, respectively. Finally, the tissue levels of glutathione peroxidase (GSH-px), superoxide dismutase (SOD) were evaluated.

Results: CCB and SMN- especially in simultaneous form of administration- significantly ($p < 0.05$) reduced VCL-induced tubular atrophy, connective tissue edema, germinal epithelium dissociation as well as RNA damage. RT-PCR results showed that CCB and CCB+SMN-treated groups showed significant ($p < 0.05$) reduction in mRNA levels of COXII and iNOS versus non-treated VCL-induced group. Meanwhile, animals in SMN alone and SMN+CCB-treated groups showed remarkable ($p < 0.05$) enhanced levels of GSH-px and SOD.

Conclusion: Our data suggest that, CCB and SMN via two different mechanisms diminished VCL-induced damages. Accordingly, CCB by down-regulating the inflammation and SMN by up-regulating the enzymatic line of antioxidant capacity protected RNA contents of germinal cells. Ultimately, CCB and SMN are suggested as appropriate compounds for medical applications in VCL.

Keywords: Varicocele, Celecoxib, Silymarin, COXII, iNOS

P-36: Evaluating The Effect of Lactobacillus Acidophilus and Bifidobacterium on Spermatogenesis Index of Testicular Tissue in Isoniazid-Induced Rats

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Background: Isoniazid is an important drug in first line of treating the tuberculosis disease. This drug can lead to damages in different parts of the body such as liver and testis. Lactobacillus acidophilus and Bifidobacterium are the probiotics that have the important innate property that inhibit from passing of Isoniazid into the blood-testis-barrier. In point of spermatogenesis indexes which includes tubular differentiation index (TDI), spermatogenesis index (SI) and repopulation index (RI) were studied.

Materials and Methods: In this study, 240 male adult Albino rats with 15 rats per each cage and four replicate for each group were used. The groups were as followed: 1. control group (Normal saline 10 ml/Kg orally); 2. Isoniazid group (were injected 50 mg/Kg IP followed for 3 weeks); 3. Isoniazid with probiotics (were injected Isoniazid 50 mg/Kg IP; administration: Lactobacillus acidophilus 2×10^9 CFU/ml; Bifidobacterium 1×10^8 CFU/ml orally followed for 3 weeks); 4. Probiotics group (administration: Lactobacillus acidophilus and Bifidobac-

terium). The testis tissues was sampled after passing the mentioned time and section providing was stained by H and E.

Results: The results showed that Lactobacillus acidophilus with Bifidobacterium significantly decreased ($P < 0.05$) RI, SI and TDI indexes compared with control group. Consumption of Isoniazid without Lactobacillus acidophilus and Bifidobacterium significantly increased RI, SI, and TDI ($P < 0.05$) compared with Isoniazid with these probiotics administered groups.

Conclusion: Our results showed that RI, SI, and TDI were significantly changed after Isoniazid and Lactobacillus acidophilus consumption. These results are caused by reducing the serum testosterone concentration and the effect of Isoniazid and Lactobacillus acidophilus with Bifidobacterium in penetration of the blood-testis-barrier. It suggests the effect of Lactobacillus acidophilus and Bifidobacterium with Isoniazid on spermatogenesis index and infertility in human which needs more investigation.

Keywords: Probiotic, Isoniazid, Spermatogenesis Index, Testis

P-37: (+)-Pulegone Reduced Cyp19 Gene Expression and Reduced Leydig Cells Steroidogenic Activity

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Background: Pulegone (PGN) is a clear colorless oily liquid, which is widely used in different areas for flavoring foods, drinks, and dental products. There are several reports indicating that the metabolites of the PGN affect cytochrome-P450 family expression and activities. The cytochromes-P450 (cyp19) are involved in Leydig Cells aromatization. Therefore, present study was performed in order to evaluate the PGN-induced changes in cyp19 gene expression and Leydig cells steroidogenesis activities.

Materials and Methods: Twenty four mature mice were randomly divided into four groups as control-sham (received 0.3mL normal saline, ip) and test groups including: low dose (25 mg/kg, ip), medium dose (50 mg/kg, ip) and high dose (100 mg/kg, ip)-PGN-received groups. After 35 days, the Leydig cells steroidogenic activity was assessed by using special staining for steroid foci under epifluorescent microscope assay. The mRNA level of cyp19 was evaluated by using semi-quantitative RT-PCR.

Results: Observations showed that Medium and high dose administration of PGN reduced mRNA expression of cyp19. However, the animals in low dose PGN-treated group showed increased level of cyp19. Moreover, the animals in PGN-treated groups exhibited a remarkable reduction in number of Leydig cells/one mm² of connective tissue with intra-cytoplasmic steroid foci. Accordingly, the animals in high dose PGN-treated groups showed the lowest reaction sites for intra-cytoplasmic steroids.

Conclusion: PGN negatively impacted the Leydig cells aromatization that in turn down-regulated the testicular endocrine status.

Keywords: Pulegone, Steroidogenic Foci, Leydig Cells, Cyp19

P-38: Effect of Systolic and Diastolic Blood Pressure on Sperm Parameters in Men Attending to Infertility Clinic

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Background: It is shown that hypertension may affect androgens. The role of blood pressure on sperm parameters is matter of debate. The main goal was to evaluate the probable association between systolic and diastolic blood pressure and sperm parameters.

Materials and Methods: Semen Sample of men (aged: 27-45 years old) from 40 infertile cases who attended at Yazd research and clinical center for infertility work-up. Subjects with history of varicocele as well as advanced age and smokers were excluded from the study. The systolic and diastolic blood pressure of men were taken. Semen analysis was done according to WHO guidelines. Sperm count and motility were assessed using Makler chamber. Papanicolaou staining method was applied for evaluation of sperm morphology.

Results: The mean age of men was 31.5 ± 4.1 . The data showed that there is a significant positive correlation between systolic blood pressure and sperm count (Pearson Correlation: 0.3, $p=0.049$) and progressive motility (Pearson Correlation: 0.3, $p=0.02$). There was insignificant correlation between systolic blood pressure and total motility and normal sperm morphology as well. Not any significant correlations were found between diastolic blood pressure and sperm parameters.

Conclusion: It seems that cardiovascular system may affect male reproductive system via alteration in testicular vessels. More studies are required to draw final conclusion.

Keywords: Systolic Blood Pressure, Diastolic Blood Pressure, Sperm Parameters

P-39: Toxic Effect of Acyclovir on Reproductive System in Male Rats

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Background: Acyclovir (ACV), a synthetic purine nucleoside analogue derived from guanosine, is known to be toxic to gonads.

Materials and Methods: In this experimental study, forty adult male Wistar rats (220 ± 20 g) were randomly divided into five groups (n=8 for each group). One group served as control and one group served as sham control [distilled water was intraperitoneally (i.p.) injected]. ACV was administered intraperitoneally in the drug treatment groups (4, 16 and 48 mg/kg/day) for 15 days. Eighteen days after the last injection, rats were sacrificed by CO₂ inhalation. After that, cauda epididymides were removed surgically. Histopathological and histomorphometrical analysis of the testis was carried out. At the end, sperm concentrations in the cauda epididymis, sperm motility, morphology, viability, chromatin quality, and DNA integrity were analyzed. Serum levels of testosterone and Lipid Peroxidation were evaluated.

Results: Male rats exposed to ACV had significant reduction in serum testosterone concentrations at 16 and 48 mg/kg dose-levels.

ACV induced histopathological changes in the testis and also increase the mean number of mast cells in peritubular or interstitial tissue in the testis at 16 and 48mg/kg dose-levels. In addition ACV caused increase of serum level of Lipid Peroxidation at 48mg/kg dose-level. ACV did not affect sperm count, but decreased sperm motility and sperm viability at 16 and 48 mg/kg dose-levels. Sperm abnormalities increased at 48 mg/kg dose-level of ACV. Further, ACV significantly increases DNA damage at 16 and 48 mg/kg dose-levels and chromatin abnormality at all doses.

Conclusion: The present results highly support the idea that ACV has adverse effect on the reproductive system in male rat.

Keywords: Acyclovir, Testis, Sperm Parameters, Testosterone, Rat

P-40: Characteristics of Gonadotrophins in Azoospermic Patients in Plateau State, Nigeria

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Background: Azoospermia is the total absence of sperm cells from a patients semen sample after centrifugation. It could be obstructive or non-obstructive and its associated causes include chromosomal defects, infections, gonadotrophins imbalance, and etc in the patients. The objective is to determine the prevalence of primary testicular failure, germinal insufficiency, obstructive azoospermia and non-azoospermia in azoospermic patients in Plateau State, Nigeria.

Materials and Methods: 73 azoospermic male patients of ages between 25-45 years old attending the infertility clinic of the Center for Reproductive Health Research, (CRHR) Jos, Nigeria, from 2008-2009 were selected for the study. Personal history were taken. Semen samples were collected after four days of abstinence and analyzed using 1999 WHO manual for semen examination. Subsequently, their 5 ml of blood samples were collected for follicle Stimulating Hormone (FSH), Luteinizing Hormones (LH) assays and using enzyme immunoassay method at 550/492nm.

Results: 73 azoospermic patients' semen and blood for FSH, LH assays were studied. 30(41%) had increased FSH and LH, 37(51%) had increased FSH and normal LH, and 6(8%) had normal FSH and LH. Also, mean increased of FSH and LH are 48.29 and 31.08($p<0.00$), mean increased of FSH and normal LH are 27.85 and 8.66($p<0.00$), and means for normal FSH and LH are 4.28 and 5.78 ($p<0.01$).

Conclusion: 92% Of studied azoospermic patients had gonadotrophins imbalance and this contributed to 41% primary testicular failure and 51% germinal insufficiency in non-azoospermic patients. Furthermore, 8% azoospermic patients had normal gonadotrophin levels which is associated with obstructive azoospermia. Endocrinopathies are common causes of azoospermia and contributed to increased percentage of primary testicular failure and germinal insufficiency in azoospermic patients in Plateau State, Nigeria. The main reason for this is not known but it could be to environmental factors, endocrine disruptors and genetic polymorphism.

Keywords: Azoospermia, Gonadotrophins

P-41: Effect of GnRH on Vincristine-Induced Spermatogenic Defects in Basal Layer

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Background: Male factors, mainly spermatogenesis disorder, are responsible for 20-30% of infertility occurred in different societies. One of the known causes of spermatogenesis disorder is chemotherapy in patients with cancer. The side effect of chemotherapeutic 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, and (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 prepared for LM, and half of testicular specimens were fixed in 2% glutaraldehyde and prepared for EM studies. The thin sections were studied with LEO 906 TEM.

Results: Observation with optic microscope in (V) group beside control group showed that thickness of germinal epithelium was reduced a lot, also the electron microscopic observations in (V) group showed that germinal cells separate out of basal lamina (BL) and exist same irregularity waviness and thickening in basal layer. Observations LM, EM in (V+C) group showed that thickness of germinal epithelium approximately 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: Anticancer Drug, GnRH Antagonist, Basal Layer (BL), Spermatogenesis

P-42: Declining Trends in Sperm Quality of Infertile Tunisian Men: A Possible Role of Genital Tract Infections

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Background: Declines in semen quality over time are not well understood. It is suspected that infectious disease may affect reproductive function. The aim of this study was to investigate semen quality in men in infertile relationships who were under investigation for couple infertility and attended a clinic for routine semen analysis over a period of 15 years.

Materials and Methods: We investigated trends in semen parameters between 1996 and 2012 in the Sfax area of southern Tunisia in a sample of 4117 men in infertile relationships. Age at semen collection, duration of sexual abstinence, volume of seminal fluid, the sperm count, percentages of motile and morphologically normal spermatozoa, and semen leukocyte concentration were determined. Linear regression was used to examine trends over time in sperm count, sperm motility,

normal morphology, and semen leukocyte concentration.

Results: Mean age and semen volume did not change between 1996 and 2012. Data adjusted for age and abstinence showed a decreasing trend in sperm count and percentage of normal morphology over the last 12 years [(R(2) = 0.8, p=0.0001, and R(2) = 0.8, p<0.0001, respectively)]. There was no significant change in sperm motility. However, semen leukocyte concentration increased significantly over time [(R(2) = 0.43, P =.01)].

Conclusion: These results coincide with the high prevalence of genital infectious diseases in the Sfax area suggesting that infection of the accessory glands lead to decreased sperm counts by functional impairment or partial occlusion of the seminal ducts. Thus, if in the general population declining sperm counts are observed, genital tract infections may be suspected as a contributing factor.

Keywords: Male Infertility, Trend, Semen Quality, Leukocyte

P-43: Stereological Evaluation of Induction of Azoospermia after The Using Busulfan in Albino Hamster Testes

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Background: Busulfan is an alkylating agent, which inhibits cell dividing by sticking to one of the DNA strands. The aim of the present study was establishment and stereological evaluation of testes of azoospermic animal model using busulfan in albino hamster.

Materials and Methods: Male adult albino hamsters were randomly assigned into 3 groups. The first group was injected one dose of busulfan (10 mg/kg, intraperitoneally, Busilvex®, France) and their testes were removed on day 35 post injection. The second group received two doses of busulfan with 21 days interval and their testes were removed on day 35 after the second injection. The testes of control group were removed without busulfan therapy. The histopathologic sections (5 µm thickness) were stained with hematoxylin-eosin. In 10 circular transverse sections of tubules, inner, outer and total diameters, calculated areas of the cellular and luminal regions and cross sectional area of the tubule, number of profiles of seminiferous tubules per unit area of testis, and numerical density of the seminiferous tubules using a systematic random scheme were measured. The testes were rated for its spermatogenic potential on a modified spermatogenic scale of 0 to 5. The data of stereological indices of seminiferous tubules were analyzed by one-way ANOVA and LSD post-hoc test (SPSS 11.5). The spermatogenesis index of seminiferous tubules was compared using Mann-Whitney U test.

Results: Lumen, cellular and total diameter, luminal, cellular and cross sectional area, number of tubules per unit area of testis, numerical density of the tubules and spermatogenesis index in hamsters that injected two doses of busulfan were more than the hamsters in one dose busulfan injected and control groups (p<0.05).

Conclusion: Two doses of busulfan injection with 21 days interval produced an appropriate animal model of induced azoospermia with comparable stereological indices of seminiferous tubules 35 days after the last injection in hamster.

Keywords: Stereology, Testes, Azoospermia, Busulfan, Hamster

P-44: Investigation The Role of MicroRNAs in Spermatogenesis and Male Infertility

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Background: One of the most remarkable processes during spermatogenesis is the drastic change in gene expression. It has been recently proposed that small RNAs such as microRNAs (miRNAs) contribute a great deal of function in regulation of the gene expression in sperm. Alterations in miRNAs function prove to be detrimental to proper spermatogenesis. As many patients suffer from idiopathic infertility, understanding the effect of miRNAs on molecular pathways would identify possible causes of certain types of infertility.

Materials and Methods: In mature sperm, about two-thirds of mRNA is partially stored in ribonucleoproteins in chromatin bodies which are thought to be reservoirs for small RNAs. As investigated, miR-218 is involved in the promotion of apoptosis while miR-34b, miR-34c, miR-449 deficiency implicates as arrest in spermatogenesis at the stage of elongation with no fully formed sperm observed. miR-122 deficiency results in histone replacement complications and leads to loose DNA packaging and increased apoptosis and reduced sperm production. It was observed that miR-888 maintains sperm flagella and mature sperm morphology. miR-19a, miR-19b, miR-372, miR-373 are involved in tumor suppression, lack of which could result in tumor formation. Lastly, a lack of miR-890 would result in improper development of the lumen.

Results: A number of miRNAs involved in spermatogenesis have been exposed. Recently, the number of studies exploring the transcripts in sperm has increased, and as a result the number of miRNAs identified is likely to increase soon.

Conclusion: Exploring the role of miRNAs in pathways interfering in spermatogenesis not only help understand the function of these small molecules, but also help understand alterations that could cause male infertility. Many of the specific functions of miRNAs are not yet known, but the genes and proteins that they interact with are a window through which their involvement can be understood.

Keywords: miRNA, Male Infertility, Spermatogenesis

P-45: Does Anti-Oxidant Therapy Add Any Extra Benefit to Standard Inguinal Varicocelectomy in Terms of DNA Damage or Sperm Quality Factor Indices: A Randomized Study

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Background: Varicocele occurs in approximately 15 to 20% of the general male population and it is the most common cause of

poor semen production and decreased semen quality. It has been demonstrated that patients with varicocele have a significantly higher DNA damage and spermatozoa with nuclear anomalies than healthy men. Previously it was documented that antioxidant therapy or varicocelectomy will improve DNA damage separately. We proposed to evaluate if antioxidant therapy adds any extra benefit to standard inguinal varicocelectomy in improvement of DNA damage by using Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) assay analysis.

Materials and Methods: We prospectively evaluated changes in sperm chromatin structure in patients with varicocele before and after varicocelectomy. A 100 men with varicocele were enrolled in this study and divided into 2 groups. Fifty men underwent inguinal varicocelectomy and second group underwent varicocelectomy and received L- carnitine 250 mg 3 times / day for 6 months. World Health Organization (WHO) semen analysis and sperm DNA damage by using TUNEL assay were assessed before 3 and 6 months post-operatively.

Results: There was no difference between two groups in age and varicocele grade at the beginning of the study. Mean percentage of DNA damage by TUNEL assay was not different between two groups at baseline. [14.08 (± 10.57%) in operation group vs. 13.97 (± 5.33%) in operation-drug group]. In analyzing the DNA damage by TUNEL, a statistically significant improvement was observed in both groups. DNA fragmentation test results showed a decreasing trend from 14.08 to 10.43 and 9.54% in the operation group and from 13.97 to 9.25 and 8.49% in the operation-drug group respectively at baseline, three and six months post-operatively; (p value=0.02 vs, respectively=0.000). A more improved slope was observed in group 2 (5.48% reduction in six months in operation-drug group vs. 4.54% in operation group) but the observed difference was not statistically different (p value= 0.255)

Conclusion: In this study, we found the positive effect of varicocelectomy on improving DNA damage but failed to show that anti-oxidant therapy can add extra benefits to this effect in terms of improving DNA damage.

Keywords: Varicocele, Anti-Oxidant Therapy, DNA Damage, TUNEL, Protamine Damage

P-46: Effects of Different Doses of Ethanol on Sperm Parameters, Chromatin Structure and Apoptosis in Adult Mice

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Background: Chronic ethanol abuse causes reproductive organ failure and infertility in both humans and laboratory animals. Since sperm has a critical role in reproductive function, the objective of this unique study was to evaluate the effects of different doses of ethanol on sperm parameters, chromatin structure and apoptosis in adult mice.

Materials and Methods: A total of 36 adult male mice were equally divided into four groups. Group 1 received ethanol (10%, v/v) containing saccharin (0.2%, w/v), group 2 received ethanol (5%, v/v) containing saccharin (0.1%, w/v), group 3 was treated with saccharin (0.2%, w/v) and group 4 served as control and fed on basal diet for 35 days. Finally, the left cauda epididymis of each

animal was cut and placed in Ham's F10 medium. Retrieved spermatozoa were used to analyze count, motility, morphology and viability. Sperm chromatin condensation and DNA integrity were assessed by five different tests including chromomycin A3 (CMA3), toluidine blue (TB), sodium dodecyl sulfate (SDS), and SCD (sperm chromatin dispersion), and sperm apoptosis was assessed by TUNEL.

Results: Following ethanol consumption, the sperm count diminished in the ethanol-treated groups. A decrease in sperm motility and an increase in the rate of morphological abnormalities (coiled and broken tails) were seen in the experimental and saccharin groups in comparison with controls. We showed that ethanol consumption can disturb sperm DNA integrity and chromatin remodeling and it may also induce sperm apoptosis. The rates of sperm apoptosis were 51.57 ± 7.45 and 42.85 ± 6.76 in the high ethanol dose and low ethanol dose groups, respectively.

Conclusion: The results showed that alcohol has negative effects on sperm parameters, chromatin/DNA integrity and apoptosis in mice. These alcohol-induced sperm anomalies may be dose-dependent.

Keywords: Ethanol, Sperm, Chromatin, Apoptosis, Mice

P-47: The Protective Effects of Green Tea Extract on Lead-Induced Testicular Toxicity

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Background: lead (Pb) is one of the most important environmental pollutants that affect the male reproductive system. The purpose of the present study was to investigate the protective effect of green tea extract (GTE) against testes damage induced by Pb.

Materials and Methods: Forty adult male mice were randomly divided into four groups (n= 10), as following: Control, GTE, Pb and Pb+GTE treated groups. Animals were given 100 g of GTE/Kg Body weight/day (IP) in the GTE and Pb+GTE treated groups while the other group received normal saline. The Pb and Pb+GTE treated groups received 1% lead (II) acetate (LA) in drinking water. After 6 weeks, the animals were sacrificed and testes were removed for microscopic evaluations. Blood samples were collected for biochemical tests. Data were analyzed using analysis of variance and Tukey posthoc tests (SPSS, p<0.05).

Results: The current study showed marked morphological changes in the form of swelling, congestion, hemorrhage and necrosis in testes of mice treated with Pb alone. However, the mice treated with Pb+GTE showed milder edema, congestion and minute foci of necrosis in the testes. The results showed that lead caused a significant decrease in body and testicular weights, seminiferous tubules diameter and height, number of Leydig cells, plasma total antioxidant capacity (TAC) and testosterone levels. Plasma Malondialdehyde (MDA) and blood Pb levels were significantly more in Pb treated group versus control. Co-administration of green tea with lead acetate significantly increased body and testicular weights, seminiferous tubules diameter and height, number of Leydig cells, plasma TAC and testosterone levels and decreased plasma MDA and blood Pb levels. There was no significant change in the number of Sertoli cells in Pb treated group in comparison with control group.

Conclusion: Treatment of mice with GTE combined with Pb enhances antioxidant/ detoxification system which reduced oxidative stress.

Keywords: Green Tea, lead, Testis, Mice

P-48: Evaluation of Sperm Chromatin Structure in Patients with Partial Globozoospermia

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Background: Globozoospermia is an uncommon disorder of sperm morphology associated with severe male infertility. Patients who contain both normal and round-headed sperm are called partial globozoospermic. It is possible that round-headed sperm may have an abnormal chromatin structure and /or DNA strand breaks. Moreover, sperm DNA fragmentation and protamine deficiency is shown to be associated with teratozoospermia. The aim of this study was to evaluate protamine content and DNA integrity of patients with partial globozoospermia who referred to Royan Institute.

Materials and Methods: In this prospective cohort study, 64 subjects including 23 infertile men with round-headed sperm (above 40%) and 41 normozoospermic men as control were recruited over a 30-month period. Semen examination was based on World Health Organization guidelines. Sperm chromatin structure assay (SCSA), TdT-mediated-dUTP nick end labeling (TUNEL), Chromomycin A3 (CMA3) assays were done for evaluation of the chromatin structure.

Results: Data analysis revealed that percentage of CMA3- positive and TUNEL-positive spermatozoa were higher in the individuals with partial globozoospermia than the men with normal spermogram. There was not statistically significant difference in sperm DNA fragmentation between two groups using SCSA method.

Conclusion: Some specific morphological abnormalities were shown to be predictive of DNA alteration. The present study demonstrated that men with round-head sperm have protamine deficiency and an elevated risk for DNA fragmentation in their sperm compared to that of control group. TUNEL and CMA3 are useful to assess DFI in globozoospermic patients but it seems that SCSA is not a suitable method in such cases. Further, confirmation in a larger scale study is needed.

Keywords: Globozoospermia, Sperm DNA Fragmentation, SCSA Assay, TUNEL Assay, CMA3 Assay

P-49: Effect of Crab Shell Hydroalcoholic Extract Induced Apoptosis in Prostate Cancer Cell Line

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Background: Prostate cancer is the second most common cancer in men worldwide and the third most common cancer in Iranian men. Chemotherapy is used to treat this cancer, but it has toxic effect. Natural compounds found in marine organisms and plants have anticancer considered. The aim of the present study was to investigate the effect of crab shell extract on induction of apoptosis in prostate cancer cells (LNCap).

Materials and Methods: Crab shell was prepared and powdered. Then the hydroalcoholic method was extracted. LNCap was used in this study. The extract was prepared 5 concentrations

(100, 200, 400, 800 and 1000 µg/ml) and within 72 hours were effective on cells. In the above study to evaluate apoptosis, cells were stained with TUNEL method, and were photographed with a fluorescence microscope. Data were analyzed by ANOVA and $p < 0.05$ was considered significant.

Results: Apoptosis was evaluated by TUNEL staining method on LNCap. Index of apoptosis in control group and 100, 200, 400, 800 and 1000 µg/ml extracts were 1.73, 3.16, 4.17, 6.86, 17.77 and 19.11, respectively. This results in 400 µg/ml group of extract in comparison with control group was significant ($p < 0.05$) and in groups of 800 and 1000 µg/ml of crab shell extract in comparison with the control group was significant ($p < 0.001$).

Conclusion: Crab shell extract induced apoptosis in LNCap dose dependently.

Keywords: LNCap Cell Line, Apoptosis, TUNEL Staining

P-50: Elongating and Elongated Spermatis Manufactured *In Vitro* from Non-Human Primate Pluripotent Stem Cells

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Background: We have recently shown that human embryonic (hESCs) and induced pluripotent stem cells (hiPSCs) can differentiate into advanced spermatogenic cells including round spermatids by *in vitro* culture (Easley et al., Direct differentiation of human pluripotent stem cells into haploid spermatogenic cells. Cell Reports 2, 440-446 2012) and also, in collaboration, that rhesus spermatogonial stem cells (SSCs) are reproductively viable after cryopreservation, thawing and transplantation into unrelated males (Hermann et al. Spermatogonial stem cell transplantation into rhesus testes regenerates spermatogenesis producing functional sperm. Notwithstanding the importance of investigating human male meiosis *in vitro*, serious bioethical limitations preclude the determination of the reproductive utility and/or constraints on these artificially manufactured gametes. While human stem cells differentiate *in vitro* into round spermatids, in humans and non-human primates, round spermatids after microinjection to mature oocytes are unable to initiate development and result in pregnancies; this is unlike the situation in mice.

Materials and Methods: To address whether *in vitro* haploid sperm-like cells have reproductive potentials, we present here significant progress in generating elongated and elongating spermatids from rhesus. Here, we confirm and extend these findings showing a non-human primate, rhesus embryonic (nhpESCs), somatic cell nuclear transfer-derived embryonic (nhpNT-ESCs), and induced pluripotent stem cells (nhpiPSCs) can be differentiated into spermatocyte stem cell (SSC) - like cells that express PLZF, a marker for stem and progenitor spermatogonia, and VASA, a known germ cell marker expressed by several germ cell types.

Results: Differentiating nhp pluripotent stem cells in SSC culture conditions also produces haploid spermatids. Most of these *in vitro* haploid cells resemble round spermatids as assessed by protamine 1, acrosin, Golgin3, and VAMP1 immunostaining as well as microtubule and centrosome marker analyses. However, round spermatids do not fertilize human or non-human primate oocytes. We have observed a small population of haploid spermatids derived *in vitro* that structurally resemble elongated spermatids, a spermatid population capable of fertilizing primate oocytes by intracytoplasmic sperm(atic) injection (ICSI).

Conclusion: Importantly, we have now generated haploid elongated and elongating spermatids from primates; this is a vital threshold for the establishment of reproductively viable male gametes *in vitro*. We are currently assessing whether these *in vitro* "elongating" haploid spermatids are capable of fertilizing rhesus oocytes. If successful, this model will provide the first evidence that functional gametes can be manufactured *in vitro* from non-human primate pluripotent stem cells and may provide insight into potentially novel methods for diagnosing and even treating human male infertility.

Keywords: Stem Cells, Pluripotency, Male Infertility, ART, Contraception, Sperm, Fertility, Meiosis

P-51: Potential Benefits of The Treatment of Leucocytospermia on The Improvement of Semen Quality and Fertility in Male Partners if Infertile Couples

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Background: To analyze the evolution of semen parameters in leucospermic men explored for a couple's infertility according to the therapeutic management of leucocytospermia. The potential benefits of leucocytospermia treatment on the natural pregnancy outcomes are also examined.

Materials and Methods: Our study was carried out in 100 leucospermic male partners of infertile couples. Leucocytospermia was routinely determined using the peroxidase technique and defined as the presence of more than 1×10^6 leucocytes per milliliter of semen in the first ejaculation according to WHO guidelines. The evolution of leucospermia was controlled by repetitive semen analysis with at least three months intervals. We excluded leucospermic men with bacterial sperm infection. All clinical and paraclinical exploration data of the couple were listed. We assess the evolution of different semen parameters and the couple's fertility according to the therapeutic management of leucocytospermia.

Results: The most frequent semen abnormalities in the first ejaculation were asthenospermia and teratospermia (62 and 74% of patients, respectively). Antibiotherapy was prescribed in 79% of patients and 21% of patients did not receive any treatment. An anti-inflammatory therapy and different vitamins and antioxidative drugs were associated with antibiotics in 63.2 and 88.6% of treated patients respectively. A total regression of leucocytospermia was observed in 49% of treated patient group. We also noted a disappearance of leucocytospermia in 38% of no treated patients ($p < 0.001$). The aggravation of leucocytospermia was significantly higher in no treated patients (52 % versus 10 % in treated group, $p < 0.001$). However, the rates of semen parameters improvement associated to regression of leucocytospermia among the treatment group was significantly greater than that of no treatment group (25% vs. 5 % respectively, $p = 0.002$). In the treated group, we noted the occurrence of spontaneous pregnancy in 25 couples (32%). No pregnancy was observed in the group of no treated patients.

Conclusion: leucocytospermia might have deleterious effects on male fertility. Etiological diagnostic approach must be proceeded to guide the optimum therapeutic management. Antibiotic therapy for such a condition could improve semen parameters and restore the

natural fertility among infertile couples.

Keywords: Leucocytospermia, Infertility, Semen, Treatment

P-52: Interest of Measuring Mitochondrial Membrane Potential of Sperm by Flow Cytometry Technique in The Exploration of Sperm Quality of Infertile Men

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Background: The fertilizing sperm potential is directly related to its mitochondria activity evaluated by measuring the mitochondrial membrane potential ($\Delta\Psi_m$). The objectives of the present study were to investigate the vitality and mitochondrial membrane potential of sperm in infertile men by the and to assess the correlations between sperm parameters and the level of sperm mitochondrial membrane potential.

Materials and Methods: Our study included 38 semen samples from patients investigated for couple infertility. Each patient underwent a semen analysis, a study of sperm vitality by flow cytometry technique after labeling with 7 -Amino- Actinomycin D (7AAD) and an assessment of $\Delta\Psi_m$ by flow cytometry technique after labeling with JC1. Having taken into account the rate of sperm vitality, we calculated the proportion of living sperm with high $\Delta\Psi_m$ and those with low $\Delta\Psi_m$ in each semen sample.

Results: We found significant and positive correlations between levels of sperm living with high $\Delta\Psi_m$ and total motility ($r = 0,639$, $p < 0.0001$) ; rapid progressive motility ($r = 0,684$, $p < 0.0001$) ; vitality ($r = 0,806$, $p < 0.0001$) ; sperm count ($r = 0,439$, $p = 0.008$) and sperm morphology ($r = 0,459$, $p = 0.007$). We divided our samples into three groups: G0: high $\Delta\Psi_m \leq 20\%$ ($n = 10$); G1 : $20\% < \text{high } \Delta\Psi_m < 50\%$ ($n = 16$) ; G3: high $\Delta\Psi_m \geq 50\%$ ($n = 9$). Comparison of sperm parameters among the three groups showed significant differences on many semen parameters : motility ($p < 0.0001$), vitality ($p < 0.001$) and sperm morphology ($p = 0.017$)

Conclusion: Evaluation of sperm mitochondrial membrane potential provides a rigorous estimate of the metabolic function of the sperm. Sperm nuclear apoptosis is preceded by severe alterations of mitochondrial functions, including alteration of $\Delta\Psi_m$. The $\Delta\Psi_m$ decline is associated to an increased production of active oxygen species responsible for a cytological and molecular loop events deleterious to sperm.

Keywords: Semen, Mitochondria, Infertility, Mitochondrial Membrane Potential

P-53: Chronic Viral Infections and Infertility in Male

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Background: The most common sexually transmitted diseases and the economic and social health problem throughout the world. However, vaccine coverage and antibiotic therapy and the development of programs to prevent and control major causes of acute and chronic diseases, pregnancy complications and infertility remains. Acute and chronic systemic viral infections can lead to infertility and permanent.

Materials and Methods: This study reviews the use of internal and external sources and is made available on the Internet.

Results: Studies have shown that viral infections of sperm with HBV, HCV, HIV, and Human Papilloma Virus (HPV) and Herpes Simplex Virus (HSV) can cause infertility in men. These pathogens can cause resistance to treatment and even be fatal infection. Chronic viral infections can infect and sperm concentration of sperms in the semen can cause damage. Findings have shown that HBV infection can sperm genetic effects of chromosomal abnormalities USOC. HBV infection can also cause damage to sperm quality. HCV negative effect on spermatogenesis and sperm function is impaired. HIV infection is caused by the deformation and failure of sperm motility. HPV causes changes in sperm motility in infertile men. HSV can cause spermatogenic cells. These viruses have negative effects on reproductive performance in male and can be transmitted to newborns and their mothers.

Conclusion: Considering that chronic viral infections cause fatal infections in infants and mothers, there is also the risk of the need for screening methods for early diagnosis and prompt treatment is felt.

Keywords: Infertility, Infection, Viral Infection

P-54: A Study on The Presence of CD52 and CD9 on Sperm in Infertile Couples Who Are Candidate for Intra Uterine Insemination

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Background: Fertilization and fecundation obviously are the phenomenon in which several factors are involved. Sperm membranous proteins are a series of these important elements. CD9, an inner acrosomal membrane protein, is one of the tetraspanin members that involved in sperm-egg fusion. CD52, is a GPI anchor protein which is expressed in the epididymal cells and during passage of sperms through epididimidis added to the sperm surface. The aim of this study was to obtain any association between the percentage of CD52 positive sperm with sperm parameters and also evaluation of correlation between the percentage of CD9 positive sperm with outcome of IUI.

Materials and Methods: A total of 120 semen samples were collected from infertile couples who were undergoing IUI treatment. Samples evaluated according to WHO 2010 guidelines. Concentration and motility parameters were measured by means of computer assisted semen analysis (CASA). Parameters recorded were motile cells, progressive motility, average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL) and linearity (LIN). Sperm washed and immunostained using antibody.

Results: This study indicates that CD9 is detected in only 19% of mature sperm. Regarding the results, the area under the ROC curve

is equal to 0.446 and CD9 is not a good predictor variable for fertility status ($p=0.482$). On sperm kinematic parameters there is a significant positive correlation between the percentage of CD52 expression with avg.VCL($r=0.282, p=0.013$), avg.VSL ($r=0.336, p=0.003$) and avg.VAP ($r=0.409, p=0.00$) and normal morphology ($r=0.866, p=0.00$). No significant correlation was found between the percentage of CD52 with avg.LIN ($r=0.020, p=0.860$) and sperm count ($r=0.125, p=0.278$).

Conclusion: The results indicated the positive correlation between the percentage of CD52 positive sperm with normal morphology and sperm kinematics. The results did not illustrated any relation between the proportion of CD9 expression and pregnancy in IUI patients.

Keywords: CD9, CD52, IUI, Sperm Parameters

P-55: Trolox (Vitamin E) Improves Sperm Quality in Oligospermia during Cryopreservation

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Background: Since the deleterious effects of reactive oxygen species (ROS) during sperm cryopreservation can be overcome by antioxidants, post-thaw human sperm quality may perhaps profit from adding antioxidants to the extender media prior to cryopreservation. This study evaluated the effects of different concentrations of Trolox supplementation to cryo-protective media on post-thaw motility and the apoptosis-like events associated with cryopreservation procedure that includes trans-location of phosphatidyl serine (PS) to the cell surface, alterations in mitochondrial membrane potential (MMP), and DNA integrity of normozoospermic and Oligoozoospermic semen samples.

Materials and Methods: Spermatozoa from 20 normozoospermic men and 20 patients with Oligoozoospermia were cryopreserved with cryo-protective agent containing 0, 20, 40, and 80 μmol Trolox. Pre and post cryopreservation period, sperm motility was evaluated by computer-assisted sperm analysis (CASA). Flow cytometry was utilized to investigate the MMP, PS externalization and DNA fragmentation before and after freezing-thawing.

Results: The results of this study indicated that addition of 40 μmol Trolox significantly ($p<0.05$) increased the percentage of post-thaw motile spermatozoa in normozoospermic semen sample compared to another group. There was no motile sperm in Trolox supplemented frozen-thawed Oligoozoospermic group. Sperm frozen in extender with Trolox had greater MMP, lower DNA fragmentation and externalization of PS in both groups, though the most effective dose of Trolox in normozoospermic and Oligoozoospermic semen samples were different.

Conclusion: These findings support the use of Trolox as freezing extender supplement to improve the quality of cryopreserved human sperm, measured in terms of early apoptosis changes and DNA integrity, before and after freezing-thawing in both normozoospermic men and Oligoozoospermic patients.

Keywords: Human Spermatozoa, Trolox, Cryopreservation, TUNEL Assay, MMP

P-56: Effect of Cissampelos Capensis Rhizome Extract on Human Sperm Capacitation and Acrosome Reaction

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Background: *Cissampelos capensis*, is commonly known by the Afrikaans name "dawidjies" and is the most important and best known medicinal plant of the family Menispermaceae used by the Khoisan and other rural people in the western regions of South Africa. Among numerous other ailments, it is taken to treat male fertility problems. The aim of this study was to investigate the effects of *C. capensis* rhizome extracts on sperm function.

Materials and Methods: Fresh *Cissampelos capensis* rhizomes were collected and an aqueous extract was produced (CRE). A total of 77 semen samples were collected. Spermatozoa were washed with human tubular fluid medium supplemented with bovine serum albumin (HTF-BSA) and incubated with different concentrations of crude CRE for 1 hour at 37°C. Sperm motility, vitality, acrosome reaction, reactive oxygen species (ROS), capacitation, annexin V-binding, DNA-fragmentation and mitochondrial membrane potential ($\Delta\psi\text{m}$) were determined. In a second part of the study, CRE was fractionated with methanol using an Oasis HLB 6cc cartridge; F1= 0% MeOH, F2= 30% MeOH, F3= 60% MeOH and F4= 100% MeOH. A total of 26 semen samples were collected. Spermatozoa were washed with HTF-BSA medium and incubated for 2 hours with 20 $\mu\text{g}/\text{ml}$ progesterone (P4) or without P4. Followed by incubation with different concentrations of fractionated CRE for 1.5 hour at 37°C. Sperm motility, ROS, capacitation were determined.

Results: While incubation of sperm with crude CRE did not affect viability, annexin V-positivity and $\Delta\psi\text{m}$, the percentages of ROS-positive, TUNEL-positive, capacitated and hyperactivated spermatozoa increased significantly and dose-dependently. After fractionation, F1 resulted in significantly higher values for ROS, capacitation and hyperactivation than F2, F3 and F4 with P4-stimulated samples generally having higher values.

Conclusion: The alkaloids present in the F1 of the extract of CRE triggered sperm intrinsic superoxide production leading to sperm capacitation and acrosome reaction induced by P4.

Keywords: Spermatozoa, Capacitation

P-57: Simvastatin Abates Doxorubicin-Induced Teratozoospermia

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Background: Therapeutic utilities of doxorubicin (DOX), an anticancer anthracycline antibiotic, are limited by its serious dose-dependent toxicity to non-target tissues such as testis. The aim of the present study was to elucidate the potential of simvastatin (SIM), a lipid lowering agent with antioxidant and anti-inflammatory activities, to attenuate DOX-induced spermatotoxicity in male mice.

Materials and Methods: Male mice, five weeks old, were randomly divided into four groups of six mice each. DOX was administered to two groups of mice in 5 equal intraperitoneal injections over a period of 4 weeks (accumulated dose of 20 mg/kg). One of these groups received 5 equal oral doses of SIM (accumulated dose of 60 mg/kg)

along with DOX. A vehicle-treated control group and a SIM control group were also included. Teratozoospermia index (TZI) was defined as the number of abnormalities present per abnormal spermatozoon.

Results: A statistically significant elevation of TZI value was observed in the DOX-treated mice as compared to control mice. In contrast, SIM co-administration provided marked normalization in the TZI value when compared to the DOX-only treated group.

Conclusion: These findings have indicated that SIM exerts protective effects against DOX-induced spermatogenic disorders. Mechanisms may involve suppressing inflammation and reducing oxidative stress induced by DOX treatment.

Keywords: Doxorubicin, Simvastatin, Teratozoospermia, Mouse

P-58: BSO-Induced Oxidative Stress Affects Testicular Ultrastructure, Levels of Oxidative Markers, Testosterone and Sperm Fertility

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Background: GSH is the main intracellular antioxidant and its level is important for protecting cell from oxidative stress. BSO inhibits GSH synthesis and cause oxidative stress. The study is designed to investigate the effect of BSO induced oxidative stress on testicular histology, semen parameters and sperm fertility.

Materials and Methods: The adult male mice were divided into two groups of control and experiment. In the experimental group the mice received 2 mmol/kg BSO for 35 days as IP injection. All mice were sacrificed at the end of the study and their testes were prepared for electron microscopy. For determination of SOD, MDA, GPX, GSH and testosterone levels, blood samples were obtained from heart. For evaluation of fertility the oocytes were obtained from superovulated mice by tubal flashing and inseminated with sperms collected from cauda epididymis. Embryo formation was considered as fertility success and compared in between groups.

Results: Electron microscopy showed that in experimental group, germ cells were detached from basement membrane and were separated from each other and sertoli cells by large vacuoles. Determination of oxidative markers and testosterone level revealed that the concentration of SOD, GPX, GSH and testosterone in experimental group, in comparison with control group, is reduced while the level of MDA was increased ($p < 0.05$). Sperm fertility, based on embryo formation, was decreased in experimental group.

Conclusion: Ultrastructural changes of mice testes in Bso-induced oxidative stress is accompanied by decreasing of antioxidants and fertility rate.

Keywords: Oxidative Stress, Buthionin Sulfoximine, Testosterone, SOD, MDA

P-59: Effect of Microsurgical Varicocelectomy on Sperm Chromatin Packaging, Miscarriage and Pregnancy Rate in Infertile Men with Varicocele

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Background: Although the pathophysiology of the testicular damage associated with varicocele remains unclear, but varicocelectomy is considered a suitable technique for the treatment of individuals with clinical varicocele. The current study was designed to assess semen parameters, chromatin packaging, and pregnancy outcome before and after varicocelectomy.

Materials and Methods: Semen samples were obtained from men with varicocele grades II and III ($n = 145$). Samples were assessed by a spermiogram, and chromatin packaging (chromomycin A3), before and after surgery microsurgical varicocelectomy was performed as part of patient management. Data was analyzed by SPSS. 11.5.

Results: Sperm parameters and chromatin packaging were improved after surgery compared to before surgery. The percentages of spontaneous cumulative pregnancies were 33.1, 42.06, 46.2, 48.9, and 55.8% after 3, 6, 9, 12 months post-surgery respectively. In addition, percentages of spontaneous cumulative miscarriage were 2.46, 4.93, 4.93, 6.17, and 6.17 % after 3, 6, 9, 12 months post-surgery respectively.

Conclusion: Microsurgical varicocelectomy in addition to improves sperm parameters and chromatin packaging, also improve the chance of pregnancy.

Keywords: Varicocelectomy, Sperm Parameters, Protamine Deficiency, Pregnancy, Miscarriage

P-60: Nano-Particle TiO2 Enhances Apoptosis in Testicular Tissue; Evidence for p53, bcl2, cyp19 Genes Expression

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Background: Nano-particle Titanium Dioxide (TiO2) is a non-combustible, odorless powder that is widely used in different fields of industries. Previous reports showed that chronic exposure to TiO2 adversely impacts the testicular tissue and down-regulates the antioxidant capacity and down-regulates the endocrine status of the testicles. Present study was designed in order to identify the role of p53, bcl2 in TiO2-induced damages. Moreover, the cyp19 gene expression and its correlation with germinal cells RNA damage in TiO2-exposed animals were evaluated.

Materials and Methods: Twenty four mature mice were randomly divided into four groups as control-sham (received 0.3mL normal saline, ip) and test groups including: low dose (10 mg/kg, ip), medium dose (50 mg/kg, ip) and high dose (100mg/kg, ip)-TiO2-received groups. After 35 days, the mRNA and protein levels of bcl2, p53 were assessed by using semi-quantitative RT-PCR and immunohistochemical analyses, respectively. Moreover, the cyp19 mRNA level was assessed by using semi-quantitative RT-PCR. The germinal cells RNA damage was evaluated.

Results: TiO2 resulted in a significant increase at p53 mRNA and protein levels and remarkably reduced bcl2 expression, in a dose dependent manner. Moreover, the animals in TiO2-received groups exhibited reduced cyp19 mRNA levels and showed intensively elevated RNA damage in germinal cells.

Conclusion: Our data suggest that TiO2 negatively impacts the germinal cells via up-regulating the p53 gene and down-regulating

the bcl2 expressions. On the other hand, TiO₂ by reducing the cyp19 expression enhances the p53-and bcl2-dependent apoptotic pathways, which in turn result in intensive RNA instability in haploid cells.

Keywords: TiO₂, p53, bcl2, cyp19, RNA Damage

P-61: Inhibitory Effect of Simultaneous Exposure to Formaldehyde Vapour and Noise on Mouse Sperm Kinematic Parameters and Sexual Hormones

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Background: This study aimed to investigate the inhibitory effects of simultaneous formaldehyde (FA) and noise exposure on the sperm kinematic parameters sexual hormones in male mice.

Materials and Methods: Forty eight NMRI adult male mice were randomly assigned to control group and experimental groups which were exposed to formaldehyde vapour (10 ppm) (F), noise (100 dB) (N), and simultaneous formaldehyde with noise (NF), respectively for 10 days (8 hours a day). Mice were sacrificed 24 hours after exposure (short-term effects) and the rest of mice were sacrificed 35 days after the end of exposure (long-term effects). Sperm analyzed by computer aided sperm analysis system. Plasma concentrations of testosterone, LH, FSH were measured.

Results: The results of short-term analysis showed that the serum testosterone in all exposure groups were decreased significantly compared to the control ($p < 0.001$). In long-term analysis, the percentages of progressive motile sperm groups N, F, and NF were 33.73 ± 3.4 , 26.65 ± 1.61 , and 14.78 ± 4.65 respectively which had a significant decrease ($p < 0.05$) compared to control group (44.47 ± 2.88). The progressive motile sperm in simultaneous exposure group was less than N and F groups ($p < 0.001$).

Conclusion: Thus, it can be anticipated that simultaneous exposure to formaldehyde and noise in workplaces can increase the possibility of damage to reproductive functions.

Keywords: Formaldehyde, Noise, Sex Hormones, Sperm Kinematic Parameters

P-62: Evaluation of Genetic Alterations in Exons 3 and 28 of SPEF2 Gene in Infertile Men with Short Tail Sperm Compared to Control Subjects

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Background: The short tail sperm defect is one of the syndromes that cause infertility in men. Characteristics of the syndrome are reduced number of spermatozoa in the semen, immotility of spermatozoa, lack of axonem or incomplete and malformation in the tail structure. One candidate gene causing the disease is SPEF2 gene. SPEF2 is expressed in all ciliated cells and is essential for correct sperm tail development and male fertility. The aim of this study is evaluation of genetic alterations in exons 3 and 28 of SPEF2 gene in two groups of control and infertile men with short tail sperm defect.

Materials and Methods: In this case-control study, 64 subjects including 23 infertile men with short tail sperm (above 80%) and 41 normozoospermic men as control was recruited. Semen examination was based on WHO guidelines. To study the genetic variations, initially primers were designed for exon 3 and 28 and PCR reaction was done. Then, DNA sequencing was performed for PCR products.

Results: Partial sequence analysis of the SPEF2 genes in patients with short tail sperm defect did not identify any mutation in exon 3 and 28 of the SPEF2 gene. However, one polymorphism (363A>C) was identified in exon 3. The GenBank sequence contains an adenine at position 363 of the coding region of SPEF2 gene, whereas our sequence analysis identified acytosine at this position in three persons of control group and four persons of patient group.

Conclusion: Although observed one missense mutation in exon 3 and one nonsense mutation in exon 28 of the mouse, our data revealed no mutation in spef2 gene in human. However, SPEF2 gene is big and has many exons, thus evaluation of other exons and promoter is necessary to make sure of involvement of SPEF2 gene in short tail sperm defect in human.

Keywords: SPEF2 Gene, Short-Tail Sperm Defect, Axonem

Animal Biotechnology

P-63: The Effect of Temperatures on Goat Epididymal Sperm Motility in Tissue Culture Media 199

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Background: There is a general deterioration in sperm quality depending on time postmortem. Refrigeration of the epididymes down to around 5°C is the best strategy to lower this damage and there are many dissimilarities between species, possibly due to differences on cold shock endurance of epididymal sperm. Our objective was to determine the effect of 5°C and 37°C on goat epididymal sperms motility during 24 hours in TCM199

Materials and Methods: Sperm were collected performing several incisions in the cauda epididymis of goat testicles, and taking the white fluid emerging into micro tube containing TCM 199 with 10% bovine serum albumin. After count (30×10^6 sperm/ml), one aliquots kept at 5°C refrigerator and the other incubated at 37°C. Sperm motility was examined at 1, 6, 12, and 24 hours after being kept at 5°C refrigerator and 37°C incubator with computer-assisted sperm analyzers (CASA). Statistical analysis were carried out using the one-way ANOVA of SPSS version 21.

Results: Till 6 hours CASA parameters; motile percentage (Class A+B+C, %), straight line velocity (VSL, $\mu\text{m/s}$), curvilinear velocity (VCL, $\mu\text{m/s}$), average path velocity (VAP, $\mu\text{m/s}$), amplitude of lateral head displacement (ALH, μm), angular displacement (MAD, D), were significantly higher in 37°C group than 5°C group ($p < 0.05$). But, at

6-12 and 24 hours., rapid progressive motility (Class A, %), progressive motility (class A+B), motile percentage, VSL, VCI, VAP, ALH, and BCF were significantly higher in 5°C group than 37°C group ($p < 0.05$).

Conclusion: Motility parameter of goat epididymal sperm can be kept at 37°C in TCM 199 only till 6 hours. But when preserved at 5°C in TCM 199, it can be kept till 24 hours.

Keywords: CASA, Goat, Epididymal Sperm

P-64: The Relationship between Polymorphism in Gene of Insulin-Like Growth Factor-I and The Serum Periparturient Concentration in Holstein Dairy Cows

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Background: One of the most important metabolic factors affecting the reproductive activity is insulin-like growth factor-I (IGF-I) concentration changes after calving. Recently, Maj et al. (2008) discovered a significant association between the IGF-I genotypes based on the 5'-untranslated region (5'-UTR) of IGF-I gene and the IGF-I blood level. The objective of this study is to investigate the relationship between polymorphism within the 5'-UTR of IGF-I gene and its periparturient concentration in Iranian Holstein dairy cows.

Materials and Methods: In this study, blood samples (5 mL, n=37) were collected by caudal venipuncture from each animal into sample tubes containing the EDTA and DNA was extracted from blood. In order to measure IGF-I concentration the collection of blood samples (n=111) was also done at 14 days before calving (prepartum), days 25 and 45 postpartum.

Results: We found evidence for a significant effect of C to T mutation in position 512 of IGF-I gene on its serum concentration in dairy cows in Iran. Cows CC genotype had significantly higher concentration (Mean \pm SD) of IGF-I at 14 day prepartum (91.8 ± 18.1) $\mu\text{g/L}$ compared to those with TT genotype (73.3 ± 14.4) $\mu\text{g/L}$ ($p=0.04$). A significant trend (quadratic) was found for IGF-I concentration, as higher in CC cows compared to ones with TT that 14 days before calving to day 45 postpartum ($p=0.01$).

Conclusion: We concluded that C/T transition in the promoter region of IGF-I gene can influence the serum concentration of IGF-I in periparturient dairy cows.

Keywords: Polymorphism, Gene of Insulin-Like Growth Factor-I (IGF-I Gene), Fertility, Dairy Cows

P-65: Effective Parameters on the Bovine Follicle Stimulating Hormone Expression in The Pichia Pastoris System

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Background: Bovine follicle-stimulating hormone (bFSH) is a heterodimer hormone that consists of a common β -subunit which non-covalently associated with the hormone-specific α -subunit. During the past 15 years, the methylotrophic yeast *Pichia pastoris* has become an important host organism for recombinant protein production because it is able to use methanol as a sole carbon and energy source. The *Pichia pastoris* expression system is being used successfully for the production of various recombinant proteins such as FSH. Optimization of conditions during expression for improving the yield of protein production is a major goal for scientists. The aim of this project is to study the effect of temperature and methanol induction yield on the expression of bFSH in the methylotrophic yeast *pichia pastoris*.

Materials and Methods: In this study, bovine FSH recombinant protein were produced in *Pichia pastoris* expression system using BMMY medium at pH=6, Temperatures of induction (15, 18, 21, 24, 27, 30, 32°C) and methanol concentration used for induction (0.5, 1, 2, 3%) in five day incubation time. The experiments were carried out in order to find the optimal temperature and methanol induction yield. The recombinant proteins were precipitate using TCA method. The protein evaluated by SDS-PAGE followed by Western blotting with specific antibody for β FSH and a ~16-kDa protein of interest was confirmed.

Results: Results showed that temperature and methanol significantly affect on expression of bFSH. The best temperature for Expression of bovine FSH is 30°C and the optimal methanol induction yield for expression system is 1%.

Conclusion: The findings demonstrate how temperature and methanol influences on production of recombinant protein bFSH in the methylotrophic yeast *pichia pastoris*.

Keywords: Bovine FSH, *Pichia Pastoris*, Temperature, Methanol Induction

P-66: Optimization of Human Luteinizing Hormone Expression in CHO Cells Culture by Stepwise Reduction in Serum Concentration

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Background: Mammalian Cell lines are the main expression system for the production of recombinant therapeutic proteins. Optimization of cell culture condition is performed via alteration in different parameter. Cell culture media plays an important role in cell cycle because of compounds such as amino acids, vitamins, inorganic salts, glucose, and serum as a source of growth factors, trace elements, and attachment factors. But serum is an ill-defined mixture of compounds

that may contain endotoxins, microbial contaminants, and growth inhibiting factors and is also expensive part of cell culture. So, efforts are directed towards reducing serum concentration in cell culture.

Materials and Methods: In this study, the recombinant CHO-C111 cells transfected with human LH (luteinizing hormone) gene were cultured in DMEM medium containing 15, 10, 5, 3 and 1% FBS (fetal bovine serum) respectively. Then, approximately equal number of cells (9×10^6) in each medium was used for protein extraction and Bradford assay. SDS-PAGE and western blotting techniques were performed to confirm protein expression. Untransfected CHO-C111 cells were used as a negative control and recombinant LH (Lutropin) as a positive control.

Results: There was no significant difference in cell growth in different concentrations of serum, because in all mediums, 9×10^6 cells were collected after about four days. Using Bradford assay, it was observed that the protein concentration was approximately equal. Despite using the equal amount of protein, western blotting showed that maximum productivity was in medium with 15% serum.

Conclusion: Higher concentrations of serum in medium caused higher level of LH expression, but because of disadvantages of serum in cell culture, it seems that the use of alternative or reduction in serum concentration is in favor of protein expression.

Keywords: Luteinizing Hormone, Optimization, CHO, Serum Concentration

P-67: Quantitative Expression of Pluripotency Specific-Genes in Mouse Blastocysts Produced by *In Vitro* Fertilization

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Background: The efficiency of *in vitro* fertilization (IVF) is still low to be developed to blastocyst stage probably because of environmental conditions. It is likely that *in vitro* environment can not exactly mimic *in vivo* environment due to differences in media, metabolic content, atmospheric composition, temperature and pH. Therefore it may affect embryo quality by changing in embryo gene expression. In this study, the expression level of pluripotency specific-genes: Oct4, Nanog and Sox2 were assessed in mouse blastocysts produced by IVF and compared with the control blastocysts flushed out of uterus on post coital day 3.5.

Materials and Methods: Matured mouse oocytes were fertilized using capacitated sperms in HTF medium. Subsequently, presumptive zygotes were cultured for 4 days in KSOM medium up to the blastocyst stage. The expression levels of three mentioned genes were assessed by real-time PCR technique in IVF-derived blastocysts as treated group and *in vivo* collected blastocysts as control group.

Results: Hatching rate of IVF-derived blastocysts was decreased significantly in comparison with the counterpart group (70.22% vs. 87.80%). Increase in Oct4, Nanog and Sox2 expression was observed in IVF versus *in vivo* generated mouse embryos.

Conclusion: *In vitro* produced embryos followed by assisted hatching can be introduced as a reliable technique to help infertile couples.

Keywords: *In vitro* Fertilization (IVF), Pluripotency Specific-Genes, Real-time PCR

P-68: Adipokines Play Major Roles in Sperm Motility and Function in Ram

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Background: Adipose tissue is not only an energy storage tissue but also an important endocrine organ that secretes protein hormones. Among them, the production and secretion of adiponectin is inversely correlated to the severity of obesity. In adipose tissue, adiponectin production is transcriptionally regulated by peroxisome proliferator-activated receptor- γ (PPAR- γ). Obesity is a major health problem. Some studies have shown a relationship between obesity and sub-fertility. Thus, the aim of the present study was to investigate the gene expression of adiponectin and PPAR- γ in ram spermatozoa and to determine whether sperm motility affects the abundance of mRNAs.

Materials and Methods: Semen samples from 6 adult rams were fractionated on a two layer discontinuous percoll gradient to separation high and low motile sperm and quantitative parameters of sperm motility were determined by CASA. Total RNA isolation was carried out on sperm cells. Total RNA was reverse transcribed into cDNA. Then, the levels of Adiponectin and PPAR- γ transcripts were determined by semi quantitative RT-PCR.

Results: We showed that ram spermatozoa express Adiponectin and PPAR γ , and the amount of expression is associated with sperm motility. There were significant ($p < 0.05$) increase of the relative amounts of Adiponectin and PPAR γ mRNAs in high motile sperm group compared to low.

Conclusion: We provided a novel evidence for the expression of Adiponectin and PPAR γ in spermatozoa which showed that ram spermatozoa express Adiponectin and PPAR γ . The amount of relative expression was associated with sperm motility. Since increase in body fat is associated with impaired sperm motility, our finding can help to find the molecular mechanisms which are involved in sub-fertility caused by obesity.

Keywords: Adipokines, Obesity, Sperm Motility, Male Fertility

P-69: Expression of Leptin Receptor mRNA in Ovine Corpus Luteum

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Background: Many hormones are involved in the regulation of reproduction. Leptin hormone which is mainly secreted by adipose tissue plays an important role in energy homeostasis and reproduction. It seems that leptin is an important linkage between body metabolism and reproductive system. Moreover, it has been shown that leptin and leptin receptor express in reproductive organs of some species. Hence, leptin has a direct effect on reproduction. The aim of

this study was to investigate the expression of leptin receptor mRNA in ovine corpus luteum.

Materials and Methods: Ovine ovaries were collected from abattoir and immediately were transported to the laboratory on ice. Corpus luteum pieces sliced and kept in -80°C. Total RNA was extracted and cDNA Synthesis was done. First, PCR reaction was done for β -actin, as a housekeeping gene, to evaluate the accuracy of molecular experiments. PCR reaction was done for leptin receptor gene using appropriate primers. Fat tissue was used as positive control.

Results: In corpus luteum samples, leptin receptor transcript was amplified with set of primers. Intron-spanning primers of β -actin were located in two exons to rule out the possibility of a DNA contamination.

Conclusion: Presence of leptin receptor mRNA in the ovine corpus luteum suggests that leptin is involved in the physiological processes of ovine corpus luteum which should be clarified.

Keywords: Leptin receptor, Ovine, Corpus luteum, RT-PCR

P-70: Evidence for Differential Gene Expression of A Major Epigenetic Modifier Enzyme, de novo DNA Methyltransferase 3b, through Vitrification of Mouse Ovary Tissue

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Background: Ovarian tissue cryopreservation is a feasible method to preserve female reproductive potential, especially in young patients with cancer or in women at risk of premature ovarian failure. Vitrification has recently emerged as a new trend for biological specimen preservation. On the other hand, gene expression that changes during vitrification can influence oocyte maturation and need to be studied. Methylation of mammalian DNA is a major epigenetic regulatory mechanism that play a special role in gene expression regulation. DNA methyltransferase3b (Dnmt3b) is responsible for de novo methylation and essential for genome stability, imprinting and embryonic development. The aim of the present study was to evaluate the effects of vitrification on mRNA expression level of Dnmt3b gene.

Materials and Methods: Ovaries of 4- to 6-week old NMRI female mice were categorized in two control and needle immersed (NIV) vitrification groups. In vitrification group, ovaries were transferred into equilibration and vitrification medium, then immersed in liquid nitrogen after loading by acupuncture needle. Parallel to vitrification process, morphology of ovarian tissues in control and vitrification group were analyzed and compared by using hematoxylin and eosin staining. Then, the expression of Dnmt3b was investigated by real-time PCR.

Results: In morphological analysis, ovarian tissue integrity was well preserved in vitrification group and was similar to the control group. However, the result of this study showed that the expression level of Dnmt3b in vitrification group was significantly higher in comparison with control group ($p < 0.05$).

Conclusion: In general we can conclude that despite normal morphology of ovarian tissue after vitrification, this process may in-

duce changes at the genetic/epigenetic level of cryopreserved tissues.

Keywords: Epigenetic, Vitrification, Ovary, DNA Methyltransferase3b

P-71: Construction of Required DNA Plasmids for Validation of Predicted MicroRNA Targets

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Background: The micro-ribonucleic acids (miRNAs) are non-coding RNA molecules that are conserved developmentally and include usually 18-25 nucleotides. miRNA regulates gene expression through mRNA degradation or inhibiting of its translation. These biomolecules contribute in cellular physiologic and pathologic processes and most of them may act as oncogenes or tumor inhibitors. Identification of miRNAs target sequences is critical for investigation of their physiology; and in this study it is considered to establish two constructs as model, for validation of bioinformatically predicted targets for miRNAs in the future.

Materials and Methods: In the present study, two expression vectors were constructed. The first one (vector A) expresses precursor of mir-101 under control of CMV promoter. The other vector contains coding strand of EGFP and Luciferase fused to each other as a reporter gene downstream of elongation factor promoter. In this vector 3'UTR region of the gene of ATP5B (as target of mir-101) was cloned between transcription terminator sequence and the poly A region. For assessment of the functionality of these two vectors, a stable cell line was obtained by transfection of HeLa cells by vector B and next, mir-101 was over-expressed in the aforementioned cell line using vector A. Difference in expression of the reporter gene was assessed after transfection of the cell line by vector A.

Results: Down regulation of the reporter gene confirmed the suitability of this system.

Conclusion: It was concluded that this system is reliable for validation of other predicted targets of any miRNA by substitution of the precursor of the mir of interest in the vector A and cloning of the related 3'UTR region in vector B.

Keywords: MicroRNA, Target Validation, Reporter Genes, DNA Plasmid, 3'UTR

P-72: Ovine Oocytes Vitrified at Germinal Vesicle Stage as Cytoplasmic Recipients for Somatic Cell Nuclear Transfer (SCNT)

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Background: The cryopreservation of immature oocytes at the germinal vesicle (GV) stage would create an easily accessible, non-seasonal source of female gametes for research and reproduction. The present study investigated the ability of ovine oocytes vitrified at the GV stage using a cryoloop to be subsequently matured, fertilized and cultured *in vitro* to blastocyst-stage embryos.

Materials and Methods: Selected cumulus-oocyte complexes obtained from mature ewes at the time of death were randomly divided into vitrified, toxicity and control groups. Following vitrification and warming, viable oocytes were matured *in vitro* for 24 hours. Matured oocytes were either evaluated for nuclear maturation, spindle and chromosome configuration or fertilized and cultured *in vitro* for 7 days.

Results: No significant differences were observed in the frequencies of IVM (oocytes at the MII stage), oocytes with normal spindle and chromatin configuration and fertilized oocytes among the three groups. Cleavage at 24 and 48 hour post insemination significantly decreased ($P < 0.01$) in vitrified oocytes. No significant differences were observed in the proportion of blastocyst development between vitrified and control groups (29.4 vs. 45.1%, respectively). No significant differences were observed in total cell numbers, the number of apoptotic nuclei or the proportion of diploid embryos among the three groups.

Conclusion: We report for the first time that ovine oocytes vitrified at the GV stage using a cryoloop have the ability to be matured, fertilized and subsequently developed *in vitro* to produce good-quality blastocyst embryos at frequencies comparable to those obtained using fresh oocytes.

Keywords: Oocyte, Vitrification

P-73: Effect of Donor Age on The Expression Stability of GAPDH as A Reference Gene for Gene Expression Analysis of Equine Adipose-Derived Mesenchymal Stem Cells

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Background: Adipose tissue is a main source for isolation of equine mesenchymal stem cells (MSCs) at different ages. It seems that characteristics of adipose-derived MSCs especially gene expression profile are changing along with age increase. A proper reference gene is required for normalizing data in gene expression analysis by qRT-PCR. This study aimed to evaluate whether GAPDH has a stable expression in adipose-derived MSCs isolated from horses with different ages.

Materials and Methods: MSCs isolated from adipose tissue of 3 mares, aged 3, 6 and 9 years old. After 3 passages, total RNA of cultured cells was isolated and mRNA reverse transcribed to cDNA. qRT-PCR was done using GAPDH primers in triplicate.

Results: Our analysis indicated that Ct of GAPDH gene is very similar in all samples. Thus, its expression was almost the same at adipose-derived MSCs of horses with different ages. Mean \pm SD of Ct was 15.53 ± 0.47 .

Conclusion: Based on the results, it suggests that expression level of GAPDH is stable in MSCs of horses with different ages. Thus, it can be considered as a suitable reference gene for gene expression analysis for adipose-derived MSCs isolated at different ages.

Keywords: GAPDH, Reference Gene, Mesenchymal Stem Cells, Adipose, Equine

P-74: Effect of Fndc5 Overexpression on Cardiac Differentiation Rate of mESCs

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Background: Fibronectin type III domain-containing 5 proteins (Fndc5), an exercise hormone, formerly known as peroxisomal protein that was cloned in 2002. Transcript profile analysis of Fndc5 revealed high degree of expression in heart, skeletal muscle and brain. Our recent studies indicated a significant increase (approximately 10 folds) in mRNA level of Fndc5 when mouse embryonic stem cells were differentiated into beating bodies.

Materials and Methods: As a step closer to identify the involvement of Fndc5 in process of cardiomyocyte differentiation, we generated a stably inducible transduced mouse embryonic stem cell (mESC) line that overexpressed Fndc5 following doxycycline induction.

Results: Our results indicate that overexpression of Fndc5 during cardiac differentiation significantly increased not only RNA levels of mesodermal markers but also the transcriptional levels of cardiac progenitors and cardiomyocyte markers. These data suggest that Fndc5 may be involved in cardiomyocyte differentiation.

Conclusion: Therefore, a new hope will be arisen for potential application of this myokine for regeneration of damaged cardiac tissues especially in cardiac failure.

Keywords: Cardiac Differentiation, Embryonic Stem Cells, Fndc5, Overexpression

P-75: Effects of Cholesterol Loaded Cyclodextrin on Cat Sperm Vitrification

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Background: Cholesterol loaded cyclodextrin (CLC) efficiently protected sperm from some species. On the other hand vitrification of sperm especially in human opened a new horizon in the field of sperm freezing. The aim of the present study was to evaluate CLC on vitrification of cat sperm.

Materials and Methods: Tom epididymal sperm (n=9) was extracted from epididymides and subjected to vitrification in TRIS base solution that was incorporated with CLC. The warmed sperm was analysed for motility and viability.

Results: Vitrification significantly ($p < 0.0001$) reduced the sperm

motility (%) in CLC-plus (1.14 ± 0.02) and CLC-minus (4.2 ± 0.02) compared to fresh samples (25 ± 0.02). Sperm viability was also decreased significantly following vitrification either with CLC (80.3 ± 0.03 ; $p=0.0063$) or without CLC (79.3 ± 0.03 ; $p=0.0159$) compared to the fresh samples ($p=0.7933$).

Conclusion: Cat sperm showed an indigenous tolerance to the vitrification procedure and CLC does not have any beneficial effect on it.

Keywords: Feline, Sperm, CIC, Vitrification

P-76: Stepwise Reduction of Fetal Bovine Serum Levels in Chinese Hamster Ovary Cells -Expressing Human Chorionic Gonadotrophin- Culture

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Background: The demand for producing recombinant therapeutic proteins by mammalian cell lines, such as Chinese hamster ovary (CHO) cells, continues to grow. Significant achievements in process optimization including development of cell culture strategies for large-scale and cost-effective production have been made. Fetal bovine serum (FBS) is an often essential growth supplement and yet most costly component for cell culture media. Furthermore, scientific and ethical concerns about the use of FBS considering its harvest and production highlight the need for replacing or reducing FBS levels in cell culture. In this study the effect of different serum concentrations on viability and productivity of a recombinant CHO cell line expressing recombinant human chorionic gonadotrophin (rhCG) was investigated.

Materials and Methods: FBS was used by 15% concentration as the reference and the levels have downward trend during one or two passages at each stage, for instance 15 to 10, 5, 3, 1%. Cells growth and morphology were studied daily by optical microscope and cell viability was determined by trypan blue exclusion. Total protein concentration was estimated using Bradford assay. The rhCG production was assessed using SDS-PAGE and Western blotting methods.

Results: The results demonstrated that cell growth and morphology were normal through gradual reduction of serum levels from 15 to 3%. Maximum cell viability observed at 15% FBS while cells longevity reached its maximum at 3% FBS. SDS-PAGE and Western blotting results showed that rhCG expression reduced from 15% FBS to 1%. Maximum productivity was seen at 15% FBS and no significant difference in rhCG production at 3 and 5% of FBS was found.

Conclusion: Remarkable amount of money could be saved by reducing FBS levels by 80%, while the viability and productivity of rCHO cells don't change significantly.

Keywords: Chinese Hamster Ovary (CHO) Cell, Human Chorionic Gonadotrophin (hCG), Fetal Bovine Serum (FBS)

P-77: Optimization of Ovine FSH Gene Expression in The Pichiapastoris System by Regulating The Culture Conditions

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Background: Ovine follicle stimulation hormone (OFSH) is a pituitary glycoprotein and belongs to the family of glycoprotein hormones. This hormone plays a key role in the function of the reproductive system: it is essential for sertoli cell function and spermatogenesis in testis and it stimulates the growth of ovulatory follicles in females. Ovine FSH hormone is a heterodimeric hormone consisting of a 92 amino acid, α subunit, and a 111 amino acid β subunit which are linked non-covalently. *Pichia pastoris* is a suitable host for the production of foreign proteins and known as the most economical eukaryotic expression system for secretion of heterologous proteins. The purpose of this study is the optimization of ovine FSH gene expression in the *pichia pastoris* system by regulating the culture conditions consist of temperature and percentages of induction of methanol.

Materials and Methods: The expression process was tested in a five-day incubation period in different temperatures (32,30,27,24,21,18,15) and different percentage of induction of methanol (0.5,1,2,3) for three times. After completing this process for each parameter, the recombinant proteins were precipitate using TCA method and then protein expression levels were evaluated by SDS-PAGE and then western blotting analysis for these two parameters. Finally, optimum temperature and concentration of induction of methanol during expression process was selected.

Results: The experiments were performed in order to find the optimal temperature and methanol induction yield. Results of SDS-PAGE and western blotting showed a ~18-kDa band of protein of interest. It is also demonstrated that the best temperature for expression of ovine FSH protein in *pichia pastoris* system was 30°C and the optimal methanol induction yield was 0.5%.

Conclusion: The findings demonstrate how temperature and methanol induction influence the OFSH gene expression in the *pichia pastoris* system.

Keywords: Ovine FSH, *Pichia Pastoris*, Temperature, Methanol Induction

Embryology

P-78: Role of Allopurinol, as An Antioxidant Factor, in Increasing The Number of Received Oocytes and Embryos, and Reduce Apoptosis after Heterotopic Transplantation Mouse Ovarian Tissue

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Background: Ischemia and reperfusion after transplantation is the main problem which decreases follicular density in the grafted ovarian. Many sources of free radicals such as xanthine oxidase were generated during ischemia. In this study, we used allopurinol as xanthine oxidase inhibitor to reduce ischemia-reperfusion injury, to increase received oocytes and embryos, and to decrease Apoptosis after ovarian heterotopic autotransplantation in mice.

Materials and Methods: Six-week-old (NMRI) mice were used in this study. Ovarian tissues were grafted into back muscle and then collected three weeks later. A total of two groups were included in this experiment: sham group (without treatment factor) and allopurinol treatment group (5 mg/kg/day). We investigated number of received oocytes and embryos as well as rate of Apoptosis in grafted ovary (immunohistochemistry assay). Data are present as the mean \pm SEM and were analyzed by one way ANOVA and Kruskal-Wallis test. A probability of $p < 0.05$ was considered to be statistically significant. The protocols approved by Ethics Committee of Royan Institute (Tehran, Iran).

Results: The number of received oocytes and embryos significantly increased in Allopurinol group compared with sham group ($p < 0.05$). Rate of Apoptosis decreased in allopurinol compared with sham group.

Conclusion: Xo plays an important role in generating superoxide anions. So allopurinol, as a XO inhibitor, reduces superoxide anions production such as O_2^- and H_2O_2 . We demonstrate that allopurinol plays an important role in order to decrease ischemia injury and to increase survival rate for follicles. Therefore, number of received oocytes and embryos are increased and apoptosis injury is decreased. These observations confirm that follicles start developing rather than remaining static after transplantation.

Keywords: Allopurinol, Oocyte and Embryo, Apoptosis, Ischemia/Reperfusion, Ovarian Transplantation

P-79: The Study of Levofloxacin Antibiotic Effects on Male Sexual Hormone in Wistar Rat

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Background: Levofloxacin is a fluoroquinolone antibacterial agent with a broad spectrum of activity against different infectious disease and Gram-negative bacteria. In more than 100 countries it has therapeutic use. This study is aimed to determine Cytotoxic of Levofloxacin effects on rat testis tissue.

Materials and Methods: For this experimental study, we used 50 male wistar rats which must be about 250 ± 1 g. The control group received no treatment, the witness group fed normal saline and treatment groups fed with 250 ml, 500 ml and 750 ml of levofloxacin ($n=10$). This stage must last 60 days long. After the examination procedure, blood sampling of rats' hearts has been done and the serum samples sent to medical diagnostic for hormonal tests.

Results: This study showed that levofloxacin in used doses has no effect in testosterone level but 500 and 750 ml doses of the drug increase LH level, and 250 and 500 ml doses of drug increase FSH

level of serum.

Conclusion: According to changes which made in this study, the levofloxacin antibiotic is one of the factors which causes disturbance in testis tissue and thereafter hormonal disorder that should be noticed in prescription time.

Keywords: Testis, Levofloxacin, Male Hormone, Rat

P-80: Histological Assessment and Oestrous Cycle Restoration after Vitrified-Warmed Rat Ovarian Tissue Autotransplantation, Using Hyaluronic Acid Hydrogel Scaffold

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Background: Follicular pool is reduced after transplantation of vitrified ovary. Therefore, methods should be improved to accelerate ovarian function revival and maintain follicular reserve. In this study hyaluronic acid hydrogel as a scaffold was used to improve restoration of ovarian oestrous cycle.

Materials and Methods: 79 mature (~8 weeks) rats having normal estrous cycles were divided into two groups: control group (vitrified-warmed ovarian transplant $n=39$) and the experimental group (vitrified-warmed ovarian transplant that encapsulated with HA $n=40$). Each group was divided into three subgroups (a: three days after transplantation, b: one full estrous cycle after transplantation and c: five estrous cycles after transplantation). In all groups, ovaries were vitrified and warmed before intramuscular autotransplantation. Histological and oestrous cycle activity of autotransplanted animals were then assessed.

Results: Our results showed that in the control group, 37 (94/87%) and in experimental group 36 (90%) of surgeries were successfully transplanted. The first full cycle and 5th full cycle were seen respectively 12-23 and 28-43 days after transplantation in experimental group. 36 (33/33%) rats had irregular cycles in this group. In the control group, these cycles completed 11-20 and 28-35 days after transplantation and 5 (26/31%) rats also showed irregular cycles. 3 days after transplantation in each group, all follicles except primordial and some primary follicles were atretic. Histological assessment of the ovarian graft with regular cycle showed that the average number of primary follicles per section increased in b subgroup of experimental group. There were no significant differences in number of other follicles between the groups.

Conclusion: Oestrous cycle restoration and ovarian follicular reserve was similar between the two groups. It seems that hyaluronic acid hydrogel is not sufficient alone to accelerate return

Keywords: Ovarian Vitrification, Ovarian Transplantation, Oestrous Cycle, Hyaluronic Acid Scaffold

P-81: Effect of Ascorbic Acid and Albumin in The Sperm Selection Medium

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Background: Several methods for sex selection of embryos have been used in research centers and infertility centers till now.

Materials and Methods: In this research, the effect of medium, by placing the mouse (NMRI) spermatozoa in the medium containing certain concentrations of albumin and ascorbic acid, separation of spermatozoa containing X and Y chromosomes and doing *in vitro* fertilization (IVF) and embryo transfer was studied. In this case study, the mentioned mouse spermatozoa which was separated and located in IVF dish, near the same race mice oocytes by the injection of pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (HCG) and were ovulation stimulated. Then, wards fetuses were transferred to the faster mother.

Results: Statistical analysis was carried out by using the software package SPSS 16. The average percentage of male infants born in experimental groups is significantly different from the control groups ($p < 0.05$). Out of fifty surrogate mother were pregnant by help of sperm separated in the presence of albumin and ascorbic acid. The achievement of questioned sex in the presence of 750 μm concentration of ascorbic acid was, 75.4% and in the control group was, 49.9%.

Conclusion: The Addition of ascorbic acid and albumin caused decrease of peroxidation of lipid in the medium and it accepts the antioxidant of the vitamin and it makes radicals free from the sperm medium. This method is suitable for sperm containing X and Y chromosomes separation and male sex selection in fertile couple and non-manipulation of fetus is a safe method.

Keywords: Albumin, Ascorbic Acid, IVF, Mouse, Sex Selection

P-82: Study The Anti-Inflammatory Effect of 1-(1-(4-Chlorobenzoyl)-6-Methoxy-2-Methyl-1H-indol-3-yl) Acetohydrazide on *In Vitro* Sperm Activation of Astheno-leuko-zoospermic Infertile Men

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Background: Different urogenital disease and damage factors, such as varicocele, inflammation, and smoking, are well known to influence the balance between radical production and antioxidant defense. The objective of the present study was to select an efficient Non-steroid Anti-inflammatory Drugs (NSAIDs) concentrations of sperm activation *in vitro* for patient complaining from astheno-leuko-zoospermia. *In vitro* sperm activation was performed on seminal fluid samples by using simple layer technique for three incubation period (30, 45, 60) in Earl's medium to select the efficient concentration and incubation period. NSAIDs inhibit the cyclooxygenase (COX) activity resulting in decreased synthesis of prostaglandin, leukotriene and thromoxane precursors.

Materials and Methods: Semen samples were obtained

from Astheno-leuko-zoospermia infertile men. This study includes 40 subfertile male partners between July 2013 to April 2014, from couples who had consulted the infertility clinic of the Babil hospital of maternity (Hilla city/IRAQ). The Semen specimens were divided into four fractions. First part was control, 0.5ml of liquefied semen mixed with 0.5 ml Earl's medium and incubated at 37°C for three incubation periods. Second part (antibiotic con.1), 0.5ml liquefied semen was mixed with 0.5ml Earl's medium supplemented with 0.02 mg/ml antibiotic and incubated at 37°C for 30,45,60 incubation periods. Third part (antibiotic con. 2), 0.5ml liquefied semen was mixed with 0.5ml Earl's medium supplemented with 0.04 mg/ml antibiotic and incubated at 37°C for 30 minutes. Fourth part (antibiotic con. 3), 0.5ml liquefied semen was mixed with 0.5 ml Earl's medium supplemented with 0.06 mg/ml antibiotic and incubate at 37°C for three incubation period. After semen specimen treated in all fractions, they were examined and assessed for macroscopic and microscopic changes.

Results: The treatment of low-level leukocytospermia with present antibiotic shows no significantly enhancement in the semen parameters among the treated patients. Low-level leukocytospermia resolved in good percentage of the treated groups.

Conclusion: The results of this study indicated that adding present antibiotic to the culture medium for *in vitro* sperm activation leads to non significant improvement in certain sperm function parameters.

Keywords: Anti-inflammatory Effect, Cyclooxygenase, Prostaglandin, Leukotriene, Thromoxane Precursors

P-83: Study The Effect of Oral Zinc Supplementation on The Stability of Sperm Membrane of Patients with Astheno-spermia

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Background: Since cellular membranes play key role in the physiological pathways of the cell, such as fertilization through almost every cellular function to cell division, it is very important to assess the structural and functional activity of the sperm membrane. In this study, a new method for measurement of sperm membrane stability measured the amount of zinc which liberated after being exposed to stress resulting from specific concentrations of mercury for a period of time. Membrane that resists or prevents liberation of zinc as a result to the existence of mercury is more stable than that which liberated zinc with high concentration due to the same effect and vice versa.

Materials and Methods: Semen samples were obtained from 60 fertile and 60 subfertile men with astheno-zoospermia between July 2011 to July 2012, from couples who consulted the infertility clinic of the Babil hospital of maternity (Hilla city/ Iraq). The subfertile group consists of the patients who were treated with zinc sulfate. Every participant took two capsules of zinc sulfate per day for three months (each one 220 mg). Semen samples were obtained (before and after zinc sulfate supplementation). After liquefaction seminal fluid at room temperature, routine semen analyses were performed. Stability of cell membrane was measured by using the method described above for a number of samples (37 fertile (age 31.4 ± 4.2 year) and 37 subfertile (age 32.3 ± 3.68 year) men with astheno-zoospermia).

Results: Stability of Sperm Membrane significantly decreases in infertile group ($83.6 \pm 4\%$) when compared with healthy donor group ($91 \pm 5\%$). The Stability of Sperm Membrane was significantly

($p < 0.005$) increases (return to normal value) after zinc sulfate supplementation ($89 \pm 7\%$).

Conclusion: Zinc Supplementation increases the Stability of Sperm Membrane.

Keywords: Zinc Supplementation, Asthenozoospermia, Stability of Sperm Membrane

P-84: Blastocysts Production and Collection in Albino Hamster Using Superovulation and Intrauterine Artificial Insemination in Non-Breeding Season

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Background: Due to the seasonality reproduction of albino hamster (April to October in the Northern Hemisphere) and the need to produce blastocysts for research applications, producing and collecting blastocysts by natural mating in non-breeding season is complex in this species. In the present study *in vivo* blastocysts production and collection using super-ovulation and intrauterine insemination was investigated in albino hamster.

Materials and Methods: Twenty young adult female albino hamsters were identified in the diestrus phase in winter (non-breeding season) using vaginal smears. Pregnant mare's serum gonadotropin hormone (25 IU, PMSG) was injected intraperitoneally. Hamsters were divided into two groups; in 48 hours and 56 hours later 25 IU of human chorionic gonadotropin (hCG) hormone was injected intraperitoneally into each hamster. After that each group was divided into two natural mating and artificial insemination subgroups. Each female hamster of natural mating group was mated with a fertile male after hCG injection and the next morning the vaginal plug was controlled. For artificial insemination, 12 hours after hCG injection, through surgical exposure of each uterine horns; artificial insemination was done in both horns separately by intrauterine injection method with 1×10^8 sperms which were collected from albino male adult hamster tail of epididymis. 3.5 days after mating or insemination, flushing was done with phosphate buffered saline solution to collect blastocysts. Blastocysts were counted and evaluated with a loop microscope.

Results: Methods of natural mating (48 and 56 hour hCG) and artificial insemination (48 hour hCG) were without blastocyst. But method of 56 hour hCG and artificial insemination lead to produce 5 ± 15 blastocyst in each albino hamster in winter. Surgery and artificial insemination has no impact on the quality of collected blastocysts.

Conclusion: This study presented a suitable timing, rapid and practical method to produce and collect blastocysts using super-ovulation and intrauterine insemination in albino hamster in non-breeding season.

Keywords: Blastocysts, Albino Hamster, Superovulation, Artificial Insemination, Non-breeding Season

P-85: Evaluation of Sperm Fertility Quality in Phenyl Hydrazine-Induced Hemolytic Anemia Impacts on Mice

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Background: Anemia and consequently low oxygen can cause changes in the function and structure of the testis and spermatogenesis. Iron released from red blood cells, followed by the increased oxidative stress, which may cause tissue iron. This study was designed to minimize the deleterious effects of hypoxia on the genitals.

Materials and Methods: Eighteen mature male mice were randomly divided into 3 groups. In order to induce experimental anemia, PHZ was administered at dose of 8 mg/kg, ip and then it was continued as 6 mg/kg, every 48 hours, ip in all groups. The test groups subdivided into non-treated PHZ-received, royal jelly-received (100 mg/kg, orally) and vitamin C-received (250 mg/kg, ip). After 35 days Semen samples were collected from epididymis for IVF study with healthy oocytes.

Results: The results of this study showed that anemia-related hypoxia resulted in a drastic reduction in quantity fertilized oocytes, The two-cell stage oocytes and blastocyst, and increase the number of stopped Oocytes.

Conclusion: These results indicate the effect of the anemia-related hypoxia on sperm Fertility quality. These results indicate the effects of oxidative stress caused by anemia (hypoxia) induced by phenylhydrazine on the in-vitro fertilization quality of mouse sperm.

Keywords: Phenylhydrazine, Hemolytic Anemia, IVF

P-86: Protective Effect of Vitamin C and Royal Jelly on Sex Hormones on Phenylhydrazine Treated Adult Male Mice

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Background: Phenylhydrazine (PHZ) is used to induce experimental anemia in animal models. On the other hand royal jelly is known as antioxidant compound. Therefore, here in present study we aimed to evaluate the protective effect of royal jelly on PHZ-induced damages in testicular tissue and compare its impact with vitamin C.

Materials and Methods: Eighteen mature male mice were randomly divided into 3 groups. In order to induce experimental anemia, PHZ was administered at dose of 8 mg/kg, ip and then it continued as 6 mg/kg, every 48 hours, ip in all groups. The test groups subdivided into non-treated PHZ-received, royal jelly-received (100 mg/kg, orally) and vitamin C-received (250 mg/kg, ip). After treatment period, blood samples were taken and their serum was separated. Testosterone was assessed by Radioimmunoassay (RIA) method, and Lutenizing (LH) and follicle stimulating hormone (FSH) were assessed by enzyme-linked immunosorbent assay (ELISA) with special kits. The data were analyzed by SPSS (Version 20; SPSS Inc., Chicago, USA) one way ANOVA and TUKEY tests. A p value of less than 0.05 was considered significant.

Results: A significant decrease in serum hormone test hormone levels (testosterone, FSH, LH) in the same control group compared with the control group and the administration of royal jelly and vitamin

C serum levels of these hormones, compared with the same control group. significant increase.

Conclusion: Toxicity in reproductive organ was performed by oxidative stress due to administration of Phenylhydrazine. According to toxic effect of Phenylhydrazine on hypothalamus-hypophysis-gonadic axis, this drug caused a notified reduction in sex hormones. Vitamin C and Royal Jelly as a potential antioxidant agent has been able to increase the sex hormone levels and protect reproductive system against the deleterious effects of oxidative stress due to Phenylhydrazine that usually cause infertility. Therefore, Phenylhydrazine through oxidative stress disturb the hormonal system and vitamin C and royal jelly as a nutrient antioxidant has protective role in toxic effects of this drug.

Keywords: Phenylhydrazine, Royall Jelly, Vitamin C, Sex Hormones, Adult Male Mice

P-87: Nicotine and Caffeine Intake in Relation to Purported Decline of Semen Quality and Ovulatory Disorder Infertility

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Background: Reproductive function and fertility are thought to be compromised by behaviors such as cigarette smoking, substance abuse, and caffeine consumption; however, the strength of these associations are uncertain. Many studies have examined whether caffeine and nicotine containing these affect fertility in men and women.

Materials and Methods: In this study we evaluate the literature concerning the relationship between Nicotine and Caffeine intake and adverse effects on fertility as well as examining the evidence for a role of Nicotine and Caffeine in the purported decline of semen quality and the ovulatory disorder infertility, as well as analyzing the data Collected, with SPSS version 16.

Results: Cigarette smoking (nicotine intake) among women and men was associated with decreased fecundability (95% confidence interval = CI). Caffeine consumption of 100 mg or less versus more than 100 mg in women and men was not associated with fecundability (95% CI). Decreases were observed among women who were coffee drinkers (95% CI) and men who were heavy tea drinkers (95% CI), regardless of caffeine content. There were 20% incident report of ovulatory disorder infertility during follow-up. Intakes of caffeine were unrelated to the risk of ovulatory disorder infertility. The multivariate-adjusted relative risk, (95% CI), p for trend comparing the highest to the lowest categories of intake were 1.11 (0.76-1.64; 0.78) for Cigarette smoking and 0.86 (0.61-1.20; 0.44) for total caffeine. The multivariate-adjusted RR (95% CI), and p for trend comparing the highest to lowest categories of caffeinated soft drink consumption were 1.47 (1.09-1.98; 0.01).

Conclusion: The association between nicotine and ovulatory disorder infertility appears to be attributable. Moderate and low caffeine intakes do not have effect on fecundability in men and women. Combined taking nicotine and caffeine are directly related to reduce fertility in both women and men.

Keywords: Nicotine, Caffeine, Semen Quality, Ovulatory Disorder, Infertility

P-88: Expression Pattern of Maturation Genes During *In Vitro* Culture of Alginate Encapsulated Preantral Follicles Derived From Frozen-Thawed Mouse Ovaries

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Background: This study was set up to evaluate the effect of ovarian tissue slow freezing on *in vitro* growth and pattern of maturation genes expression in mouse preantral follicles encapsulated within alginate hydrogel.

Materials and Methods: Ovaries of 12-14 days old female NMRI mice were randomly allocated into control and slow freezing groups. In slow freezing group, ovaries were equilibrated in the cryoprotectant solution using dimethyl sulfoxide (DMSO), and then were cooled in a programmable freezer according to Min Xu et al., 2009. Ovaries were thawed in a stepwise manner and their morphology was studied histologically (HandE staining). Then in both control and experimental frozen-thawed groups, pre-antral follicles were mechanically isolated from ovaries and cultured for 12 days in 0.7% alginate hydrogels. Survival rate, follicular growth, antrum formation and relative expression of maturation genes (Bmp15, Gdf9, Fgf8, Igf1, Kit, Kit-l) was assessed after 1, 8 and 12 days of culture and finally maturation rate of oocytes was studied.

Results: Morphological integrity of ovarian tissue was similar in both groups. However after *in vitro* culture significant lower survival and antrum formation rates were observed in slow freezing group compared to the control one (p<0.05). At the end of culture period, no significant difference reported in oocyte maturation rate (p>0.05). After 12 days of culture, Preantral follicles in cryopreserved group showed similar pattern of maturation genes expression compared to the control group. The pattern of Bmp15, Gdf9, Fgf8, Kit and Kit-l were down regulated during culture. Also, the expression of Kit and Kit-l in slow freezing group was lower than control group at day 8 of culture.

Conclusion: Although slow freezing of ovarian tissue reduces the survival and antrum formation rate of encapsulated cultured pre-antral follicles, it could not modify the expression pattern of maturation genes and oocytes maturation capacity.

Keywords: Maturation Genes, Preantral Follicle, Slow Freezing of Ovarian Tissue, Alginate

P-89: Role of Nutrition Diet with and without Mineral-Vitamin Elements on Superovulation of Mice

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Background: The quality and quantity of the diet has undeniable effects on the reproduction of organisms. We examined the effects of two diets (with and without mineral supplementation-Vitamins) on super-ovulation in two strains of mice (B6D2F1 and NMRI) in Royan Institute, Tehran.

Materials and Methods: For this purpose, 40 female mice (21 days) from each strain were divided into four experimental groups (n=10) including: (1) NMRI mice with diet supplements, (2) in NMRI without supplements (3) B6D2F1 mice with diet supplements and (4) B6D2F1 without supplements. The animal received food and water ad libitum in controlled standard conditions. At the age of six weeks, mice were treated (IP) with 7/5 IU PMSG and 7/5 IU hCG. The next morning, after cervical dislocation, all of the oocytes were counted. Data collected for each group included the total numbers of oocytes and the numbers of dead oocytes. The data were analyzed using the software SPSS16.

Results: At the beginning of the period (21 days), there was no significant difference in weight between different strain (Average Weight 10/8 g). Weight differences between the strains that started from age 42 days to 49 days continued (end of period) (P <0/05). NMRI (mean weight at the end of period 25 g) compared with B6D2F1 (mean weight end of period 18/5 mg), despite identical initial weight and eating, at 49 days of age were significantly more weight. On the other hand, the rate of ovulation between experimental groups were similar, but the rate of degeneration of oocyte in NMRI with diet supplements (mean = 2/5) were significantly less than NMRI without supplements (mean = 6/1) and B6D2F1 with diet supplements (mean = 6). Also, rate of degeneration in B6D2F1 without supplements compared with other groups (3/5) were no difference.

Conclusion: Result of this study demonstrates that significantly decreased degeneration of oocyte in NMRI mice than in B6D2F1 can be interpreted on the basis of genetic differences and differences in nutritional requirements.

Keywords: Diet, Superovulation, Mice

P-90: Effect of Phosphodiesterase Type 3 Inhibitor, Cilostamide, on The Developmental Competence of Ovine Oocytes Isolated by Glucose 6-Phosphate Dehydrogenase Activity

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Background: The developmental competence of oocytes matured *in vitro* (IVM) is yet far below than *in vivo* counterparts. Recent studies suggest that the asynchrony between nuclear/cytoplasmic maturation and the initial low/heterogeneous quality of oocytes

are the most important factors affecting IVM success. We investigated whether selection of growing oocytes (based on their glucose 6-phosphate dehydrogenase (G6PDH) activity) and chemical induction of nuclear/cytoplasmic synchrony (through transient inhibition of meiosis resumption with cilostamide) can improve developmental competence of sheep oocytes

Materials and Methods: Abattoir-derived oocytes were stained with 26µM BCB for 45 minutes to isolate growing (high G6PDH activity) and fully grown (low G6PDH) oocytes according to their differential capacities in breaking BCB and retaining colorless (BCB-) or blue (BCB+) ooplasm, respectively. Then, BCB- and BCB+ oocytes were incubated with 1 µM cilostamide for 6h before culture in normal IVM medium. Then, matured oocytes were used for embryo development assessment using parthenogenetic activation

Results: Cilostamide delayed meiotic progression in BCB- ovine oocytes. The cleavage, blastocyst and hatching rates in BCB- oocytes that treated by cilostamide were higher than that of control group, although these increases were not statistically significant

Conclusion: We concluded that increase of ovine oocyte cAMP concentration during two-step culture partially improves yield and quality of *in vitro* embryo. This also may suggest that phosphodiesterase type 3-mediated inhibition of cAMP activity is not the only mechanism that controls the process of nuclear maturation in ovine oocyte

Keywords: Ovine, BCB-Oocytes, Phosphodiesterase, Two-Step Culture System, Embryonic Development

P-91: The Effects of Mesenchymal Stem Cell- Conditioned Medium on The Fertilization Rate and Embryo Development in NMRI Mice

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Background: Normal growth of oocytes, embryos and also successful implantation depend on environmental factors such as the secretion of cumulus cells and composition of follicular and tubular fluids. Therefore, addition of serum, conditioned medium (CM) to the culture medium and also co-culture with somatic cells improve mammalian embryo development. So, in the respect to the secretion of different growth factors and cytokines by mesenchymal stem cells (MSc), the present study shows the effects of MSc-derived CM on fertilization rate and early embryo development.

Materials and Methods: 60 female and 10 male NMRI mice were used to obtain the MII oocytes and capacitated sperm for IVF in the following groups: The basic fertilization medium (BFM) in control group contained T6+BSA 15mg/ml, for treated groups 1-3 was added respectively 25,50 and 75% of MSc-derived CM to BFM. The fertilized oocytes transferred to embryo developmental medium (T6+BSA 4mg/ml) for 72-hour. All data was analyzed by ANOVA in SPSS 16.0 software.

Results: The study showed statically a difference on fertilization rate in the treated group 3 in comparison with other ones. The results didn't show any difference on embryo developmental rate.

Conclusion: It concluded that adding MSc-derived CM (50%v/v) may improve early embryo cell division.

Keywords: *In Vitro* Fertilization, Conditioned Medium, Mesenchymal Stem Cell

P-92: Assessment of Stem Cells in Adult Mouse Ovaries during Estrous Cycle

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Background: The concepts of reproductive biology were changed by stating the ovarian reserve in postnatal mammalian females is replenished. The aim of this study was to investigate the expression of OCT4 in the mice ovarian tissue during different stages of mouse estrous cycle.

Materials and Methods: The mice were considered as pro-estrous, estrous, met-estrous and di-estrous based on the cell type of the vaginal smear. Real time PCR of complementary cDNA of pluripotency marker mRNA OCT4 of ovarian tissue was performed to evaluate the expression pattern.

Results: The results showed that the rate of OCT4 gene expression was significantly lower in the pro-estrous than other phases.

Conclusion: Our results may suggest that adult mice ovaries accommodate cells carrying stem cell features.

Keywords: Ovarian Stem Cells, OCT4, Estrous Cycle

P-93: Understanding The Different Kinds Factors of Infertility in Men and Women in order to Improve The Treatment of Infertility

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Background: Infertility in couples who fail to get pregnant after one year of sexual intercourse without contraception is called. approximately 40% of infertility problems in men, 40% women and 10% of androgynous, 10% is unknown. Therefore, the awareness of the different kinds of infertility treatment is effective.

Materials and Methods: Study overview, the present Internet search at timescales between 2000 - 2013 the databases Iranian and International Scopus, ScienceDirect, PubMed, Cochran Library, ISI, magiran, Springer, Elsevier, SID iranmedex, EMBASE, Uptodate with a gathering of 126 articles that ultimately 88 articles with keyword: infertility, male factor, female factor, treatment of infertility, promotion examined.

Results: Factors for male infertility include a path blocks sperm, sexually transmitted infection, chronic diseases, impaired erection or impotence, impaired sperm production, Ray exposure, destructive and harmful substances, genital trauma, varicocele, azoospermia, Klinefelter syndrome and other congenital anomalies including abnormalities in women with uterus and cervix system disorders, disorders of the hypothalamus, pituitary, adrenal, thyroid, fallopian tube obstruction due to inflammatory disease or endometriosis, ovulation disorders, obesity, mental stress, high levels of the hormone prolactin, early menopause and ovarian agenesis, disease and sexually

transmitted infections, Turner syndrome, malignant tumors, immune system problems noted.

Conclusion: The review and follow-up causes of infertility is necessary to use a variety of methods of assisted reproduction. the age, duration of infertility, ovulatory status, underlying disease, sperm tests for deciding which treatment to use and effective reproductive health.

Keywords: Infertility, Male Factor, Female Factor, Treatment of Infertility, Promotion

P-94: The Effect of Calcium Ionophore A23187 and Ethanol on Parthenogenetic Activation of Mouse Oocytes in Presence of Hydrostatic Pressure and Cytochalasin B

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Background: Parthenogenetic activation of mammalian oocytes using artificial stimuli is commonly used in various reproductive bio-techniques. Calcium ionophore is known to elevate intracellular calcium levels in the cytoplasm of oocytes through the influx of calcium from extracellular spaces. Ethanol promotes a single intracellular Ca²⁺ increase of greater and longer amplitude than the initial increase observed at fertilization. Hydrostatic pressure can act as a mechanical stimulator that rearranges egg contents. In this study, we investigated the effect of calcium ionophore and ethanol on parthenogenetic activation of mouse oocytes in presence of hydrostatic pressure.

Materials and Methods: 6 to 8-week-old female NMRI mouse were super-ovulated by an injection of 10 IU of PMSG, followed by 10 IU HCG 48 hours later. Metaphase II oocytes were collected from oviduct 14 hr after HCG injection. Oocytes transferred to T6 medium supplemented with different concentration of calcium 0, 1.7, 3.4 mM (treatments, II), and then treated with temporal sequential combinations of 2 chemical activators [5µM of calcium ionophore (CI) for 5 minutes (group 1), and 7% ethanol (ET) for 5 minutes (group 2)] followed by exposure to 20 mmHg pressure for 20 minutes. Oocytes from two groups were transferred to T6 medium supplemented with different concentration of calcium and 5µg/ml CB for 4 hours. Oocytes were cultured for 72 hours and embryo development was assessed.

Results: In 1 and 2 group treatments, II, the percentage of cleavage was 20.42, 82.78, 51.6, 18.51, 69.63, 45.36%, respectively. Cleavage rate in 1 group was higher than 2 group (p<0.05). The highest cleavage rate associated with treatment II which were significantly different with treatments, and 2 group (p<0.05).

Conclusion: Exposure of oocytes to HP, followed by exposure to CI could improve embryonic development and by effecting calcium channels leads to increase rate of cleavage in the mouse oocyte, probably.

Keywords: Parthenogenetic Activation, Hydrostatic Pressure, Calcium Ionophore, Ethanol, Cytochalasin B

P-95: Influences of Metabolic and Nutritional Status on IVF Outcomes

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Background: The aim of our study was to determine the metabolic profile: glucose, triglycerides (TG), high density lipoprotein (HDL) and cholesterol (CHT) in women undergoing ART (Assisted reproductive technology) cycles and estimate its effect on *In Vitro* Fertilization (IVF) outcomes

Materials and Methods: Forty three women undergoing IVF treatment were included in the study. Patients were allocated into three groups according to body mass index (BMI), normal weight, overweight and obese. Blood samples were collected at the time of ovum pick up to determine metabolic profile. Higher levels of TG in obese women is negatively correlated with embryo quality and fertilization rate ($p=0.032$ and $p=0.0196$). CHT levels are also negatively correlated with oocyte maturity and quality. Sperm and oocytes preparation techniques for intracytoplasmic sperm injection and evaluation of embryo quality were interpreted according to WHO criteria.

Results: Dyslipidemia is the responsible for impaired oocyte quality by the increased production of reactive oxygen species unfavorable to the development of preimplantation embryo. Hyperglycemia was found in obese women and is negatively correlated with oocyte maturity, fertilization rate and segmentation rate. This Hyperglycemia-induced apoptosis of progenitor cells in the embryo can affect differentiation of the remaining cells, manifesting later as malformations or miscarriages. Potential influences of maternal nutritional status in obesity are indicated by reports showing that periconceptual exposure to high energy substrates such as fatty acids and glucose results in perturbed oocyte and embryo mitochondrial metabolism. Altered oocyte and early embryo mitochondrial metabolism may be responsible for poor reproductive outcomes frequently reported in obese women.

Conclusion: Encouraging weight loss in obese patients before responding to a request for medical assistance to procreation, should definitely integrate our practices.

Keywords: Body Mass Index, Glucose, IVF, Cholesterol

P-96: Appositional Expressions of Cyclin D1 and E2F1 Gene Machineries in Mycoestrogen Zeralenone-Induced Apoptosis in Testicular Tissue of Rats

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Background: Zearalenone (ZEA) is known as a nonsteroidal oestrogenic mycotoxin produced by different species of *Fusarium* fungi. ZEA is known for its competitive effects with the natural 17- β estradiol to bind with the specific binding sites of the estrogen receptors (Ers). On the other hand, the cyclin family (especially cyclin D1) and E2F1 genes are the checkpoint genes involved in cell cycle. The Ers are directly involved in controlling the checkpoint genes expression. The present study was designed to uncover the effect of ZEA

on cyclin D1 and E2F1 genes expression.

Materials and Methods: Thirty mature male rats were divided into four groups including; control-sham (2 mL, normal slain, ip), low dose ZEA-treated (1mg/kg, ip), medium dose ZEA-treated (2mg/kg, ip), high dose ZEA-treated (4mg/kg, ip). All animals received chemicals for 21 continuous days. The mRNA levels of cyclin D1 and E2F1 were analyzed using semi-quantitative RT-PCR. The cyclin D1 protein expression was evaluated in germinal epithelium by using immunohistochemical analyzes. The cellular apoptosis in testicular tissue was evaluated by using DNA laddering test.

Results: Low and high dose ZEA-treated animals showed cyclin D1 and E2F1 over-expression, while the animals in medium dose ZEA-treated group exhibited a remarkable reduction in cyclin D1 expression. However, the E2F1 over-expression was manifested in medium dose ZEA-received group. ZEA, in dose dependent manner, resulted in intensive DNA fragmentation.

Conclusion: Our data showed that, ZEA affects the cyclin D1 and E2F1 genes expression. Accordingly, cyclin D1 promotes the apoptotic pathway in low and high doses and competes with natural 17- β estradiol in low dose of administration and in turn provokes cellular arrest. However, by different mechanism followed by severe DNA damage, over/reduced-expression of E2F1 stimulates cellular cycle arrest in ZEA-induced testicles.

Keywords: Zearalenone, Cyclin D1, E2F1, DNA Ladder, Cell Cycle

P-97: A Novel Staining Method of Mouse Ovarian Tissue for Comparison of Two Different Methods of Vitrification

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Background: Different cryoprotectants are used for Cryopreservation of ovarian tissue in patients who are at risk of infertility. Ethylene glycol (EG), dimethyl sulfoxide (DMSO) and propanediol (PROH) were chosen as the basic permeable cryoprotectants due to their less glass-formation characteristic compared to the others. In the present study, the effects of two different vitrification methods on whole mouse ovarian tissue by using a novel staining method (Trypan Blue) was evaluated.

Materials and Methods: Ovaries of 8 days-old NMRI mice were isolated and distributed into control, Vitrification 1 (Vit1) and Vitrification 2 (Vit2). Vit1 and Vit2 solutions were composed of (α -MEM+ 20% FBS + 15% EG + 15% DMSO) and (α -MEM+ 15% FBS +20% EG + 20% PROH) respectively. Vit1 and Vit2 procedures were done in 4°C and room temperature respectively. Warming was performed in α -MEM+ 20% FBS that supplemented with 1M sucrose in Vit 1 group and α -MEM+ 15% FBS and descending concentration of sucrose (1,0.5,0.25 M) in Vit2 group. Control and vitrified warmed Ovaries were put in α -MEM that supplemented by 4% trypan blue for 15 minutes, then stained ovaries were fixed in bouin's fixative, serially sectioned in paraffin wax and finally quantitatively evaluated under light microscope.

Results: The highest percentage of primordial follicles was observed in control group (59.73 ± 5.87) and a significant difference was seen between control and Vit1, also between Vit1 group (26.27

± 3.04) and Vit2 group (45.98 ± 6.38, p<0.05). No significant difference was observed in primary and preantral follicles between control and vitrification groups.

Conclusion: Trypan blue staining is a faster and easier method for evaluation of the ovarian tissue and also vitrification 2 protocol is better for preservation of follicles reserve in ovary.

Keywords: Ovarian Tissue, Vitrification, Trypan Blue Staining

P-98: Study of The Protection Effect of Ascorbic Acid on Human Sperm's Chromatin Quality with Acridine Orange Test, After Mobile Phone Radiation

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Background: Human and animal studies have suggested a relationship between mobile phone use and semen quality and high level of sperm DNA damage due to RF-EMW have been reported. In the present study a fluorescence method has been used to search for abnormal chromatin condensation and the protection effect of ascorbic acid on human ejaculated semen sperms was performed.

Materials and Methods: Semen samples were obtained from fertile males. Followed by liquefaction, each sample was divided equally into 3 parts: One aliquot fresh semen as control, second aliquot was exposed to cellular phone radiation for 10 minutes continuously, and third aliquot exposed to cellular phone radiation for 10 minutes continuously treated with ascorbic acid (10 µg/ml). A drop of semen was spread on the glass slides and allowed to air-dry and fixed in carnoys fixative (methanol/acetic acid, 3:1) overnight. The slides were then stained with 2-3 cc acridine orange solution for 15 minutes and gently rinsed with PBS. The sperms were evaluated with fluorescence microscope (Olympus IX 71, Japan). Three types of staining patterns were identified: green sperms (double-stranded DNA), yellow and red sperms (single-stranded DNA). Data were analyzed by one-way ANOVA using SPSS version 16 software.

Results: Our result showed that exposure group revealed a significant decrease the percentage of yellow and red sperms in the second exposed groups as compared to control groups (p≤0/05). In addition, ascorbic acid induced significant increases the percent of acridine orange positive sperm in third groups as compared to the second mobile phone exposed groups (p≤0/05).

Conclusion: Mobile phones cause changes in DNA fragmentation and ascorbic acid protecting sperm chromatin against cell phone radiation.

Keywords: Cell Phones Exposure, Ascorbic Acid, Acridine Orange and Sperm Chromatin

P-99: The Effects of Zinc in Vitrification Medium on In Vitro Growth of Follicles Derived from Vitrified-Warmed Mouse Ovary

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Background: The development of a reliable method for the cryopreservation of mammalian ovary would be an important advancement in the field of reproductive biology for the preservation of genetic resources. The cryopreserved follicles have the potential to develop in-vitro; however, the developmental rate is lower than that of fresh follicles. Researchers have used that of different cryoprotectants and various techniques to improve the cryopreservation of ovaries despite significant recent progress, the efficiency of ovary cryopreservation is still low. There are many reports on adding some materials such as calcium, ascorbate and antioxidants to freezing medium. The necessity of inorganic elements in biological systems is well established. Zinc is an integral component of hundreds of enzymes, transcription factors, and other molecules involved in a variety of biological functions, and the homeostasis of this trace element is tightly regulated. Zinc, in particular, has gained attention as key agent in cellular signaling processes. The present study was designed to determine whether different zinc concentrations in the ovary vitrification solutions could improve the developmental growth and competence of follicles derived from vitrified ovaries.

Materials and Methods: In this experimental study, the ovaries of 2-4 week-old NMRI mice randomly assigned to following groups :V0(vitrified-warmed ovaries without any zinc in vitrification solution),V1,V2,V3 (vitrified warmed ovaries with 100,150 and 200 µg/dl zinc concentration in vitrification solution,N-v (none vitrified ovaries). Ovaries in the vitrified groups were vitrified sequentially by immersion into two vitrification solutions VS1: 7.5% ethylene glycol (EG) + 7.5% DMSO in holding medium (α-MEM + 20% FBS) for 7 minutes and VS2: 15% EG + 15% DMSO for 3 minutes in holding medium and vitrified by straw and were kepted in LN2 tank for a week. After one week, the ovaries were thawed at temperature room in 1.0,0.5 and 0.25 M sucrose. Vitrified ovaries as well as non-vitrified ovaries were serially sectioned and examined histologically. Pre-antral follicles (specified with a diameter around 100-130 µm) were mechanically isolated from vitrified-warmed and fresh ovaries and cultured for 9 days.

Results: The results show that the presence of zinc in vitrification solution is effective and can reduce the traumatic effects of vitrification. Follicle viability, growth and survival rate was better preserved in the 200 µg/dl zinc concentration group in comparison to other vitrified-warmed groups. Nevertheless, it was less than that of none vitrified group (Anova, p value<0.05). Vitrification by using EG and DMSO is an efficient procedure for cryopreservation of ovaries.

Conclusion: The results of this study demonstrate that zinc supplementation of vitrification medium in a time and dose manner improved the follicle growth in follicles derived from vitrified-warmed ovaries.

Keywords: Vitrification, Zinc, Follicle Growth, Ovary, Mouse

P-100: Improved Efficiency of Somatic Cell Nuclear Transfer in Sheep by Perse-cution of Recipient Oocytes with Brilliant Cresyl Blue Staining

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Background: The most important factor affecting the efficiency of somatic cell nuclear transfer (SCNT) is the initial quality of the oocyte. Since the abattoir-derived oocytes are heterogeneous in quality, selection of cohort fully grown/competent oocytes is necessary for proper development of SCNT embryos.

Materials and Methods: Glucose-6-phosphate dehydrogenase (G6PDH) is actively expressed in growing oocytes, but decreased in activity as oocytes developing to fully grown state. We selected growing and fully grown sheep oocytes based on G6PDH reaction with brilliant cresyl blue (BCB); growing oocytes (unfinished growth phase with abundant G6PDH) degrade BCB and remain a colorless cytoplasm (BCB-) while fully grown oocytes (with reduced G6PDH activity) are unable to degrade BCB leaving a blue cytoplasm (BCB+) after incubation with 26 μ M BCB for 45 minutes. BCB- and BCB+ oocytes were separately matured *in vitro* and their reprogramming capacity and developmental competence were compared after SCNT by using a handmade method.

Results: The percentage of cleavage was significantly higher in cloned embryos reconstituted with BCB+ vs. BCB- oocytes (99.33 \pm 0.66% vs. 86 \pm 1.15%, respectively). Notably, cloned blastocyst development rate was significantly higher in BCB+ vs. BCB- oocytes (15.66 \pm 1.2% vs. 6.33 \pm 0.88%, respectively).

Conclusion: BCB staining test of immature oocytes is a useful method for selecting competent oocytes and improve SCNT in sheep through increasing the reprogramming ability of the cytoplasm.

Keywords: Brilliant Crysyl Blue, Oocyte, Sheep, SCNT

P-101: Developmental Competence of Bovine Vitrified GV Oocyte Selected by BCB

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Background: Cryopreservation of farm animal embryos is nowadays a widely used routine technique. Success in cryopreservation of farm animal depends on many factors including: freezing medium, types of cryoprotectants, melting process, lipid content in ooplasm and oocyte quality. It has been found that immature Oocytes with reduced reproductive performance or slaughtered at the end of their use are heterogeneous in quality. It is generally believed that glucose-6-phosphate dehydrogenase (G6PDH) protein is active in the growing oocyte, but its activity is decreased in oocytes that have finished their growth phase. Then, to have achieved developmental competence. It seems the enzyme G6PDH can degrade brilliant cresyl blue (BCB). Thus, oocytes yielding decreased G6PDH (finished growth phase) showing a blue cytoplasm (BCB+) after BCB staining, while growing oocytes (unfinished growth phase) have abundant G6PDH and a colorless cytoplasm (BCB-).

Materials and Methods: The oocytes were exposed to 26 μ M BCB and classified according to their cytoplasm coloration. After selection of Oocytes from each group, they were then vitrified by using a crytop. Cumulus-enclosed oocytes (3-5) were suspended in equilibration solution (HM containing) ethylene glycol (EG) and

dimethylsulfoxide (DMSO)) for 10 minutes to allow initial shrinkage and recovery. Following equilibration, they were transferred to the vitrification solution (HM containing EG, DMSO and 0.5 M sucrose) for 45-60 seconds. After thawing, Oocytes were then matured, fertilised, and cultured *in vitro* for 7 days. Viability following vitrification and warming, fertilisation events following IVF and subsequent re-implantation embryo development were evaluated

Results: Significant differences were observed in survival rates between BCB+ and BCB- oocytes in both vitrified. Cleavage was significantly higher BCB+ vitrified oocyte ($p < 0.05$) in in compared to BCB- oocytes (65.38 \pm 3.8 vs. 34.5 \pm 3.2% respectively). Blastocyst development rate was significantly higher in BCB+ vs. BCB- oocytes (13.76 \pm 1.2 vs. 2.4 \pm 0.88%, respectively).

Conclusion: Therefore, the BCB test is a useful method for selection of more competent immature bovine oocytes for oocyte cryopreservation

Keywords: Oocyte Cryopreservation, Vitrification, Brilliant Cresyl Blue (BCB), Bovine, Blastocyst

P-102: Evaluation of G6PDH Enzyme Activity in GV Oocytes Cytoplasm to Recommend The Effects of Vitrification Process

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Background: Process of oocyte freezing, seriously damages the molecular structure of the oocyte into the life and impair its ability to develop. It seems Oocytes can partially recompense those damages. The pentose phosphate pathway (PPP) constitutes the major source of NADPH required for the neutralization of reactive oxygen species (ROS). Glucose-6-phosphate dehydrogenase (G6PDH) is the first enzyme of the oxidative part of the PPP. This study was conducted to determine whether BCB staining performed on immature bovine oocytes to assess G6pdh level has an impact on quality bovine embryos product from GV vitrified oocyte.

Materials and Methods: We selected growing and fully grown bovine oocytes based on G6PDH reaction with brilliant cresyl blue (BCB); Immature oocytes were exposed to 26 μ M BCB concentration and classified according to their cytoplasm coloration as grown BCB+ (blue cytoplasm) and growing BCB- (colourless cytoplasm) After selection, oocytes were vitrified by using a crytop. GV oocytes (3-5) were suspended in equilibration solution (HM containing) ethylene glycol (EG) and dimethylsulfoxide (DMSO) for 10 minutes to allow initial shrinkage and recovery. Following equilibration, they were transferred to the vitrification solution (HM containing EG, DMSO and 0.5 M sucrose) for 45-60 seconds. After oocyte thawing the oocytes cultured in maturation media. For this study, biochemical and morphological events which were occurring during early embryonic development, parthenogenesis has been used. In order to evaluate the quality of blastocyst inner cell mass (ICM) and trophectoderm (TE), cell number between BCB+ and BCB- were determined by using a differential staining protocol.

Results: Cleavage was significantly higher BCB+ vitrified oocyte ($p < 0.05$) in compare BCB- oocytes ($53.6 \pm 6\%$ vs $40.7 \pm 4.2\%$ respectively). The percentage of Parthenogenesis blastocyst development was higher in BCB+ and was significantly different between BCB+ (15.01 ± 9.03) and BCB- (3.19 ± 7.48) oocytes. Total cell number, TE cell number, ICM cell number: TE ratio of blastocysts were significantly higher in the BCB+ group compared with the BCB- group.

Conclusion: The cytoplasm of BCB+ vitrified oocyte show more competency to repair damage of verification process and it make improve efficiently of gamete cryopreservation.

Keywords: Vitrification, G6PDH, Parthenogenesis, Differential Staining, Bovine

P-103: Connection of The G6PDH Activity on Fertilization and Developmental Competency in The Bovine

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Background: This study was conducted in order to determine whether BCB staining performed on immature bovine oocytes to assess G6pdh level has an impact on ivf/ivp technology in bovine embryos. Both spermatozoa and oocytes metabolize glucose through the pentose phosphate pathway (PPP), and NADPH appears necessary for gamete fusion. Activity of glucose 6-phosphate dehydrogenase (G6PDH), the key enzyme of the PPP, have a high in growing oocytes, while grown oocytes have low G6PDH activity. In additional it seems grown oocyte have more ability to developmentally competency, have the more rate of Sperm-oocyte fusion too.

Materials and Methods: Immature compact cumulus-oocyte complexes were stained with brilliant cresyl blue (BCB), used to indicate G6PDH activity. Based on their colouration, oocytes were divided into BCB (-) (colourless cytoplasm, high G6PDH activity) and BCB (+) (coloured cytoplasm, low G6PDH activity). The MII oocytes and complex sperm oocyte 16-18 hour post insemination were stained with DAPI to evaluate percentage of maturation and fusion of sperm and oocytes, and other oocytes were then matured, fertilized, and cultured *in vitro* for 7 days. Fertilization events following IVF and subsequent pre-implantation embryo development were evaluated. Embryo quality were assessed with deformational staining.

Results: The rate of mature oocytes at the MII stage differed significantly between BCB+ (85.5%) and BCB- (67.8%). Sperm-oocyte fusion rate were also significantly higher in the BCB+ group compared with the BCB- group. The percentage of blastocyst development was higher in BCB+ and was significantly different between BCB+ (39.25 ± 2.4) and BCB- (20 ± 7.48) oocytes. Total cell number, TE cell number, ICM cell number: TE ratio of blastocysts were significantly higher in the BCB+ group compared with the BCB- group.

Conclusion: These results confirmed that the staining of bovine cumulus oocyte complexes with BCB before *in vitro* maturation may be used to select developmentally competent oocytes for IVF.

Keywords: Sperm-Oocyte Fusion, IVF, Oocyte Quality, G6PDH, Embryo Quality

P-104: Effects of Prenatal Exposure to Electromagnetic Fields on Fertility of F1 Generation in *In Vitro*

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Background: Electromagnetic fields interact with human tissues and may have adverse effects on fertility and reproduction. During the last decades there has been a growing concern on the effects of electromagnetic radiations on biological systems in general. Radiation risks from electromagnetic fields generated by processes such as reduced fertility and a significant decrease in implantation rates and live births and incidence of congenital anomalies have been reported. This study evaluated the effects of electromagnetic fields in the prenatal period on fertility of F1 generation in *in vitro*.

Materials and Methods: In treatment group pregnant Wistar rats were exposed 3mT EMF for 21 days, 4 hours per day, in sham group pregnant rats were kept in similar condition but off the electromagnetic field and in the control group pregnant rats were maintained in the room condition. After the pups were kept until maturity, then *in vitro* fertilization (IVF) in Universal IVF Medium was performance, fertilized eggs were preserved in oven CO₂, 37°C. After 24 and 48 hours the number of multicellular embryos were assessed by light microscope.

Results: The results showed percentage of multicellular embryos in treatment group compared with the control were decreased, but no difference observed between sham and control groups.

Conclusion: Infertility is one of the major problems in match's life. The results of present study showed that rats exposed to magnetic fields during the development period at time puberty have impaired fertility and their fertility decrease. Due to fertility decrease were observed in this study, pregnant maternal exposure in magnetic field may be cause of sperm DNA damage that it regarded as a potential risk factor to the development of normal embryos leading to impaired embryonic development.

Keywords: Electromagnetic Field, Prenatal, Fertility, F1 Generation

P-105: Prenatal Effects Exposure to Extremely Low Frequency- Electromagnetic Field (ELF-EMF) on Pathology of Testis in Newborn Rats

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Background: Human beings are unavoidably exposed to ambient electromagnetic fields (EMF) generated from various electrical devices and from power transmission lines. The effects of extremely low-frequency electromagnetic fields (ELF-EMF) on the biological functions of living organisms represent an emerging area of interest for human health. One of the critical issues is that EMF may adversely affect the reproductive system. The aim of this work is to evaluate the effects of intrauterine exposure to 50Hz electromagnetic field (ELF-EMF) on testicular development.

Materials and Methods: Pregnant wistar rats were exposed to 3mT, EMF for 21 days (4 hours/day). Pregnant rats under the same condition of treatment group, but off the field as a Sham group intended and pregnant rats were used as control in the room. After delivery, testis were removed from male pups, fixed and prepared for light microscopic studies.

Results: Microscopic results revealed seminiferous tubules in treatment group were widely separated from each other, in this group in seminiferous tubules vacuolization, detachment of gonocytes from each other, heterochromatic gonocytes and decreasing in interstitial tissue in comparison with the control and sham groups was found. In sham and control groups the seminiferous tubules and interstitial tissue appeared normal.

Conclusion: Early pregnancy exposure to magnetic field affects gonads in prenatal male fetuses and postnatal testicular development. EMF can influence the mechanism of apoptosis and also lead to increased apoptosis rate of germ cells. In general, as a result of the exposure to EMF during early developmental period, morphological changes in testicular development were evident that may well extend till adult stage and may affect fertility.

Keywords: ELF-EMF, Testicular Development, Newborn, Rat

P-106: The Computer-Assisted Vacuolated Sperm Analysis and Zygote Quality

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Background: Digital image processing and analysis are used for computer assisted evaluation of sperm morphology. Sperm head morphology has been identified as a characteristic that can be used to predict a male's semen quality. On the other hand, zygote competence analysis is an importance tool to select embryo(s) for transfer. The aim of the present study is to determine zygote quality resulted from vacuolated sperms evaluated use of computer-assisted sperm analysis techniques.

Materials and Methods: The first to fourth stages are the image preprocessing stage and removing of image noises, the detection and extraction of individual spermatozoa, segmenting of the spermatozoon into various region of interest such as sperm head, midpiece and tail, and classifying of Spermatozoa as normal or abnormal. Two hundred and twenty motile spermatozoa were observed, immotiled and selected for Intracytoplasmic sperm injection (ICSI). The selected sperms were assessed and divided based on the presence/ absence vacuole, and two pronucleus (2PN) scores of resulted zygotes were studied.

Results: No statistically significant differences were observed between 2 groups with regard to fertilization rate. The high frequency of scores of Z1 and Z2 zygotes were observed by using of intact sperm. The Z3-1 and Z3-4 scores increased in injected oocytes with

the vacuolated sperms.

Conclusion: The evaluation of sperm vacuole is a prognostic tool in the prediction of ICSI success and zygote quality.

Keywords: Sperm, Vacuole, Zygote Quality, ICSI, Automated Assessment

P-107: Intrinsic Apoptosis Pathway Involvement in Mouse Testicular Tissue Degeneration after Vitrification and Short-Term Culture

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Background: Although testicular tissue cryopreservation could be advantageous for fertility preservation in cancerous children, the possibility of apoptosis incidence should not be ignored. Our aim was evaluation of the apoptosis incidence in mouse testicular tissue after vitrification and short-term culture.

Materials and Methods: Testes of 7days old NMRI male mice were isolated, divided and distributed into control and vitrification groups. Vitrification was performed in 3 steps with combination of EG and DMSO. Fresh and vitrified-warmed testis pieces were cultured in RPMI and 10% KSR for 20 hours. Just after vitrification (0h) and after 3 and 20 hours of culture real-time PCR, Flow cytometry and light microscopy were performed to study apoptosis gene expression, apoptosis incidence and morphology.

Results: Totally, tissue degeneration was increased during culture period. Late apoptosis was higher in vitrification group in 0 and 3 h of culture compared to the control, this rate became similar at the end of the culture period. During culture period, BAX expression was significantly increased, and BCL-2 expression was significantly decreased in vitrification group compared to the control one. Also, P53 expression was lower (p<0.05) in vitrification group at 0 hours of culture compared to the control and it became the same as the control at the end of the culture period. Through whole culture period BAX and P53 had lower expression in 3 hours of culture compared to 0 and 20 hours of culture in both vitrification and control groups. 3 hours post culture, BCL-2 expression was higher compared to 0 and 20 hours of culture in vitrification and control groups.

Conclusion: It seems that intrinsic pathway and P53 gene are involved in testicular tissue apoptosis followed by culture and also vitrification.

Keywords: Vitrification, Apoptosis Incidence, Testicular Tissue

P-108: Stepwise Pattern of Reactive Oxygen Species during In Vitro Development of Cloned Goat Embryos

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Background: The unique feature of embryo metabolism is the production of reactive oxygen species (ROS). It is well established that during *in vitro* culture, ROS level increases over normal ranges observed in *in vivo* derived embryos. This study evaluated stepwise pattern of ROS production during *in vitro* development of goat embryos produced by a zona-free method of somatic cell nuclear transfer (SCNT).

Materials and Methods: Zona-free oocytes, and SCNT embryos at different stages of *in vitro* development (2, 4, 8, 16-cells, morula, and blastocyst) were used for assessment of ROS production using 2,7-dichloro dihydro fluorescein diacetate (DCHFDA) probe. Grade 1 and 2 blastocysts were also transferred into recipients in order to assess post-implantation developmental competence.

Results: ROS level of MII oocytes (29.85 ± 1.17 arbitrary unit) slightly, but not significantly, changed during early stage of post reconstitution [2 (22.73 ± 1.6) and 4 (21.97 ± 1.59) -cells]. There was a gradual increase in ROS production from 4-cells stage onward which resulted in significantly higher ROS level for embryos reaching the zygote genome activation stage [morula (35.49 ± 2.4) vs. MII oocytes (29.85 ± 1.17), $p < 0.05$]. Importantly, blastocyst formation was coincided with a sharp increase in ROS production (84.04 ± 3.8) with a significant difference compared to all of the previous stages. Upon transfer of 45 developed blastocysts into synchronized recipients ($n=10$), early established pregnancy was 33.3% from which 66.7% continued and resulted in the live viable birth of two clone kids, indicating efficiency of SCNT procedure in goat.

Conclusion: obtained results suggest that the gradual pattern of increases in ROS is concomitant with embryo progression in development and indicated that the blastocyst stage is the critical point of ROS production during *in vitro* development of cloned goat embryos. This further emphasizes the special need of cloned embryos to external sources of antioxidants.

Keywords: Somatic Cell Nuclear Transfer, Reactive Oxygen Species, DCHFDA, Goat

P-109: A Histomorphometrical Study of the Protective Effect of Grape Seed Hydroalcoholic Extract against Fluxetine Toxicosis in Testis Tissue of Male Mice

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Background: Fluoxetine (FLX) belongs to the selective serotonin reuptake inhibitor group of antidepressants, which effectively alleviate symptoms of a wide spectrum of mood disorders. GSE contains mainly flavonoids, which involved in ameliorating the oxidative stress *in vitro* and *in vivo*. Thus this study was designed to investigate Protective effect of GSE hydroalcoholic extract against fluxetine-induced histomorphometrical damage on testicular.

Materials and Methods: In present study, 24 mature male mice were used. The animals were divided into four groups as, control, FLX (20 mg/kg), FLX (20 mg/kg) + GSE (100 mg/kg) and GSE (100 mg/kg) orally for 42 days. after the last gavage, animals were sacrificed. The tissues were fixed in 10% formalin, processed and blocks were made in paraffin wax. 5-7 μ m thick sections were cut

and stained with haematoxylin and eosin. Diameter of the seminiferous tubules (DST), Seminiferous tubules epithelial height (GEH), Testicular capsule thickness (TC), interstitial testicular tissue thickness (ITT), were examined in the light microscope under high magnification (X 400) by Scaled lens.

Results: Histomorphometrical results showed that the male mice exposed to fluxetine had significantly ($p < 0.05$) reduced in (GEH) and significantly ($p < 0.05$) increased in (ITT) in comparison with the control, GSE and FLX+ GSE groups. On the other hand (DST) and (TC) was not statically significant between all groups.

Conclusion: Fluxetine can cause infertility in male mice, Grape seed extract combined with fluxetine can reduce the negative effects of fluxetine.

Keywords: Fluxetine, GSE, Mice, Histomorphometrical

P-110: Effect of Oral Zinc Supplementation on Total Antioxidants Status and Antiperioxidant Activity in Spermatozoa of Patients with Oligoasthenospermia

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Background: Oligoasthenospermia (OAS) is a condition characterized by low sperm count associated with low sperm motility. Medical treatment is not known in allopathic system for treatment of oligoasthenospermia. Homeopathic medicine is effective in more than 95% patients. Oxidative stress and decreased antioxidant levels have been projected as a potential factors involved in the pathophysiology of diverse male infertility types, including oligoasthenospermia. Oxidative stress plays a key role in male infertility. For this reason, it is essential to estimate the level of oxidative stress for determination if it is a considerable contributor to sperm dysfunction in a given patient. In the present study, the effect of zinc supplementation on the quantitative and qualitative characteristics of semen along with total antioxidants status and antiperioxidant activity in the seminal plasma of oligoasthenospermic patients.

Materials and Methods: Semen samples were obtained from 25 fertile (age 27.5 ± 5.5 year) and 25 subfertile (age 28 ± 7.2 year) men with asthenozoospermia between July 2012 to July 2013, from couples who had consulted the Infertility Clinic of Babylon Teaching Hospital of Maternity (Hilla city/ Iraq). The sub-fertile group treated with zinc sulfate. Every participant took two capsules of zinc sulfate per day for three months (each one 220 mg). Semen samples were obtained (before and after zinc sulfate supplementation). After liquefaction, seminal fluid at room temperature, routine semen analyses were performed. Total antioxidants status, antiperioxidant activity and various sperm parameters were compared among fertile controls and infertile patients (before and after treatment with zinc sulfate).

Results: Compared with healthy controls, the value of total antioxidants status and antiperioxidant activity was found to decrease significantly in seminal plasma and spermatozoa of patients with oligoasthenozoospermia compared with that of healthy controls. Zinc supplementation restores total antioxidants status and antiperioxidant activity in spermatozoa and seminal plasma of asthenozoospermic subjects to normal ranges. Volume of semen, progressive sperm motility percentage, and total normal sperm count were increased after zinc sulfate supplementation.

Conclusion: Zinc sulfate supplementation is an effective treatment to male suffer infertility.

Keywords: Oligoasthenozoospermia, Zinc Supplementation, Oxidative Stress/ Total Antioxidants Status, Antiperoxidant Activity

P-111: L-Carnitine's Role in Fertility

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Background: L-Carnitine is a naturally occurring nin-essential amino acid that is synthesized through two essential amino acids called lysine and methionine. several amino acids also support hair growth, maintain cartilage (treating arthritis), improve circulation (e.g. in cases of Erectile Dysfunction) and promote male fertility. Sperm cells have to travel farther than any other single human cell-and they need a tremendous amount of energy in order to make the trek. That's what makes carnitine such a vital nutrient for men with poor sperm quality. Carnitine is a vital transporter molecule whose function is to carry high-energyfat compounds into mitochondria, where they are "burned" to release their energy. This helps give sperm the boost they need if they are going to have a chance at fertilizing an egg. This is important because weakly-swimming sperm (asthenozoospermia) is one of the most important reasons for male factor infertility.

Materials and Methods: This is review article that were identical by computer search of medline (2000-2014)

Results: L-carnitine therapy was effective in increasing semen quality, especially in groups with lower baseline levels. Many studies have demonstrated that L-carnitine taken daily enhance sperm motility by 40% and sperm count by 15%.

Conclusion: Supplementation with L-carnitine and/or acetyl-L-carnitine has proven benefits on sperm quality. Doses of 2000-3000 mg/day of L-carnitine, and 500-1000 mg/day of acetyl-L-carnitine have produced increases of sperm count, motility, straight-swimming ability However, these results need to be confirmed by larger clinical trials and *in vitro* studies.

Keywords: L-carnitine, Sperm, Infertility

P-112: Vitamin D and Fertility

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Background: Vitamin D has been well-known for its function in maintaining calcium and phosphorus homeostasis and promoting bone mineralization. There is some evidence that in addition to sex steroid hormones, the classic regulators of human reproduction, vitamin D also modulates reproductive processes in women and men.

Materials and Methods: This is a review article that were identical by computer search of medline (2000-2014).

Results: The vitamin D receptor (VDR) and vitamin D metabolizing enzymes are found in reproductive tissues of women and men. Vdr knockout mice have significant gonadal insufficiency, decreased sperm count and motility, and histological abnormalities of testis, ovary and uterus. Moreover, these studies present the evidence that vitamin D is involved in female reproduction including IVF outcome (clinical pregnancy rates) and polycystic ovary syndrome (PCOS). In PCOS women, low 25-hydroxyvitamin D (25(OH)D) levels are associated with obesity, metabolic, and endocrine disturbances and vitamin D supplementation might improve menstrual frequency and

metabolic disturbances in those women.

Conclusion: Moreover, vitamin D might influence steroidogenesis of sex hormones (estradiol and progesterone) in healthy women and high 25(OH)D levels might be associated with endometriosis. In men, vitamin D is positively associated with semen quality and androgen status. Moreover, vitamin D also increases levels of testosterone, which may boost libido.

Keywords: Vitamin D, Fertility, Hormones

P-113: Survey of *In Vitro* Effect of Resveratrol on Nitric Oxide Secretion of Human Endometrial Epithelial Cells

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Background: Resveratrol is a natural polyphenolic compound, synthesized by plants as a phytoalexin and protects against ultraviolet radiation and fungal infection. Nitric oxide is formed from L-arginine through nitric oxide synthases (NOS), a group of enzymes that structurally resemble cytochrome P-450 reductase. In females, circulating NO is increased during follicle development and decreased immediately after ovulation. The aim of present study was to determine *in vitro* resveratrol effect on nitric oxide (NO) secretion by human endometrial epithelial cells.

Materials and Methods: In this experiment study, human endometrial biopsies (n=8) were chopped in sterile conditions. Epithelial cells, were isolated after enzymatic digestion (collagenase 2 mg/ml) and cell filtration through filter mesh. Cell viability was defined by trypan blue staining. The cells were divided into five groups: control, 1, 10, 20 and 50 μ M resveratrol concentration for three culture periods (24, 48, 72 hours). Nitric oxide secretion were assessed by Griess assay. Data was analyzed by one way ANOVA and $p < 0.05$ was considered significant.

Results: Difference between NO amount were significant in control, 1, 10, 20 and 50 μ M resveratrol concentration were 9.08 ± 1.6 , 9.62 ± 1.7 , 11 ± 1.6 , 12.78 ± 2.1 , $20.2 \pm 5.1 \mu$ M at 24- hour culture period ($p=0.08$), 11.94 ± 1.3 , 13.61 ± 1.06 , 15.91 ± 1.2 , 16.64 ± 1.5 , $25.63 \pm 5.4 \mu$ M at 48 hours ($p=0.04$), 13.81 ± 1.83 , 16.06 ± 1.14 , 16.43 ± 1.08 , 17.45 ± 1.6 , $26.74 \pm 5.6 \mu$ M at 72 hours ($p=0.00$) respectively.

Conclusion: Resveratrol increased endometrial epithelial cells NO secretion in dose dependent manner. Its higher doses showed significant effect.

Keywords: Human Endometrium, Epithelial Cells, Nitric Oxide, Resveratrol

P-114: The Study of 9-10 Days Mouse Embryo Forelimb Bud and Immature Oocyte Coculture on *In Vitro* Maturation

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Background: Because of the problems of achieving to mature oocytes, ability to deliver the oocytes to mature outside of ovaries was a big discovery that somewhat could reduce the risk of ART's.

Because of the low percentage of *in vitro* oocyte maturation, research for improving the IVM condition and increasing the oocyte maturation rate is to be continued. The aim of this research is the study of the effect of the factors released from mouse embryo forelimb formation on IVM conditions.

Materials and Methods: 48 hours after intraperitoneal injection of PMSG on NMRI female mice, ovaries immature oocytes were transferred to the base medium (α MEM supplemented with 20% FBS). Three experimental groups were considered to: 1. Base medium + 1 IU/mL HCG, 2. Base medium coculture with 9-9.5 days mouse embryo forelimb bud and 3. Base medium coculture with 12-13 days mouse embryo forelimb. Oocytes after 24 hours of incubation, were identified and counted in four groups as: MII, GVBD, GV and Degenerated.

Results: Percentage of matured oocytes in control, experimental groups 1, 2 and 3, was $23.61\% \pm SE$, $39.24\% \pm SE$, $60\% \pm SE$ and $48.34\% \pm SE$ respectively. Oocytes maturation rate in experimental groups than the control group showed a significant increase ($p < 0.005$).

Conclusion: The coculture of oocytes with forelimb bud has significantly increased the maturation rate of unmaturation oocytes. It is probably that the released paracrine factors during of forelimb formation has had significant effect on oocyte maturation and probably is most effective than the effect of presence of HCG endocrine factors in maturation culture medium during IVM process. It may be possible to recommend the use of forelimb co-culture or paracrine factors that release during forelimb formation for improving the IVM conditions.

Keywords: IVM, Forelimb Bud, Coculture

P-115: Excitatory and Inhibitory Effects of Nitric Oxide on Development The Cardiomyocyte of Rat Embryos

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Background: According to increased consumption of supplements especially during pregnancy and the teratogenic impact some they on evolution the vital systems of embryos. The purpose of this study to improve the health of the fetus and prevention of cardiovascular abnormalities as the most common cause of infant mortality. We examined the effects of L-Arginine and L-NAME administration on fetal rat heart.

Materials and Methods: Forty Wistar rats, weighing 200-250 g with a mean age of 8 weeks, were divided into 5 groups after making sure that the experimental rats were pregnant. The first group received 2 mg/kg normal saline and the others received respectively 200 mg/kg L-Arginine, 20 mg/kg L-NAME and a mixture of the same doses of L-Arginine and L-NAME on 8, 9, 10 and 11th gestational days via intraperitoneal. The control group did not receive any injection. The fetal hearts were removed on 18th gestational days, after tissue preparation and staining method (E and H) histological changes were studied by Light microscopy Olympus Cx31 and software Image tools III.

Results: In L-NAME group despite the significant decrease ($p < 0.05$) in the diameter of cells and their nuclei, irregularities in heart muscle structure and intercalated disc were observed. L-Arginine group was also associated with severe Myogenesis and Angiogenesis.

Conclusion: Results of this study showed that Nitric oxide (NO) in the amounts of higher or lower than normal is associated with con-

genital heart diseases. Therefore, drugs and supplements should be used with caution especially during pregnancy.

Keywords: L-NAME, L-Arginine, Fetal Heart, Rat

P-116: The Catalase Effect on LDH Enzyme Leakage of Buffalo Epididymal Sperm During Culture in Human Tubal Fluid Media

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Background: Mammalian spermatozoa are highly susceptible to lipid peroxidation, which leads to structural damage to the sperm cell, the enzymatic leakage from spermatozoa accompanied by lowered motility and metabolism. Catalase, an enzyme that detoxify hydrogen peroxide and reduce the enzyme leakage from sperm cell

Materials and Methods: For this study, buffalo bull testicles (20 pairs) were picked up in the march till September 2013 in Urmia local slaughterhouse and transported to the laboratory in a cool container (filled with 5°C ice pack). Epididymis were dissected and cleaned from connective tissue. Sperm were collected performing several incisions in the cauda epididymis with a surgical blade, and taking the white fluid emerging from the cut tubules. Five levels of catalase (50-100-200-400-800 IU) were added into human tubal fluid media (HTF) containing sperms (30×10^6 sperm/ml), with 10%, bovine serum albumin and were incubated for 36 hours at 37 °C. Sperm LDH enzyme assay was performed at 1-4-12- 24 and 36 hours based on "Deutsche Gesellschaft Fur Klinische Chemie (DGKC)" protocol. After collection of data, Statistical analyses were performed with procedures available in ANOVA of SPSS version 20.

Results: The results showed that, adding catalase enzyme on buffalo epididymal sperm cells increase LDH enzyme leakage.

Conclusion: Catalase has no effect on sperm cell membrane integrity during culture in HTF media.

Keywords: Buffalo, Epididymis, Sperm, LDH, Catalase

P-117: Methamphetamine Can Induce Sperm Chromatin Abnormality and Apoptosis in Mice

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Background: It is showed that methamphetamine has harmful effects on male reproductive system. The aim of this study was to investigate the effects of methamphetamine administration on testis weight, sperm count, sperm morphology, and chromatin status as well as sperm apoptosis followed by the cytochemical tests in a mouse model.

Materials and Methods: Twenty four NMRI male mice were divided into four groups. First, second and third groups were administrated a dose of 4, 8 and 16 mg/kg methamphetamine, respectively. Fourth group was administrated saline and considered as control. Methamphetamine was injected intraperitoneally for

thirty days (one spermatogenesis period in mouse). The mice were killed and the testes were weighted. Caudal epididymis was cut to obtain sperm smears. Sperm concentration was evaluated with Makler chamber. Papanicolaou staining method was done to evaluate sperm morphology. TUNEL assay was applied for detection of sperm cell apoptosis. Also toluidine blue and aniline blue were used to evaluate sperm chromatin abnormality.

Results: Data showed that administration of methamphetamine may not affect testes weight. The sperm count showed a decreasing trend in experiment groups compared to controls but the difference was insignificant. Rate of normal sperm morphology showed significant decrease in second and third groups compared to controls ($p < 0.001$). TUNEL assay analysis showed significant increase in third group compared to control group (14.17 ± 7.9 and 2.67 ± 2.1 , respectively). Toluidine blue and aniline blue staining techniques showed that there is significant increase in sperm with abnormal chromatin in third group compared to others ($p < 0.001$).

Conclusion: Administration of methamphetamine could have toxic effect on male reproductive system. It seems methamphetamine can reduce rates of sperm count and normal morphology and it could have detrimental impacts on sperm DNA integrity.

Keywords: Methamphetamine, Apoptosis, Chromatin Abnormality

P-118: Stepwise Morphometric Changes during *In Vitro* Maturation of Goat Oocyte in Relation with Nuclear Remodeling and Developmental Competence

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Background: Successful *in vitro* maturation (IVM) of oocytes is an important step for production of livestock through assisted reproductive techniques including somatic cell nuclear transfer (SCNT). Obtaining an optimal duration for maturation of oocyte is essential, since over exposure to maturation medium results in oocyte aging, while under exposure leads to production of immature incompetent oocytes. Therefore, this study by assessing stepwise changes of morphometric parameters, nuclear and microtubule remodeling, hopes to obtain the optimal duration for IVM of goat oocytes.

Materials and Methods: In experiment 1, cumulus oocyte complexes were fixed at 2 hours intervals from 14 to 32 hours of culture to assess oocyte nuclear maturation and morphometric parameters. The curve for the percentage of oocytes at metaphase II (MII) reached a plateau by 20 hours of IVM culture (64.9% MII) and did not show a significant change thereafter. Also ooplasm diameter, zona pellucida thickness, volume of ooplasm and perivitelline space (PVS) at different interval of *in vitro* maturation were examined and results show declining trend in diameter and volume of ooplasm and an increase in volume of PVS. But there variations were not significant. In experiment 2, the effects of duration of IVM culture (20, 24, and 30 hours) on the fertilizability of goat oocytes were evaluated. After 20 hours IVM and 20 hours of IVF culture, cleavage and blastocyst rates did not differ from other groups (24 and 30 hours) but rate of morula formation significantly increased. Also the quality of blastocysts did not differ between groups.

Results: These results suggest that morphometric parameters

cannot be applied as prognosis factor in oocyte maturation outcome in IVM program, as no relation were observed between morphometric parameters and nuclear statuses. The best organization of microtubules and arrangement of chromosomes on spindles were observed at 20 hours post maturation.

Conclusion: Based on this observation, we proposed that 20 hours may be considered as the optimal time for IVM in goat

Keywords: Morphometric, Nuclear Remodeling, Goat, Developmental Competence

P-119: *In Vitro* Assessment of Sucrose for Preservation of Buffalo Epididymal Spermatozoa

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Background: Epididymal spermatozoa have been used for production of offspring from genetically important animals after they are dead. To attain optimal fertility with epididymal spermatozoa, they should be preserved in a suitable medium in 37°C. Preservation media provides requirement for spermatozoa and affects its motility, viability, and fertility. Sucrose is one of the important components of semen extenders. The beneficial effect of sucrose on preservation (cold and freeze condition) of semen has been examined in other species. While this has not been studied in buffalo epididymal spermatozoa. In this study we evaluate buffalo epididymal sperm parameters to find optimal concentration of sucrose in preservation media.

Materials and Methods: Thirty testes from 15 mature buffalo bulls were collected from Urmia slaughterhouse and transported to laboratory in cool condition. Spermatozoa were obtained from caudal epididymis and pooled together. The samples were diluted in tissue culture media (TCM) at concentration of 20×10^6 sperm/ml and divided to five groups containing sucrose at concentration of 0, 2.5, 5, 7.5 and 10 mM. Sperm motion characteristics, viability, and cytoplasmic droplet were evaluated by computer assisted sperm analyzer and one steps eosin- nigrosin stain respectively at 1, 6, 12, and 24 hours of incubation at 37°C. Mean of data were compared using Duncan's multiple range tests by significant level of 0.05.

Results: The addition of sucrose in preservation media did not affect motility, viability, and velocity patterns of epididymal spermatozoa until 6 hours of incubation ($p > 0.05$). While supplementation of sucrose at 5 and 7.5 mM significantly improved motility, viability, and curvilinear velocity of spermatozoa following 12 and 24 hours of incubation ($p < 0.05$). Cytoplasmic droplets were reduced when 5 mM of sucrose was added in preservation media at 6 and 12 hours of incubation as compared to control group ($p < 0.05$).

Conclusion: We conclude that addition of 5 mM sucrose in preservation media of epididymal buffalo spermatozoa results in the release of cytoplasmic droplets and improves motility and viability of spermatozoa during 24 hours preservation.

Keywords: Buffalo, Epididymal Sperm, Sucrose, Motion Characteristics, Cytoplasmic Droplets

P-120: Evaluation of the Antioxidant Effects of N-Acetylcysteine on Ischemia Damage, Apoptosis Incidence and Restoration of Ovarian Activity Following Mice Ovary Heterotopic Autotransplantation

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Background: Ovarian tissue transplantation is now considered as a procedure to preserve the fertility of young woman patient undergoing cancer therapy. An essential strategy to improve the efficiency of ovarian transplantation is to overcome the initial ischemia reperfusion injury and free radicals production that lead to a significant follicular loss. The aim of this study was to investigate the effects of N-acetylcysteine (NAC) as a potent antioxidant and antiapoptosis factor on hormonal changes, apoptosis incidence and oxidative damage in mice ovary heterotopic autotransplantation.

Materials and Methods: The NMRI mice (4-5 weeks) were divided into three groups (n=6): control; auto transplanted +NAC (150 mg/kg i.p, one day before surgery till 7days after transplantation), and auto transplanted + saline. 28 days after transplantation, the total volume of ovary, cortex and medulla were estimated stereologically. DNA fragmentation, plasma levels of malondialdehyde (MDA), progesterone and estradiol were also evaluated. Data were analyzed with SPSS using one way ANOVA and means were considered significantly different at p<0.05.

Results: A significant increase was seen in the mean total volume of ovary and cortex in the auto transplanted +NAC group compared to the auto transplanted + saline ones. There was no significant difference in the percentage of apoptotic follicles among the NAC-treated and the control groups (p>0.05). The concentration of MDA significantly reduced in the NAC treated group compared to the saline treated group (p<0.05). The level of progesterone did not differ in both of the graft groups (p>0.05) while the level of estradiol increased significantly in the grafts+ NAC compared to the saline-treated grafts (p<0.05).

Conclusion: NAC could effectively inhibit ischemia reperfusion injury and improve the restoration of transplanted ovaries function by reducing the oxidative stress and apoptosis.

Keywords: N-acetylcysteine, Autotransplantation, Ischemia Reperfusion, Ovary, Mouse

P-121: Which Developmental Stage is Suitable for Re-vitrification? Compact Stage Re-vitrification or Early Blastocyst Stage Re-vitrification

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Background: The aim of this study was evaluation of developmental rate and Bax, Bcl-2 and ErbB4 genes expression following re-vitrification in compact and early blastocysts stages.

Materials and Methods: 5-8 cell embryos were collected from female mature mice, 60-62 hours post hCG injection. The embryos were divided to five groups including: fresh, vitrified at 5-8 cells, vitrified at blastocyst stage, vitrified 5-8 cells and re-vitrified at compact or early blastocyst stage. Embryos were vitrified by using cryolock, as described by Kuwayama et al. Development of embryos were recorder daily. Results were analyzed by using Chi square. Q-PCR has been used to examine the expression of Bax, Bcl2 ErbB4 genes in derived blastocysts. The data was analyzed with ANOVA.

Results: Blastocyst formation rates showed no significant differences between vitrified and re-vitrified groups, although there was significant difference between re-vitrified and fresh embryos (p<0.05). Also it showed no significant differences between two re-vitrified groups. Our data showed that expression of Bax, Bcl-2 and ErbB4 genes had a significant difference (p<0.05) between re-vitrified and fresh embryos. Although there was no significant difference between re-vitrified and vitrified embryos regarding genes expression. Expression of Bax, Bcl-2 and ErbB4 genes showed no significant difference between two re-vitrified groups.

Conclusion: Based on our study, re-vitrified embryos in compact and blastocyst stage are nearly equal in developmental rate and expression of Bax, Bcl2 and ErbB4 genes, although this amount of genes expression is altered significantly compared with fresh embryos. Therefore, we can re-vitrify embryos in compact or blastocyst stage.

Keywords: Vitrification, Blastocysts Stage, Apoptotic Genes

P-122: Effects of Cobalt Chloride and Chromium Chloride on Development of Mouse Testes

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Background: Cobalt (Co) is an essential trace element for mammals required for the synthesis of vitamin B12. It is not a cumulative toxin but chronic exposure induces negative effects on the organism. It was proven that cobalt passes via placenta appearing in the fetal blood and amniotic fluid and it is shown to possess an embryotoxic effect. Also, chromium (Cr) is recognized as a trace element essential for both animal and human nutrition. Chromium has been shown to have antioxidative properties *in vivo*. In the present study, we focused on the effects of chronic exposure to cobalt chloride and chromium chloride on development of mouse testes.

Materials and Methods: The mice were acclimatized for seven days prior to coupling and were housed in an air conditioned animal house at 22 ± 2°C with exposure to 10-12 hours of day light. Day zero of pregnancy was determined by vaginal plug test. Pregnant mice were administered cobalt chloride and chromium chloride intraperitoneally at the concentrations of Co-60, Co-50, Co-40, Co-10, Cr-50, Co-10/Cr-50 and Co-40/Cr-50 mg/Kg bw on day 12-14 pregnancy. After pup's delivery, male pups were sacrificed on day 35 and testes were removed, fixed in 10% neutral buffered formalin and embedded in paraffin wax. Serial sections (5µm) of the medullary area were obtained in each group and stained with hematoxylin-eosin. The sections were observed under light microscopy. For all experiments, at least 6 to 8 replicates were performed. The data were

analyzed statistically using Chi square test and analysis of variance (ANOVA). A level of ($p < 0.05$) was accepted as significant.

Results: Our results showed that single dose ip injection of cobalt chloride caused miscarriage in dose dependent manner (60, 50 and 40 mg/kg). Also, cotreatment of cobalt chloride and chromium chloride improved rate of successful pregnancy in Co10/Cr50 and Co40/Cr50 mg/kg bw. Histological comparisons of control and treatment groups showed that cobalt chloride in 10 and 40 mg/kg bw decreased the number of spermatogonia, primary spermatocytes cells, sertoli cells and sperm. Chromium chloride in 50 mg/kg bw compensated toxic effects of cobalt chloride. Tubules number and tubules diameter decreased after exposure to cobalt chloride and cotreatment of cobalt chloride and chromium chlorid compensate this reduction.

Conclusion: Cotreatment of cobalt chloride and chromium chlorid compensate the toxic effects of cobalt chloride on development of mouse testes in a dose dependent manner.

Keywords: Cobalt Chloride, Chromium Chloride, Development, Testis, Mouse

P-123: The protective Effects of The Vitamin E on Sperm DNA Damage Induced by Mobile Phone Radiation

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Background: There has been a growing claim that cell phone usage may have a detrimental effect on sperm chromatin. Infertile men possess anomalies in the composition of their sperm nuclei, displaying higher levels of loosely packaged chromatin and damaged DNA. In this study we examined the relationship of cell phone radiation and the protection effect of alpha_tocopherol on human sperm DNA.

Materials and Methods: Semen samples were collected from 12 patients presenting to the infertility clinic. Each samples was divided in two aliquots: control group (sample not exposed) and exposure group (sample exposed to cell phone). Exposure group was divided in two parts, the first was exposed to the cell phone radiation directly, the second group was mixed with 5mM alpha_tocophrol and emitted to cell phone radiation. Smears from each group were fixed in Carnoy's fixative (methanol: acetic acid, 3:1) overnight. The slides were stained for 15 minutes and gently rinsed with PBS. The green and red fluorescence sperms were observed by fluorescence microscope.

Results: The AO showed that the exposure group samples had a greater DNA damage if compared to control group. Furthermore adding alpha_tocophrol in semen during mobile phone exposure showed protection effect compare control and exposed group only significantly.

Conclusion: Genotoxic effect of RF_EMW on sperms is either through ROS production or through direct clastogenic chromatin breaking effect. The addition of alpha_tocopherol in the semen could be useful to prevent human sperm chromatin after mobile phone radiation.

Keywords: Cell Phone, Alpha Tocopherol, Sperm Chromatin, Radiation

P-124: Protective Effect of Capper Hydroalcoholic Extract on Monosodium Glutamate-Induced damages on The Ovary

of Rats; Evidence for Hsp70-2, p53 Genes Expression

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Background: Monosodium glutamate (MSG), the sodium salt of glutamate, is commonly used as a flavor enhancer in modern nutrition. It is known to have some adverse effects in humans and experimental animals various cell types. On the other hand, Capper contains high amounts of antioxidant agents. Therefore, present study was designed to evaluate the protective effect of Hydroalcoholic Extract of capper on MSG-induced damages. Moreover, the MSG-induced changes on Hsp70-2 and p53 proteins expression levels as well as capper's protective effect were assessed.

Materials and Methods: For this purpose, eighteen mature female Wistar rats were divided into three equal control and test group. The animals in test group received 4 mg/g bw of MSG and MSG + 300 mg/kg from Hydroalcoholic Extract of capper, intra-peritoneally for 14 days (n=6 for each group). The Hsp70-2 and p53 proteins expression was evaluated by immunohistochemical analyses. The total and normal follicles were counted per one ovary.

Results: Observations demonstrated MSG up-regulated p53 gene expression and down-regulated Hsp70-2 expression in follicular cells. Moreover, the animals in capper-treated group showed a significant enhancement in total follicles and exhibited remarkably ($p < 0.05$) higher normal follicles content versus non-treated animals.

Conclusion: In conclusion, our data suggest that hydralcoholic extract of capper reduced the MSG-induced derangements via up-regulating the Hsp70-2 and down-regulating the p53 proteins expression.

Keywords: Monosodium glutamate, Capper, p53, Hsp70-2

P-125: The Pregnancy Success Rate of IVF/ICSI with and without The Use of PGS in Female Partner over 35 Years Old Referred to Tehran Infertility Centers

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Background: Preimplantation genetic aneuploidy screening (PGS) has been performed during the last decade as a way of enhancing embryo selection in patients with an increased incidence of embryonic numerical chromosome abnormalities (advanced maternal age, recurrent miscarriage, and recurrent implantation failure). It has been proposed that the replacement of euploid embryos in these patients would result in a higher implantation and pregnancy rate and a reduced miscarriage rate. Additionally, the transfer of fewer embryos could reduce the chances for multiple pregnancies in all IVF patients. Advanced maternal age (AMA) is an important parameter that negatively influences the clinical pregnancy rate in IVF, in particular owing to the increased embryo aneuploidy rate. Although, to date, multiple studies have addressed this issue, contradictory results have been encountered. The purpose of this study was to test whether or not employing preimplantation genetic screening (PGS) in AMA patients would increase the clinical pregnancy rate.

Materials and Methods: In our study, couples with female

partner over 35 years of age were participated in a randomized controlled trial (RCT). Results of embryo transfer following PGS using FISH technique for chromosomes 13, 18, 21, X/Y were evaluated and compared with those in control group.

Results: 150 couples with female partner over 35 years old were included in this study. Of these, 50 couples underwent PGS and the remaining were used as control group. Pregnancy rates obtained in PGS and control group were 30 and 32%, respectively.

Conclusion: These two groups showed no significant difference in comparison with each other and therefore in conclusion PGS for chromosomes 13, 18, 21, X and Y does not elevate IVF/ICSI success rate in women over 35 years of age.

Keywords: Preimplantation Genetic Screening, Aneuploidy, IVF/ICSI, Advanced Maternal Age

P-126: Assessment of Some Trace Elements and Other Criteria of Infertile Patients

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Background: The aim of present study was to estimate the levels of some Trace element (Lead, Copper, Cobalt, Chromium, and Cademium) concentrations in Asthenospermia and Normospermia by atomic absorption method, in comparison with control (Fertile), Estimate concentration of (MDA) Malondialdehyde and Total protein concentration in semen specimens. Also relationships between mentioned components and some semen parameters were investigated.

Materials and Methods: This study was performed on human semen specimens obtained from Asthenospermic patients (35 specimens) Normospermic males (40 specimens), and (20 specimens) Fertile Control group, who were attending to the laboratories of Fertility center in ALSader Hospital of AL-Najaf AL-Ashraf city during the period extended from 1-9-2013 to 30-1-2013.

Results: The results revealed significant increase ($p < 0.05$) in the concentration of examined trace elements in Asthenospermia and Normospermia in comparison with control, and the studies showed significant increase ($p < 0.05$) in total protein concentration, also in MDA concentration in semen specimens.

Conclusion: That there was an increase in concentration of trace elements and it influenced on blance and parameter of seminal fluid. Also there was the correlation between trace element and Protein in semen. All of this leads to arise of cases of Asthenospermia and Normospermia infertile patients.

Keywords: Trace Elements, Asthenospermia, Normospermia, Cobalt, Chromium

P-127: The Effect of Supplementation of In Vitro Culture Medium with FGF2 on Ovine Embryo Development

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Background: Fibroblast growth factors are a great family of growth factors that exert variety of functions in vertebrates including cell growth, survival, differentiation and migration. FGFRs are transmembrane tyrosine kinase receptors transmitting FGF signals. Four types of FGFRs (1/2/3/4) exist in vertebrates. FGF2 is one of the FGFs detected in bovine and ovine uterus flushes, supposing that it has effects on embryo development.

Materials and Methods: *In vitro* produced ovine embryos via IVF were removed at different developmental stages (zygote, 2-4cell, 8-16cell, morula, and blastocyst) from day 1 to day 7 and positioned in RLT for investigation of FGFRs expression pattern by Real-Time PCR. In the case of FGF2 treatment effect, from day1, embryos were divided into two groups (control or 500ng/ml FGF2) in serum-free conditions. FGF2 was present in treatment group medium whole days and developmental rate were assessed during development until day8. Finally, expanded and hatched blastocysts differentially stained and cell numbers recorded by using fluorescence microscope.

Results: According to our data all four types of FGFRs evinced dominant expression at morula stage while in the other stages FGFRs expression were in low levels. FGF2 supplementation did not significantly change the blastocyst formation rate and total cell numbers in FGF2 supplemented group compared to the control until day8. even though FGFRs were present in ovine embryos, their expression were in low levels except in morula. It is unclear whether FGFRs are in adequate levels to transduce FGF2 signals in ovine embryos. However, it does not seem that FGF2 exerts any proliferative or developmentally amending effect on ovine embryos up to day 8, according to our data. It is not known whether proliferative respond to FGF2 will appear from hatched blastocyst stage onward, one in which high proliferation rates prevail.

Conclusion: We found neither a prominent effect of FGF2 on blastocyst formation rate nor proliferation in ovine embryos up to day 8 post fertilization.

Keywords: FGF2, FGFR, Ovine Embryo

P-128: The Effect of Purmorphamine on Nuclear and Cytoplasmic Maturation of Oocytes In Vitro

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Background: Oocyte quality and the media compounds are two main factors in development of the embryo. To date, different protocol with diverse factors such as BDNF, EGF, LIF for the oocyte maturation have been studied. However, the efficiency of *in vitro* oocyte maturation is very low in compared to that of *in vivo* maturation. In this study, the effects of different concentrations of Purmorphamine on the nuclear and cytoplasmic maturation of sheep oocytes were considered.

Materials and Methods: Ovaries were obtained from local abattoir and cumulus-oocyte complexes were aspirated, cultured in 50µl droplet containing FBS, TCM199, LH, FSH and estradiol (as a control group) and different concentrations of purmorphamine (250

and 500ng) (as a treatment groups). After 24 hours maturation, oocytes stripped from cumulus cells and stained with Hoechst and cell tracker blue for assessment of nuclear and cytoplasmic maturation respectively, fluorescent microscope was used for photograph, taken digital images analyzed by image j software and spss.

Results: The results showed that 250ng concentration of Purmorphamine induce nuclear maturation and about 81 percent of oocyte continued cell division until meiosis II.

Conclusion: This is a first study about the effect of SHH agonist (Purmorphamine) on nuclear and cytoplasmic maturation of oocyte. According to result, it seems that 250 ng concentration of Purmorphamine (in contrast with other reagents) induces nuclear maturation of oocyte and is a good reagent for oocyte maturation *in vitro*.

Keywords: Oocyte, Nuclear Maturation, Cytoplasmic Maturation, Sheep, Purmorphamine

P-129: Prunus Cerasus Effects on Progesterone Receptor Expression in Cumulus Cells

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Background: Infertility is defined as inability to achieve pregnancy after one year of unprotected regular intercourse. It has been reported that about 10-15% of young couples suffer from infertility. There is evidence that antioxidants improve the fertility rate. Prunus cerasus contains strong antioxidant activity. The aim of this study is to evaluate Prunus cerasus effects on expression of progesterone receptor in cumulus cells. Expression of progesterone receptor is of essential factor for cumulus expansion. That disorder in expression of progesterone receptor can lead to infertility.

Materials and Methods: In this study, 30 adult female mice were randomly divided into control and experiment groups. Prunus cerasus was added to the diet of female mice for 14 days. Ovulation was induced using HMG/PMSG (10 IU) and HCG (10 IU) that injected intraperitoneally then mice were dissected and ovaries were separated. Oocytes were collected and cumulus oophorus cells were isolated. Real time-polymerase chain reaction (RT-PCR) was performed to confirm the expression of progesterone receptor in cumulus cells.

Results: Data revealed that Prunus cerasus increased the expression of progesterone receptor in cumulus cells.

Conclusion: The recent study reported different results of the effect of antioxidant on fertility. In the present study, we found that Prunus cerasus can increase the expression of progesterone receptor in cumulus cells. As regard study on oocyte in environment rich in antioxidant is antithesis. There are similar studies regarding the correlation between antioxidant and fertility. Antioxidants such as Prunus cerasus can be used as an important medicine to improve oocyte quality and open new opportunities for treatment of infertility.

Keywords: Prunus Cerasus, Cumulus Cells, Gene Expression, Infertility

P-130: Efficacy of Commercial *In Vitro* Maturation Medium Is Similar to Home-made Medium for *In Vitro* Maturation of Human Oocytes

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Background: *In vitro* maturation (IVM) of immature oocyte is a promising treatment option for infertile women. IVM procedures attempt to mimic the natural conditions of the follicular environment. The technique of IVM of oocytes has been successful after the first trial using culture of human oocytes in follicular fluid. The crucial part of this technique is the maturation process itself, and the choice of the base medium for human IVM is considered particularly difficult. The aim was to compare the efficacy of two culture media namely, home-made human follicular fluid (HFF) medium and commercial IVM medium in maturation of human oocytes.

Materials and Methods: The oocytes were obtained from stimulated ovaries of infertile patients who underwent ICSI. 101 immature oocytes and then were cultured in HFF medium containing Ham's F10 supplemented with LH+FSH, and 85 immature oocytes were cultured in commercial IVM medium supplemented with LH+FSH. They were checked for maturity 24-40 hours after culture at 37°C/ 5% Co₂. Finally, the maturation rates were compared between two groups of oocytes.

Results: The rate of maturation in HFF medium group was 59.5%, whereas in commercial IVM medium group was 65.8% (p=0.364, OR=0.74, 95%CI=0.40-1.35). The rate of degeneration in HFF and commercial medium group was 3 and 4%, respectively.

Conclusion: HFF medium, although widely used for embryo fertilization and maintenance in ART techniques, has similar efficacy as commercial IVM medium for the maturation of human oocytes.

Keywords: IVM, Human Oocytes, Culture Medium

P-131: Effects of Heat Shock during Early Stage of Oocyte Maturation on Meiotic Progression and Subsequent Embryonic Development and Gene Expression in Ovine

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Background: Heat shock may affect different aspects of oocyte maturation and its subsequent development to the blastocyst stage. A series of *in vitro* experiments was performed to determine whether physiologically heat shock (41°C) disrupts the progression of the ovine oocytes through meiosis, activation and blastocyst formation.

Materials and Methods: The cumulus-oocyte complexes (COCs) were aspirated from 2-6 mm follicles and cultured at 38.5°C (control) or 41°C (heat shock) for the first 12 hours of maturation. The oocytes were incubated at 38.5°C during the last 10 hours of maturation and 8 days after activation.

Results: Results showed that most of the oocytes matured in

heat shock condition remained at GVBD stage and they showed an aberrant chromatin configuration. After heat shock, oocyte diameter and time spent for zona pellucida dissolution increased ($p < 0.05$). Heat shock condition decreased ($p < 0.05$) cleavage rates (56.19 vs. 89.28%) and morula formation (26.85 vs. 37.81%). However, there was no significant difference in blastocyst formation and percentage of hatched blastocysts. 12 hours heat shock had an adverse effect on embryo quality and reduced inner cell mass number ($p < 0.05$). Quantitative gene expression analysis showed a greater transcripts ($p < 0.05$) for Na/K-ATPase mRNA in heat shocked oocytes

Conclusion: To sum up, heat shock has disruptive effects on ovine oocyte maturation and it can impair cellular and molecular factors which are important for embryo development.

Keywords: Heat Shock, Ovine Oocyte, Nuclear Maturation, Embryo, Blastocyst

P-132: The Effect of Different Fetal Bovine Serum Levels on Recombinant Chinese Hamster Ovary Cells, Expressing Human Follicle Stimulating Hormone

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Background: Follicle stimulating hormone (FSH) is one of the most common and effective gonadotropin hormones used in infertility treatment. Production of FSH as a drug is affordable as long as it is done with minimal cost. FBS is the most expensive component in medium culture furthermore; it's being controversial to cause unnecessary suffering for the unborn calf for collection their serum. In this study, the effect of different amount of FBS on the cell viability and the expression of this protein, in the CHO cell line was investigated.

Materials and Methods: The FBS of cell culture medium was reduced from 10% as reference to 5, 3 and 1% gradually and was carried out for 2-3 passage for each step. The cell viability was assessed by trypan blue staining method. The morphology of the cells characterized using microscope. Total Protein concentration was measured by Bradford's technique and then SDS-PAGE and Western blotting used for detection of rFSH.

Results: The results showed that, the maximum cells productivity was at 10% FBS. Increase in cell Longevity indicated at 3% FBS. Moreover, reductions of FBS to 1% lead to change in morphology of the cells. The result of SDS page and Western Blotting demonstrated reduction in the expression of FSH following the decline in the concentration of FBS. No significant difference was found in the expression of protein at 3 and 5% of FBS.

Conclusion: Using 3% of FBS for FSH production is economically advantageous. This concentration is appropriate for the growth and the viability of the cells. In addition it's good enough for production of FSH.

Keywords: Follicle Stimulating Hormone, Fetal Bovine Serum, Infertility

P-133: Effects of Dietary Earthworm (Eisenia Fetida) Meal and Ethanolic Extract

of Sperm Quality Parameters of Native Iranian Roosters

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Background: Avian spermatozoa are characterized by high proportions of poly unsaturated fatty acid, associated with increased susceptibility to Reactive Oxygen Species and lipid peroxidation. Recent advances in poultry reproduction have focused on the potential of Reactive Oxygen Species as one of the prime mediators of infertility. Oxidative stress is the result of imbalance between the Reactive Oxygen Species production and antioxidants in the body which can lead to sperm damage, deformity, and eventually male infertility. Constituents in earthworm (*Eisenia fetida*) have high phenolic compounds *in vitro* models. These compounds are secondary metabolites and act as free radical scavenging molecules. This experiment was conducted to determine the antioxidant effect of dietary earthworm on sperm quality parameters of 24 Iranian native roosters.

Materials and Methods: The measured parameters were related to sperm velocity and motility. Semen samples were collected twice a week from all roosters during the whole period of experiment (12 weeks). Dietary treatments were as follows: 1. control diet, 2. Control diet + 150 IU vitamin E, 3 and 4. control diet + 1 and 2% earthworm meal respectively, and, 5 and 6. control diet + 1 and 2% earthworm ethanolic extract respectively.

Results: Results indicated that in all measured traits, the highest and lowest values belong to the groups receiving meal (2%) and ethanolic extract (2%) of earthworm, respectively. Velocity of Average Path and Velocity of Straight Line were significantly improved by ethanolic extract of earthworm (2%) compared to control group ($p < 0.05$). However, dietary supplementing of 2% earthworm meal had lower parameter values than that of controls.

Conclusion: It seems that antioxidant properties of ethanolic extract of earthworm (2%) improved some of the sperm quality parameters during spermatogenesis.

Keywords: Earthworm, Sperm Quality, Native Roosters

P-134: Anti Fertility Effects of Amaranthus Caudatus Extract in Female Rats

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Background: Persistent oral administration of Amaranthus extract could decrease contractile response and enhance relaxation response in aortic tissue of diabetic rat through affecting endothelial related agents. This may be beneficial in prevention of long-term

vascular complications of diabetes.

Materials and Methods: In this experimental study, *Amaranthus caudatus* rats (160-220 g) were used in three experiments. In each experiment, animals were divided into two subgroups (n=10): control group which received distilled water (2 cc/kg) and case group which received herb extract (100 mg/kg) intra-peritoneally. In the first experiment, animals received single dose of extract or DW. In the second experiment, female rats received extract or DW in the per-implantation period during day 1 to 5 of pregnancy and day 5 to 7 of pregnancy in the third experiment. Implantation sites and neonates were counted in all the three experiments.

Results: All the three experiments showed significant differences between control and case groups in implantation sites and neonates number. These differences were more prominent in the first and third experiment.

Conclusion: *Amaranthus caudatus* extract showed anti fertility effect in female rats.

Keywords: Anti Fertility, *Amaranthus Caudatus*, Rat

P-135: The Protective Effect of Green Tea Extract on Lead-Induced Oxidative Stress, Apoptosis and Morphometric Changes in Male Mouse Epididymis

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Background: Lead (Pb) is one of the most important environmental pollutants that affect the male reproductive system. The aim of this study was to investigate the protective effect of green tea extract on lead-induced oxidative stress, apoptosis and morphometric changes in male mouse epididymis.

Materials and Methods: Forty adult male mice were randomly divided into four groups (n= 10), as follows: Control, GTE, Pb and Pb+GTE treated groups. Animals were given 100 mg of GTE/Kg body weight/day (ip) in the GTE and Pb+GTE treated groups, while the other groups received normal saline. The Pb and Pb+GTE treated groups received 1% lead (II) acetate (LA) in drinking water. After 6 weeks, the animals were sacrificed and the body of epididymis was removed. Blood samples were collected for biochemical tests. Data were analyzed using analysis of variance (ANOVA) and Tukey post hoc tests (SPSS, p<0.05).

Results: The histological evaluations indicated that lead increased vacuole formation in epithelial thickness of epididymis and significantly reduced epithelial cells height and diameter of epididymis in comparison with that of control group (p<0.001). TUNEL staining showed that lead induces apoptosis in epithelial cells of epididymis (p<0.001). Lead caused a significant decrease plasma testosterone level and increased plasma Malondialdehyde (MDA) concentration (p<0.001). However, co-administration of GTE with lead acetate significantly increased epithelial cells height and diameter of epididymis and decreased number of apoptotic cells compared to Pb treated group (p<0.001). Moreover, combined treatment significantly increased plasma testosterone level and decreased plasma MDA concentration versus Pb treated group (p<0.001).

Conclusion: GTE is potentially reducing Pb toxicity and tissue damage of epididymis by enhancing the antioxidant/detoxification system

Keywords: Green Tea, Lead, Epididymis, Apoptosis, Mice

P-136: High Concentrations of Noscapiene Induces Apoptosis in Stromal Cells of Endometriosis In Vitro

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Background: Endometriosis is a benign hormone-dependent disease in women of reproductive age. There is no cure for eradicating the disease and there is need for new drugs. The aim of this study was to investigate the *in vitro* effect of noscapiene as inducer of cell death on endometriotic stromal cells in patient's endometriosis *in vitro*.

Materials and Methods: Endometrial biopsies from patient's endometriosis (n=8) were digested by enzymatic method (collagenase, 2 mg/ml). Stromal cells were collected by sequential filtration through nylon meshes (70 and 40 µm) and ficoll layering. The cells were divided to five groups: control and 10, 25, 50, and 100µM concentration of noscapiene, and cultured for 48 hours. Cell viability was assessed by MTT assay; and cell morphology analyzed with Acridine orange (AO)-ethidium bromide (EB) double staining, and cell death was assessed by TUNEL assay. Data was analyzed by one way ANOVA.

Results: Viability of endometrial stromal cells were 94.3, 88.4, 86.7, 86.3, 76.9% in control, 10, 25, 50 and 100 µM noscapiene concentration in 48 hours respectively. Cell death increased in high concentrations of noscapiene and TUNEL positive cells were increased in 10, 25, 50 and 100 µM noscapiene concentration (7.48, 12.56, 14.44, 16.11% respectively) compared to control group (4.46%) in 48 hours (p<0.05).

Conclusion: Noscapiene increased apoptotic index in endometriotic stromal cells in dose and time dependent manner. It can suggest for endometriosis treatment.

Keywords: Endometriosis, Noscapiene, Apoptosis, Stromal Cell

P-137: Effect of Different Equilibration, Freezing and Thawing Rate on Cryopreserved Buffalo Spermatozoa Diluted in Tris- Egg Yolk Extender

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Background: Artificial insemination (AI) has been used for increasing genetic potential of animals in production while application of AI has been restricted in buffalo due to low freezing ability and fertility of frozen-thawed buffalo spermatozoa. So improvement in buffalo sperm cryopreservation may enhance the breeding programs

of this valuable farm animal. Semen processing is one of the major factors which affect post thawed sperm quality. Therefore the aim of this study was to evaluate sperm functional and structural characteristics in order to find optimal equilibration, freezing, and thawing rate of buffalo semen diluted in tris- egg yolk extender.

Materials and Methods: Twenty ejaculates of four buffalo bulls were diluted in tris-egg yolk extender. Semen processing was done as follow: equilibration (2, 4, 8 and 16 hours), freezing (5, 10, 15 and 20 minutes), and thawing (30 seconds at 37°C, 15 seconds at 50°C and 6 seconds at 70°C). Post thawed motility characteristics, membrane integrity, Acrosome intactness and chromatin stability were assisted with CASA, HOS solution, Giemsa dye, and sperm chromatin structure assay respectively.

Results: The post thaw sperm motility, plasma membrane integrity (PMI), and normal apical ridge (NAR) in equilibrium 4 and 8 hours were higher than the rest of the equilibration times. Ten minute freezing time significantly increased motility and progressive motility of spermatozoa compared to others freezing times ($p<0.05$). The motility and velocity patterns were superior in 70°C for 6 seconds as compared to 37°C and 50°C ($p<0.05$). DNA integrity of frozen-thawed spermatozoa remained unaffected following different equilibration and freezing rates while sperm DNA damages were slightly increased when semen thawed in 70 °C for 6 seconds.

Conclusion: The results showed that, 4 hours equilibration, 10 minute freezing time and 6 sec thawing in 70°C were suitable for buffalo sperm cryopreservation.

Keywords: Buffalo, Cryopreservation, Semen Processing, Sperm Parameter

P-138: Effect of Short Term Storage on Functional Parameters of Post-Thawed Buffalo Spermatozoa

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Background: Detection of sperm parameters based on conventional methods, immediately after thawing, have been revealed sperm capacity to *in vivo* and *in vitro* fertilization. But this evaluation is not fully reliable because of long journey of sperm during female genital tract to reach the oocyte and fertilize it. Therefore, analyzing post-thawed sperm parameters after incubation is more beneficial for estimating sperm fertilizing ability. Because of high precision, repeatability and ability to assist large numbers of cells in a short period of time, recent techniques such as computer assisted sperm analyzer (CASA) as well as flowcytometry have been partially substituted for routine subjective analysis. Therefore, the aim of this study is to evaluate sperm functional and flowcytometric parameters of buffalo frozen-thawed spermatozoa after thawing and following a four-hour incubation

Materials and Methods: Twenty ejaculates of 4 buffalo bulls were collected twice a week according to standard artificial insemination procedures. The semen were diluted in Bioxcell extender (IMV France) and frozen in liquid nitrogen. The samples were thawed and sperm motility characteristics, membrane integrity, superoxide

anion, and DNA intactness were evaluated with CASA (SCA, Spain), hypo osmotic solution test, dihydroethidium, and sperm chromatin structure assay respectively after thawing and four hours incubation in 37 °C. Mean of data were compared with paired-sample t test by significant level of 0.05.

Results: Sperm motility, kinematic parameters, and membrane integrity were reduced after four hours incubation which were not statistically significant. The sperm motility, curvilinear velocity, and straight line velocity were 69.72 %, 73.97 $\mu\text{m/s}$, and 41.09 $\mu\text{m/s}$ respectively after thawing and 41.20 %, 40.01 $\mu\text{m/s}$ and 20.43 $\mu\text{m/s}$ after 4 hours of incubation. In contrast Reactive oxygen species (52.11 vs. 66.08, $p>0.05$) and DNA damage (5.32 vs. 7.87, $p<0.05$) were increased after four hours of incubation.

Conclusion: Our results showed that among the sperm functional parameters, DNA integrity was more prone to oxidative damages during 4 hours of incubation.

Keywords: Buffalo, Functional Parameters, Post Thawed Spermatozoa, Incubation

P-139: Ram Sperm Motility Following 48 Hours of Refrigeration: Evaluation of Different Extenders for The Preservation of Ram Semen in Liquid State

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Background: The application of laparoscopic method for intrauterine insemination is limited in sheep industry by the high cost of equipment and low pregnancy rate of frozen-thawed spermatozoa. Both disadvantages could be decreased if chilled ram semen is transported to the farms and inseminated through cervix. Semen extender provides the requirements for sperm and affects its motility, viability, and fertility during preservation in chilled condition. In this study we evaluated the motion characteristics of ram sperm which was diluted in egg yolk, milk, and soybean based extenders to find a suitable extender for chilled preservation of ram semen.

Materials and Methods: Twenty ejaculates of 5 mature Zandi rams were collected once a week according to standard artificial insemination procedure. The samples were diluted in Tris-egg yolk, milked based (Inra; IMV France) and soybean based (Bioxcell; IMV France) extenders with concentration of 20 million/ml and preserved in refrigerator (4°C) for 48 hours. Sperm motion characteristics were evaluated in 1, 24, and 48 hours after refrigeration by Sperm class analyzer (Microptic, Barcelona Spain). Mean of data were compared using Duncan's multiple range tests by significant level of 0.05.

Results: Result showed that all of the kinetics parameters were reduced in time depend manner. The mean of motility were 88.17, 71.30 and 52.34 after 1, 24, and 48 of refrigeration respectively. Sperm motility, progressive motility and velocity patterns were significantly higher in Bioxcell extender at 1 and 24 hours compared than others groups. While after 48 hours of refrigeration milk based extender showed higher motility and velocity patterns compared with other two extenders. Egg yolk based extender indicated lower kinematic characteristics compared with others in all of the evaluated times.

Conclusion: Our result showed milk based extender have more potential to preserved motion characteristics of chilled ram sperma-

tozoa and were suitable to long term preservation of ram semen.

Keywords: Ram Sperm, Milk, Egg Yolk, Soybean, Chilled Preservation

P-140: Protective Effects of Imedeon on Oxidative Stress Induction Spermatogenic Disorders and Fertility Potential of Cyclophosphamide-Treated Male NMRI Mice

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Background: Imedeon tablets are revolution in anti-aging skin-care. This tablets contains Biomarin complex (rich in natural proteins and Marin polysaccharides) and Lycophence GS complex rich in grape seeds extract and lycophence antioxidant, helps to give the skin protection against both direct and indirect damage that caused by free radicals. Cyclophosphamide as an anticancer agent has been known as a male reproductive toxic agent. Therefore the purpose of this study was to investigate whether Imedeon as an established antioxidant and anti-toxicant might have protective effects on reproductive toxicity of CP by assessment of histological characteristics in male mice or not.

Materials and Methods: In this experimental study, 60 mature male mice were assigned to six groups, 10 mice in each. The animals allocated to control group, CP treated in 12 mg/kg/day, Imedeon treated in a first dosage 111 µg/kg/day, Imedeon treated in second dosage 222 µg/kg/day, CP treated and Imedeon treated in first dosage and the last group CP treated and Imedeon treated in second dosage. After 35 days animals were sacrificed. Histological and histomorphometrical analysis of testis was carried out. Serum levels of testosterone were evaluated.

Results: In CP treated group the histological and histomorphometrical study of testis confirmed the percentage of seminiferous tubules with positive tubular differentiation (TDI), repopulation (RI) and spermatogenesis (SPI) indexes were significantly ($p < 0.05$) less than those of control and Imedeon treated groups and there was an obvious structural changes such as appearance of vacuolated edema in the interstitial connective tissue between the seminiferous tubules with increased hypertrophic mass of Leydig cells, while Imedeon use diminished the adverse effects of CP ($p < 0.05$).

Conclusion: The present results highly support the idea that CP can adversely damage the testicular tissue, but Imedeon could effectively prevent these adverse effects by inhibiting of oxidative processes and efficient scavenging of free radicals.

Keywords: Cyclophosphamide, Imedeon, Histological, Spermatogenesis, Mouse

P-141: Impact of Oxidative Stress Induced with BSO on Development and Differentiation of Ovarian Follicles in Mice

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Background: Whenever the balance between Antioxidants and reactive oxygen species (ROS) is disrupted towards an over-abundance of ROS, oxidative stress (OS) occurs. There is evidence that glutathione as an intracellular antioxidant plays an important role against ROS in female reproductive system. BSO is a selective inhibitor of glutathione peroxidase. The aim of the present study is to investigate BSO induced OS effect on development and differentiation of ovarian follicles in mice.

Materials and Methods: In this study, 30 female and 15 male mice were used. The female mice were divided into 3 groups of control, sham and experimental ones. 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, 6 and 7 days old new born were sacrificed and their ovaries were prepared for light and electron microscopic studies. The number of follicular nests and diameter of primordial and primary follicles were determined using Motoc software and compared with control values.

Results: Light microscopy and morphometry showed that follicular nests are distinguished on the 2nd and 3rd days old newborn and follicles on 6th and 7th days. Morphometric studies revealed that the number of follicular nests were similar in control and sham groups, but significantly ($p < 0.001$) reduced in experimental groups in comparison to control group. However, the number and diameters of primordial and primary follicles in experimental and controls were not significantly different. TEM showed that cytoplasmic organelles in experimental group had preapoptotic features.

Conclusion: The result indicates that oxidative stress suppresses follicular differentiation at early stages but does not affect the development of already differentiate follicles.

Keywords: Oxidative Stress, BSO, Differentiation, Ovary, Follicle

P-142: Effects of Main Substrate of Nitric Oxide in The First Half of Pregnancy on Testis in Mice Embryos and Offspring

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Background: Nitric oxide (NO) is an important molecular regulator in biology of reproductive systems. In addition, critical level of NO is essential for Implantation and embryo's development. However, increased levels of NO can have a wide range of adverse effects. This molecule is synthesized from L-Arginine (L.A) by Nitric oxide Synthetase (NOS) activity. In the current study, the effects of increased dose of L.A in pregnant mice on their embryo and offspring's testis tissue have been investigated.

Materials and Methods: Twenty-four female NMRI mice were selected and randomly divided into three groups of eight upon observing the vaginal plaque that has been considered day zero in pregnancy. L.A was used at a dose of 200 ml/kg (i.p.) for three days respectively on 3rd, 4th, and 5th days of pregnancy. One day before

and seven days after delivery, histological changes in testis were assessed by Histotechnique method and HandE staining.

Results: Our data showed that using L.A treatment in pregnant mice is led to reduced number of seminiferous tubules and decreased diameter Tunica albuginea in embryos that were dissected one day before delivery in comparison with that of control and normal saline groups. In addition to the mentioned changes, although they were more severe in embryos, dissemination was observed in offspring group that were dissected seven days after their birth.

Conclusion: The results suggest that excess dose on NO generated by L.A induction in blood of pregnant mice, affect testis tissue and structure in their embryos and offspring, that can interfere with normal function of the mentioned organ. However, these changes seem to be improved during their growth, which needs more experiments.

Keywords: Nitric Oxide, L-Arginine, Testis Tissue, Mice Embryo, NMRI Mice

P-143: Histomorphometric Evaluation of Small Intestine during Pregnancy in Rat

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Background: Food intake of rats increases during pregnancy. This requires changes in the structure of the small intestine to absorb additional food. The aim of the present study was to investigate the morphological changes in the layers of small intestine in rats during pregnancy.

Materials and Methods: Duodenum, jejunum and ileum of 18 pregnant Sprague-Dawley rats (day 7, 14 and 21 pregnancy) were collected. Villous height and width and thickness of tunica submucosa, tunica muscularis entirely and separately (circular and longitudinal layers) were measured on transverse sections. The data were analyzed by one-way ANOVA and Tukey post-hoc test (SPSS 11.5). $P < 0.05$ was considered significant.

Results: Villi length in duodenum decreased until day 14 of pregnancy and then increased near parturition ($p < 0.05$). In the jejunum this item increased during growth of fetuses ($p < 0.001$). In the ileum, the length of villi decreased with age until day 14 of pregnancy and did not increase until parturition ($p < 0.05$). Villi width in duodenum and jejunum increased during pregnancy, but in ileum decreased until day 14 and then increased in day 21 ($p < 0.05$). Lamina propria and tunica muscularis diameters in duodenum did not have any remarkable changes during pregnancy ($p < 0.05$). However, in jejunum, they were increased until day 14 pregnancy ($p < 0.05$) and remained unchanged until the end of pregnancy. Most diameters of them were in day 14 of pregnancy in ileum and then decreased until day 21 of pregnancy ($p < 0.05$).

Conclusion: More capacity of duodenum to digest food intake during pregnancy was observed by increasing villi length and muscular layer thickness. Furthermore, along with the progress of gestation greatest histomorphologic change in small intestine was observed in the jejunum to increase absorption of nutritional needs during pregnancy. While, the reduction in the ileum histomorphologic indices during pregnancy, control the absorption of excess produced amino acids and glucose by hyperphagia.

Keywords: Pregnancy, Small Intestine, Histomorphometry, Rat

P-144: Evaluating The Role of Long-Term

Administration of Methamphetamine on Sperm Motility, Acrosome Reaction and Chromatin Packaging

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Background: This study investigated the probable effects of daily administration of methamphetamine (MA) on sperm motility, acrosome reaction (AR), acridine orange (AO), sperm chromatin dispersion (SCD) test and chromomycin A3 (CMA3) in mice.

Materials and Methods: Twenty four NMRI male mice were administrated three different doses of MA intraperitoneally ($n=6$ in each group). Different doses of MA were administrated thirty days to different groups as follows: low, medium and high dose, 4, 8 and 16 mg/kg, respectively. Control group was only received saline instead. The mice were weighed before and after MA administration. Sperm motility and AR were analyzed accordingly. The effects of MA on sperm DNA integrity were assessed using AO, SCD and CMA3 tests.

Results: There was a significant decrease in body weight in high dose group compared to other groups. Sperm analysis showed that percentage of progressive motility was decreased in medium and high dose groups compared to controls (24.67 ± 6.32 , 20.17 ± 4.1 and 46.4 ± 3.8 , respectively, $p < 0.001$). A significant increasing level in AR and SCD analysis was seen in high dose group ($p < 0.001$). The results of AO and CMA3 exhibited a significant increase in high and medium groups compared to control group ($p < 0.0001$).

Conclusion: It appears that MA may significantly reduce sperm progressive motility. The sperm DNA integrity seems to be affected by MA administration in a dose-dependent manner as well.

Keywords: Methamphetamine, Sperm Motility, Acrosome Reaction, Sperm Chromatin Integrity

P-145: Efficacy of Simvastatin in The Prevention of Doxorubicin-Induced Testicular Morphometric-Stereological Alterations in Mice

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Background: Doxorubicin (DOX), a widely used antineoplastic compound, is associated with testicular damage and infertility. In this study, we intended to determine the possible preventive effects of simvastatin (SIM), a lipid lowering agent with antioxidant and anti-inflammatory activities, on testicular morphometric-stereological changes due to DOX treatment in mice.

Materials and Methods: Male mice, five weeks old, were randomly divided into four groups of six mice each. DOX was administered to two groups of mice in 5 equal intraperitoneal injections over a period of 4 weeks (accumulated dose of 20 mg/kg). One of these groups received 5 equal oral doses of SIM (accumulated dose of 60 mg/kg) along with DOX. A vehicle-treated control group and a SIM control group were also included. Morphometric measurement was determined by using calibrated ocular micrometer.

Results: Histomorphometrical examinations revealed significant reductions in the seminiferous tubules diameters and their epithelial heights along with an increase in testicular capsule thickness and interstitial space widening in DOX-treated mice. All above-mentioned parameters were recovered significantly by SIM co-administration.

Conclusion: These results have indicated that SIM may have a preventive role on morphometric-stereological changes caused by DOX in mouse testes.

Keywords: Doxorubicin, Simvastatin, Testis, Mouse

P-146: Fertility and Flow Cytometric Evaluations of Frozen-Thawed Rooster Semen in Cryopreservation Medium Containing Low Density Lipoprotein

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Background: Frozen-thawed rooster semen is not reliable for use in artificial insemination in commercial stocks. Low density lipoprotein (LDL) has been assessed for effectiveness as a cryoprotectant in the extender to improve the quality of frozen-thawed rooster semen. Although LDL has been evaluated in a few studies in other species for semen cryopreservation, to date, no study has been conducted that examines this cryoprotectant for freezing fowl semen. Thus, this study aims to analyze the effects of different concentrations of LDL (0, 2, 4, 6, and 8%) in a Beltsville extender for cryopreservation of rooster spermatozoa.

Materials and Methods: In experiment one, motion parameters, membrane integrity, acrosome integrity, apoptosis status, and mitochondria activity were assessed after freeze-thawing. The highest quality of frozen-thawed semen was selected to be used for evaluation of the fertility rate in experiment two. Semen was collected from six roosters, twice weekly, then extended in a Beltsville extender that contained different concentrations of LDL as follows: 0 (control), 1% (BLDL1), 2% (BLDL2), 4% (BLDL4), 6% (BLDL6), and 8% (BLDL8).

Results: Supplementation of the Beltsville extender with 4% LDL produced the most significant percentage of motility (43.1 ± 1.3), membrane integrity (59.4 ± 2.1), mitochondria activity (49.1 ± 1.19), and viable spermatozoa (45 ± 2.28) compared to control treatment with the results of 22.7 ± 1.3 , 38.4 ± 2.1 , 40.25 ± 1.19 , and 37.8 ± 2.28 for motility, membrane integrity, mitochondria activity, and viability, respectively. In experiment two, more significant percentage of fertility rate was observed for frozen-thawed semen in the extender supplemented with 4% LDL (49.5 ± 1.6) compared to the control (29.2 ± 2.9). Progressive motility and acrosome integrity were not affected by LDL levels in the extenders

Conclusion: The results showed that supplementation of the Beltsville extender with 4% LDL resulted in higher quality of frozen-thawed rooster sperm.

Keywords: Rooster, Cryopreservation, LDL, Apoptosis, Fertility

P-147: Effect of Phoenix Dactylifera Pit

Powder on Reproductive Physiology in Female Rat

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Background: The pit of Phoenix dactylifera contains different compounds such as saturated and unsaturated fatty acids, Zinc, Cadmium, Calcium and Potassium which could improve steroidogenesis. In this research, effect of phoenix dactylifera pit powder was evaluated on female rat reproduction.

Materials and Methods: In this experimental study, 50 female wistar rats were divided in 5 groups of 10. In order to set them in the same sexual cycle, sex hormones were used. Phoenix dactylifera pit powder was given for 28 days orally. Experimental groups received 150, 300 and 600 mg/kg of the powder. The control group received nothing and the sham group received solvent. The blood samples were taken at the end of the 28th day and serum concentration of LH, FSH, estrogen and progesterone was measured. These data were analyzed by Anova and Tukey statistical method.

Results: The result showed that the serum concentration of LH and FSH in the experimental groups were not significantly different compared to that of control group. The received groups 300 and 600 mg/kg phoenix dactylifera pit showed increasing amount of estrogen and progesterone.

Conclusion: According to the findings of this study, phoenix dactylifera pit increases estrogen and progesterone by steroidogenesis improving but has no effect on gonadotropins.

Keywords: Phoenix dactylifera Pit, Steroidogenesis, Gonadotropin, Rat

P-148: Effect of A Low Glycemic Index Diet on Insulin Resistance and Reproductive Hormones

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Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disease in women affecting 5-10% of those of childbearing age. The majority of women with PCOS, regardless of weight, have a form of insulin resistance that is intrinsic to the syndrome. Obese women with PCOS have an added burden of insulin resistance related to their adiposity. Given the association of obesity and insulin resistance with PCOS, weight management is recommended as first-line management for women with PCOS. In general population, a diet lower in GL from reducing GI or carbohydrate is associated with greater weight loss and maintenance proposed to

be a result of reduced appetite and energy intake from increasing protein, reducing carbohydrate intake or lowering GI, or related to a diuretic effect with an acute low-carbohydrate diet. Because the majority of women with PCOS show a marked compensatory hyperinsulinemia after carbohydrate ingestion, there may be specific advantages of diets with a low GI in this group. The aim of this review study was to investigate the effects of a low-glycemic-load diet on reproductive hormones, inflammatory markers, lipids, glucose, and insulin levels in obese women with PCOS.

Materials and Methods: This review article was prepared by studying of articles obtained from Google, pub med sites with key words such as polycystic ovary syndrome; insulin sensitivity; dietary intervention, glycemic index, reproductive hormones.

Results: Barr et al. (2013) studied the efficacy of an isocaloric low-GI dietary intervention on insulin sensitivity, in women with PCOS. A non randomized 12-week low-GI dietary intervention, preceded by a 12-week habitual diet control phase and proceeded by a 12-week follow-up phase was conducted. Measures of insulin sensitivity and nonesterified fatty acid improved after intervention. Marsh et al. (2010) assigned overweight and obese women with PCOS (n= 96) to consume either an ad libitum low-GI diet or a macronutrient-matched healthy diet and followed the women for 12 months or until they achieved a 7% weight loss. They reported increased menstrual regularity for 95% of women after a low-GI ad libitum weight-loss diet compared with 63% of women following a standard healthy ad libitum weight-loss diet. Pregnancies did not differ by diet composition. Both the low-GI and the conventional diets led to similar improvements in blood lipid, androgenic hormones concentrations, and markers of inflammation. Among the biochemical measures, only serum fibrinogen concentrations showed significant differences between diets. Herriot et al prescribed a reduced glycaemic load diet, with energy reduction in overweight PCOS patients. At the follow-up appointment, BMI and waist circumference significantly decreased in overweight patients. 71% of women self-reported hypoglycaemia initially; this was reduced to 13% at follow-up. Galletly et al. (2007) examined Twenty-five overweight women with PCOS. They were randomly allocated to the low protein - high carbohydrate (LPHC) or high protein - low carbohydrate (HPLC) diet for 16 weeks. The HPLC diet was associated with significant reduction in depression and improvement in self-esteem. There was no change in any psychological measures for the LPHC group. There was no difference in weight loss between the groups. HPLC diets may be associated with better compliance and hence be more successful in the long term treatment of obesity. Douglas et al. (2006) conducted a 16-day acute weight-maintenance intervention. Three diet compositions were compared (a monounsaturated fatty acid (MUFA) enriched, a conventional healthy diet (STD diet) and a low-carbohydrate diet with a total energy intake of approximately 2,000 kcal/day. The results indicated that fasting insulin was lower following the Low CHO diet relative to the STD diet. The change in mean body weight during the Low CHO dietary intervention was significantly greater than that for the MUFA dietary intervention and tended to be greater than that for the STD dietary intervention. It is possible that the greater weight loss in this study is related to the lower GL in the low-carbohydrate diet, because all diets were of equivalent energy and protein intake.

Conclusion: In conclusion, low-GI diet may provide an additional advantage over and above that of a conventional healthy diet in managing the underlying insulin resistance, cardiovascular risk, and irregular menstrual patterns in women with PCOS who are overweight.

Keywords: Polycystic Ovary Syndrome, Insulin Sensitivity, Dietary Intervention, Glycemic Index, Reproductive Hormones

P-149: Antioxidant Effects of Dietary Rosemary (*Rosmarinus Officinalis*) Extract on The Rooster Semen Performance and Sperm Quality

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Background: Exposure of sperm membranes to Reactive Oxygen Species induces lipid peroxidation causing membrane breakdown and loss of function. A rosemary extract as dietary additive was assayed for antioxidant effectiveness to improve the quality of rooster semen. Although rosemary extract has been evaluated for *in vitro* study of semen preservation, up to date no study has been conducted to examine this vegetable substance in rooster ration during spermatogenesis. The objective of this study was to evaluate the antioxidant effects of dietary rosemary extract on rooster semen quality.

Materials and Methods: Sixteen roosters were divided into equal four groups consuming diets supplemented with 0, 50, 100 or 200 mg of rosemary extract/kg of feed. Semen parameters including motility, progressive motility, membrane integrity and lipid peroxidation were assessed at the 0, 14, 28, 42 and 56 days of experiments.

Results: Results show that inclusion of 100 and 200 ppm of Rosemary extract significantly improved the total motility and membrane integrity of sperm at the end of experiment compared to other treatment. However, concentration, progressive motility and lipid peroxidation of sperm were not affected by the dietary rosemary extract

Conclusion: It seems that rosemary extract with antioxidant properties seems to be suitable additive for improving the sperm preservation during spermatogenesis.

Keywords: Rosemary Extract, Rooster, Sperm, Lipid Peroxidation

P-150: Effect of Vitrification on High Magnification Morphology and Fertility Potential of Human Spermatozoa Cell

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Background: Many sperm characteristics including, motility and morphology are important for normal spermatozoa- oocyte interaction. These features may be altered during vitrification. Motile sperm organelle morphology examination (MSOME) is an unstained real time high magnification technique that analysis viable sperm morphology instantly. This study aimed to investigate the influence of vitrification on human sperm vacuolization by MSOME technique and fertility potential by zona binding assay (ZBA).

Materials and Methods: 30 normozoospermic ejaculates were prepared by direct swim up technique. After swim up, the supernatants were divided into two parts: fresh and vitrified groups. For vitrification, sperm suspension was mixed with equal volume (1:1) of Hams' F10+HSA+0.5 M sucrose, and 30 µl sperm suspension was dropped directly to LN. Warming was performed by quick submerging spheres one by one into pre-warmed 5ml Hams' F10 with

5% HSA at 37°C accompanied by gentle vortexing for 5-10s. Sperm motility and MSOME were evaluated for each sample. For MSOME, we loaded 2µl sperm suspension into 4 µl polyvinylpyrrolidone (PVP) in different culture dishes (GWST 5040; Will Co, Wells BV) and covered with oil. 2×10^6 spermatozoa in each 25µl droplet containing four oocytes were performed for ZBA.

Results: Our formula is (Normal head score=2)+ (Lack of vacuole score=3)+ (Normal base score=1)= (Total score=6) for a morphologic "normal top" spermatozoon. For MSOME analysis, 3 grades were considered: normal spermatozoa with no vacuoles (grade1), ≤ 2 small vacuoles (grade2), and at least one large vacuole or > 2 small vacuoles and morphometrically abnormal spermatozoa (grade3). There was no significant difference between the rates of MSOME in class1 (1 ± 2.7), class2 (-0.5 ± 3.1), class3 (-1.11 ± 3.6) and fertility potential before and after vitrification

Conclusion: Vitrification has adverse effect on sperm parameters. This technique, however, neither causes vacuolization of sperm head nor alters fertility potential.

Keywords: Sperm Vitrification, Motile Sperm Organelle Morphology Examination (MSOME), Polyvinylpyrrolidone (PVP), Liquid Nitrogen (LN)

P-151: Optimal Condition of Introducing Green Fluorescent Protein (GFP) Gene into The Rainbow Trout (*Oncorhynchus mykiss*) Sperm by Electroporation Method

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Background: Many techniques have been developed to increase DNA molecules into sperms called sperm mediated gene transfer (SMGT) and one of them is electroporation. Sperm mediated gene transfer is an ideal method for mass gene transfer in organisms with external fertilization. Gene transfer is applied to rainbow trout sperm and the effects of electroporation on sperms and rate of gene uptake by sperms were examined.

Materials and Methods: In this study, sperms were collected from ten male rainbow trout at the age of 3 years. Through the method of hand stripping, average of 1.5-5 ml sperms were collected from each; then sperms were counted and the amount always have been diluted to $2 \times 10^6/\mu\text{l}$. The condition of electroporation such as field strength pulse duration, medium in use, concentration of gene, and effect of incubation after electroporation were optimized. Current study has many groups with variety of conditions in each stage. Therefore, before each stage, the group and condition have been explained. Sperms were electroporated in the existence of linear pIRES2-EGFP plasmid at 50-100 ng/µl, with field strength between 100 to 1500 v/cm.

Results: Present studies indicated a direct relationship between DNA concentration and rate of gene uptake and also pulse length and intensity of gene uptake. By the way, if electroporated sperm/gene was incubated after electroporation, the rate of gene uptake will be increased. On the other hand, if the seminal plasma is used as diluent, the viability and gene uptake will be significantly increased.

Conclusion: Compared to the non-electroporated group, these result showed that electroporation enhanced the capacity of spermatozoa to uptake plasmid DNA so that ultra violet microscope showed

93.5% of electroporated sperm (in the best and optimal condition) absorbed the plasmid DNA with different intensity.

Keywords: Electroporation, SMGT, Rainbow Trout Sperm, GFP

P-152: The Effect of Hops (*Humulus lupulus L*) Extracts on Hormone Levels and Sexual Cells in Adult Male Mice

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Background: Hops Plant (*Humulus lupulus L.*) plants is very beneficial has many industrial and medical applications. Hops has protein, amino acids, lipids, fatty acids, sugars such as glucose and fructose, pectin, salts, wax and estrogenic compounds. So the aim of this study was to evaluate the effect of hops extracts on hormone levels and sex cells in adult mice.

Materials and Methods: 40 adult male mice used in research are divided into four main groups, each consisting of five sub-groups of 8 groups were divided into of control, experiment 1, 2 and 3. The experimental group received daily 50, 100 and 150 mg / kgBW Hops extract was given orally. At the end of 35 days, using the ether imbued cotton in a jar of anesthesia, after anesthetized, blood from the left ventricle was performed, and the testes removed, the tissue sections were prepared for evaluation of tissue changes. Samples stained with hematoxylin - eosin was performed. Levels of testosterone and progesterin hormones estrogen and sex cells also were examined. And independent sample t test and ANOVA were used ($p \leq 0/05$).

Results: The results of this study indicate that extracts of hops increases testosterone and estrogen in the experimental group, While they do not show a significant effect on progesterone. It also increases the number of, spermatogonia cells, and spermatid in experimental group.

Conclusion: This study showed that extracts of hops can affect on hormone levels and sexual cells in adult male mouse and this plant can be useful in treatment of infertility.

Keywords: Hops, Mice, Sexual Cell, Sperm, Testis Sexual

P-153: Hyaluronic Acid Hydrogel Scaffold Containing VEGF and bFGF Affect Oestrous Cycle Restoration after Rat Ovarian Tissue Autotransplantation

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Background: The functional longevity of ovarian transplant at

present is one of the most challenging questions regarding ovary transplantation. So, we used hyaluronic acid (HA) hydrogel scaffold with combination of VEGF and bFGF for restoration of cyclical ovarian function after ovarian tissue transplantation in a rat model.

Materials and Methods: A total of 25 Wistar female rats weighing between 90 and 100 g were used. Vaginal cytology was used to assess ovarian function. All these animals had normal estrous cycles according to vaginal cytology and were randomly divided into 3 groups for ovarian tissue autotransplantation including: group A (ovarian tissue without HA, VEGF and bFGF), group B (ovarian tissue encapsulated with HA) and group C (ovarian tissue encapsulated with HA with VEGF and bFGF). To mimic the clinical situation of sterility, a bilateral ovariectomy was performed. All grafts were transplanted into latissimus dorsi muscle in diestrus stage. From the fourth day of post-surgery, ovarian function was monitored by taking vaginal smears and continued till restoration of first cycle.

Results: Daily vaginal smear showed that the first estrous cycle began on the average of 14.7, 13.4 and 10.1 days after transplantation in group A, group B and group C respectively. Statistical analysis showed the significant ($p < 0.05$) difference between group A and C as well as between B and C but not about group A and B.

Conclusion: The findings suggest that host treatment with HA hydrogel improves oestrous cycle restoration specially when combined with VEGF and bFGF. This protocol can be applied in ovarian autotransplantation for fertility restoration.

Keywords: Ovarian Transplantation, bFGF, VEGF, Hyaluronic Acid, Oestrous Cycle

P-154: Comparison of Ovine Matured Oocytes Quality in SPOM Medium Supplemented with Different Doses of Oocyte-Secreted Factors

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Background: In this study we evaluate the effects of different doses of GDF9 with fixed dose of BMP15 on quality of *in vitro* matured sheep oocytes.

Materials and Methods: Good ovine Cumulus-oocyte complexes (COCs) were first transferred to Pre-IVM medium, and then to three IVM mediums supplemented with 10%v/v BMP15 and: 1) 175 ng/ml 2) 131 ng/ml or 3) 219 ng/ml GDF9. After maturation period, MII oocytes with normal first polar body were counted. Oocyte viability was determined by means of the TUNEL technique. Immunofluorescence microscopy staining were used to evaluate cell meiotic spindle and redistribution of cortical granules (CGs). In addition, fertility rate in group 1 and 2 was determined by means of IVF. Statistical analyses were carried out by ANOVA and significant differences between means were determined using Tukey HSD test for comparison of multiple means.

Results: Maturation rate of group 3 was significantly lower than group 1 and 2 ($75.57\% \pm 2.74$ vs. $93/56\% \pm 0.56$ and $90.67\% \pm 4.09$,

respectively, $p < 0.05$). Spindle, CGs, and TUNEL staining results indicated no significant difference between groups. MII oocytes with normal polar body in group 1 was significantly higher than group 2 and 3 ($71.39\% \pm 0.75$ vs. $25.80\% \pm 1.09$ and $31.68\% \pm 2.36$, respectively, $p < 0.001$). Finally, the blastocyst formation in group 1 was significantly higher than group 2 ($39.86\% \pm 0.38$ vs. $28.73\% \pm 1.77$, $p < 0.01$).

Conclusion: These results demonstrate that SPOM medium supplemented with 175 ng/ml GDF9 and 10% BMP15 enhance oocyte developmental competence.

Keywords: IVM, Ovine, GDF9, BMP15

P-155: Studies of Cytotoxic Effects of CdSe Quantum Dots on Fetal Development Testis and Epididymis in Albino Mice

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Background: Quantum dots (QDs) are among the most promising items in the nanomedicine toolbox. These nanocrystal fluorophores have several potential medical applications including nanodiagnostics, imaging, targeted drug delivery. Nevertheless, *in vivo* cytotoxicity of these nanoparticles has not been highly considered. For this reason, the cytotoxic effects of CdSe QDs on testis and epididymis embryonic development are presented in this study.

Materials and Methods: In this work, 10, 20 and 40 mg/kg doses of CdSe QDs were injected to some female mice in day 8 gestation. Structural and optical properties of QDs were studied by XRD, UV-Vis absorption spectrum and Scanning Tunneling Microscopy, and the number of cells in seminiferous tubes of various groups were analyzed using SPSS 16 programme (one way Anova test).

Results: Histological studies of testis tissue in adult offspring didn't show any effect of QDs on morphology seminiferous tubular, lamina propria, Interstitial tissue, germinal layer thickness and in number of spermatogonia, spermatocytes. Histological study of epididymis tissue also showed no significant effect of QDs on morphology and structure of tube and in the lumen content.

Conclusion: This study showed no significant effect of QDs on embryonic development of testis and epididymis tissues, even in high doses and considering lack of literature review in this field. This study can be an introduction to researches of toxicity effect of QDs on development of male reproduction system.

Keywords: CdSe, Quantum Dots (QDs), Fetal Development, Testis

P-156: A Study about Toxicity of CdSe Quantum Dots on Male Sexual System of Mice and Controlling This Toxicity by ZnS Coverage in Immature Mice

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Background: Quantum dots are commonly composed of cadmium contained semiconductors. Cadmium is potentially hazardous but toxicity of such quantum dots is not yet systematically investigated. On the other hand, *in vitro* studies have shown almost complete control of CdSe induced cytotoxicity by ZnS coverage. Toxicity of CdSe quantum dots and controlling this toxicity by ZnS coverage in immature mice are presented in this study.

Materials and Methods: Mentioned quantum dots were injected in 10, 20, and 40 mg/kg doses to some male mice and structural and optical properties of quantum dots were evaluated using XRD.

Results: One month after CdSe:ZnS injection, Histological studies of testis tissue showed high toxic effect of CdSe:ZnS in 40mg/kg. Histological studies of epididymis didn't show any effect of quantum dots on morphology and tube structure. Mean concentration of LH and testosterone and testis weight showed also high toxicity of CdSe:ZnS in 40 mg/kg dose. But FSH hormone and body weight didn't show any difference with control group.

Conclusion: Although it has been reported that *in vitro* coverage controls CdSe highly, this study showed high toxicity of *in vivo* CdSe:ZnS (even more than CdSe) which can show that coverage has caused increase in induced toxicity by quantum dots. Considering lack of any previous study in this category, our study can be an introduction to more studies about effects of quantum dots toxicity on development of male sexual system.

Keywords: CdSe, Male Sexual, ZnS Coverage

P-157: Polymorphic Core Promoter GA-repeats Alter Gene Expression of The Early Embryonic Developmental Genes

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Background: We examine the GA-repeat core promoters of MECOM and GABRA3 in human embryonic kidney-293 cell line and show that those GA-repeats have promoter activity, and those different alleles of the repeats can significantly alter gene expression. We propose a novel role for GA-repeat core promoters to regulate gene expression in the genes involved in development and evolution.

Materials and Methods: The core promoter sequence of human MECOM and GABRA3 genes was amplified by PCR. Three different alleles ranging the longest, medium, and shortest allele size were selected followed by sequencing, and were used for cloning and expression studies. The fragments cloned into pGL3 basic luciferase vector and transfected into HEK-293 cells. 24h after transfection, luciferase activities was measured.

Results: The MECOM and GABRA3 core promoter GA-repeats are polymorphic in humans and We detected significant difference in gene expression activity as a result of different alleles across MECOM and GABRA3 core promoters in the HEK-293 cell line.

Conclusion: Protein complexes that bind to GAGA'DNA elements are necessary to replace nucleosomes to create a local chromatin environment that facilitates a variety of site-specific regulatory responses. We have previously identified human protein coding gene core promoters that are composed of exceptionally long GA-repeats. The functional implication of those GA-repeats is beginning

to emerge in the core promoter of the human SOX5 gene, which is involved in multiple developmental processes. In the current study, we analyzed the functional implication of GA repeats in the core promoter of two additional genes, MECOM and GABRA3, whose expression is largely limited to embryogenesis. Similar to the SOX5 gene, MECOM and GABRA3 are involved in key developmental processes. Null mutations within the SOX5 and MECOM genes are lethal in the embryo, whereas hemizygosity for the GABRA3 deleterious mutations result in disorders within the brain. Across-species homology check revealed that the GA-repeats in the core promoters of the three genes, SOX5, MECOM, and GABRA3 are highly conserved across evolution, which further strengthens a role for those repeats in gene expression and evolution.

Keywords: MECOM, GABRA3, GA-repeat, Core Promoter, Expression

P-158: Effect of Occupational Exposures on Male Fertility

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Background: Occupational exposures do have a negative impact on the male reproductive system, but sometimes it is difficult to isolate a single insult. Workers can be exposed to a number of harmful physical, chemical and psychological factors in their working environment. During recent years, the various diseases and disorders caused by these stressors have drawn the attention of a number of researchers throughout the world. The effect of certain working hazards on the human reproductive system is one of the areas that have been studied and a number of reports concerning this have been published in this field.

Materials and Methods: Through a descriptive study 1572 males who referred to the Infertility Research Center in Iran were selected. According to participants' occupations and considering similar occupational exposures, twelve occupational categories were derived. The study consisted of questionnaires completed by trained interviewers to provide information about demographics, marital status, type and duration of infertility, occupational history including job title and task, and exposure to occupational physical hazards. The semen analysis method in this study was computer-assisted semen analysis (CASA). The relationships between semen parameters, age and body mass index (BMI) were investigated using Spearman non-parametric correlation. Analysis of variance was used to compare semen parameters between occupational groups. Data analysis was done by SPSS 19 software.

Results: There were no statistically significant differences in the mean sperm count or sperm morphology between occupational categories. Assessment of the differences in the frequency of sperm motility classes between occupational categories revealed a significant difference only in the frequency of sperm with class B motility. The following physical exposures were shown to deteriorate sperm

parameters radiation (both ionized and microwaves) and heat. Our findings support the results of previous studies.

Conclusion: This study could help hazardous jobs and factors to be recognized (both chemical and physical) in association with infertility in men and can be referred to as a resource for other analytical studies concerning all factors influencing infertility. Workers' safety and commitment to the safety principles in the workplace can keep infertility factors at a minimum level.

Keywords: Reproductive System, Physical Exposures, Sperm Parameters, Occupational Hazards

P-159: Effect of Silymarin on Viability, Motility and Mitochondrial Membrane Potential of Ram Spermatozoa Treated with Aluminum Chloride

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Background: Aluminum, as an environmental pollutant, can exert oxidative stress on spermatogenesis and sperm morphology. Silymarin, an effective substance extracted from *Silybum marianum*, is a potent antioxidant which inhibits oxidative stress. Due to the damaging effects of aluminum on the male reproductive system as well as sperm and the role of silymarin, this study was performed to investigate if silymarin can prevent the adverse effects of aluminum chloride on viability, motility and mitochondrial membrane potential in ram sperm.

Materials and Methods: Farahani's ram sperm is divided into five groups: 1. sperm at 0 hour, 2. sperm at 180 minutes (control), 3. sperm treated with aluminum chloride (0.5 mM), 4. sperm treated with silymarin (0.5 µM) + aluminum chloride (0.5 mM), and 5. sperm treated with silymarin (0.5 µM). The treatments were done for 180 minutes. In order to evaluate viability and mitochondrial membrane potential in the sperm, MTT ((3-4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Rhodamine 123 staining were used respectively. Sperm motility was done according to World Health Organization (WHO) protocol. Data were analyzed with one way ANOVA followed with Tukey's test.

Results: The percentage of sperm viability, progressive motility and intact mitochondrial membrane potential were significantly decreased in aluminum chloride group compared to the control. In silymarin + aluminum chloride group, silymarin could compensate the adverse effects of aluminum chloride on the sperm parameters compared to aluminum chloride group

Conclusion: Aluminum chloride has toxic effect on ram sperm viability, motility and intact mitochondrial membrane potential and silymarin is able to compensate the adverse effect of aluminum chloride on these sperm parameters.

Keywords: Aluminum, Silymarin, Ram Sperm, Viability, Motility and Mitochondrial Membrane Potential

P-160: The Effects of Vanadium in Vitrification Medium on In Vitro Growth of Follicles Derived from Vitrified-Warmed Mouse Ovary

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Background: Cryopreservation of ovarian tissue is an important option in order to preserve the fertility of cancer patients undergoing chemotherapy and radiotherapy. There are many reports on adding some materials such as calcium, ascorbate and antioxidants to freezing medium. Vanadium is one of the minerals, which is very important as a trace element for normal cell function as well as development. Vanadium, affects enzymes behavior and imitates and regulates the activities of development factors. In this study, the effects of vanadium in vitrification medium on *in vitro* growth of follicles derived from vitrified-warmed mouse ovary were examined.

Materials and Methods: In this study, the ovaries of 2-4 week-old NMRI mice randomly assigned to non-vitrified and vitrified-warmed in the presence of different concentrations (0, 10, 100 and 250 µM) of ammonium metavanadate in vitrification medium. Ovaries in the vitrified groups were frozen sequentially by immersion into two solutions, equilibration solution: 7.5% EG, 7.5% DMSO in holding medium (α-MEM+10%FBS) for 7 minutes and vitrification solution: 15% EG, 15% DMSO, 0.5 M sucrose in holding medium (α-MEM+10%FBS) with different concentration of ammonium metavanadate for 3 minutes in room temperature. The ovaries were transported to the straw and kept in a nitrogen tank for a week. After this period, ovaries were transferred to thawing medium with falling concentration of sucrose (1, 0.5, 0.25) at 37°C. After thawing ovaries were examined histologically and then pre-antral follicles with diameter around 100-130 µm mechanically were isolated from ovaries in order to assess the follicle growth and survival rate were cultured for 9 days.

Results: The results show that the presence of ammonium metavanadate in vitrification solution is effective and can reduce the traumatic effects of vitrification on ovarian tissues. In addition, follicle growth rate was higher in the presence of 100µM ammonium metavanadate compared to the other vitrified-warmed groups; nevertheless, it was lower than non-vitrified group (ANOVA, p-value<0.05).

Conclusion: This study demonstrated that vanadium supplementation of vitrification medium has a positive effect on the follicle growth in follicles derived from vitrified-warmed ovaries.

Keywords: Vitrification, Ammonium Metavanadate, Follicle Growth, Ovary, Mouse

P-161: Deleterious Side Effects of Methotrexate on In Vitro Fertilization and Protective Role of Cornus Mas Extract in Adult Mice

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Background: Because of stress oxidative side effect of MTX on reproductive system, current study was aimed to evaluate protective effects of cornus mas fruit extract (CMFE) in mice treated with methotrexate (MTX).

Materials and Methods: 48 young mature male mice were classified in 4 groups. Control group received only normal saline orally (0.2 ml/day), and the second group was administered

MTX (20 mg/kg/week, IP). The third group received CFME orally (1000 mg/kg/day) along with MTX. The fourth group was only given CFME with a dose of 1000 mg/kg/day orally. The study period for all groups was 35 days. Then, the animals were anesthetically euthanized and the sperms were separated from epididymis. DNA damage level, the amount of malondialdehyde (MDA) as well as *in vitro* fertility was evaluated.

Results: In groups receiving CFME, DNA damage level, and MDA amount suggested a decrease in comparison to MTX group ($p < 0.05$). Also, *in vitro* fertility and embryonic development in MTX-treated group was significantly lower than control group, and the level of embryonic arresting was higher ($p < 0.05$). In groups which received CFME, *in vitro* fertility, and embryonic development was higher than MTX group ($p < 0.05$) and the embryonic arresting showed a decrease.

Conclusion: This study suggested that cornus mas is able to ameliorate the side effects of MTX.

Keywords: Methotrexate, Cornus Mas, Sperm Quality, DNA Damage, Mice

Ethics and Reproductive Health

P-162: Comparing The Prevalence of Sexual Dysfunction in The Fertile and Infertile Women Referring to Alzahra Hospital in 1391-1392.

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Background: Female sexual dysfunction (FSD) is defined as sexual desire disorders, sexual arousal disorders, orgasmic disorders and sexual pain disorders that result from anatomical, physiological, medical and psychological multiple factors and cause personal severe discomfortability and affect quality of life and relationship between individuals. The aim of this study is to compare the prevalence of sexual dysfunction in the fertile and infertile women referring to Alzahra clinical training center of Rasht.

Materials and Methods: This study was compared prevalence of sexual dysfunction on the 149 fertile women and 147 infertile women referring to Alzahra hospital of Rasht in 1391-1392 that used FSFI questionnaire.

Results: Sexual desire disorder in fertile group was 69/8% and infertile group was 54/4%, sexual arousal disorder in fertile group was 63/8% and infertile group was 47/6%, lubrication disorder in fertile group was 22/1% and infertile group was 19/7%, orgasm disorder in fertile group was 23/5% and infertile group was 13/6%, satisfaction disorder in fertile group was 15/4% and infertile group was 8/2%, pain disorder in fertile group was 43% and infertile group was 46/9%, female sexual dysfunction in fertile group was 74/5% and infertile group was 68%.

Conclusion: Female sexual dysfunction in fertile women was more than infertile women. Evaluation of the infertile patients in other aspects may be beneficial.

Keywords: Female Sexual Dysfunction, Female Sexual Function Index Questionnaire, Infertility

P-163: Study of Awareness among Pregnant Women Referred to Imam Ali Hospital AZNA City about The Benefits of Cord Blood Stored in 1392

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Background: Cord blood stem cells are a source of very young. Away from moral hazard problems, autoimmune reactions, cancer, and adults have the benefits of both a source of embryonic stem cells.

Materials and Methods: In this cross-sectional study, 100 pregnant women with the range 45-19 years of age participated. The questionnaire included 15 questions based on information and knowledge factors were collected. They were analyzed by SPSS software and descriptive and inferential statistics.

Results: The results are as follows: 74% of poor knowledge and 24% moderate, and 2% are good

Conclusion: The level of awareness of the benefits of cord blood is an acceptable level. Ongoing information and periodic assessment in this area are recommended.

Keywords: Cord Blood, Stem Cell, Pregnant Women

P-164: Comparing the Socio-Cultural Beliefs of Recipients and Non-Recipients of Assisted Reproductive Donation Procedures

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Background: Using assisted reproductive donation procedures increase hope in infertile couples. However large part of the world population do not recognize these procedures. One of the important steps in recognition of infertile couples' problem, whose only therapeutic option is using assisted reproductive donation procedures, is investigating their Socio-cultural Beliefs about these procedures in line with other infertile couples. This study therefore aimed to compare the Socio-cultural Beliefs of recipients and non-recipients of assisted reproductive donation procedures in 2010 in Mashhad, Iran.

Materials and Methods: This descriptive-comparative study was carried out on two groups of infertile couples with primary infertility (50 recipients, 180 non-recipients) using convenience sampling at the Montaserieh Infertility Center in Mashhad. Data was collected using demographic and knowledge questionnaire, also valid ($\alpha = 0.887$) and reliable self-structured questionnaires to measure Socio-cultural Beliefs about donation procedures, and data was analyzed using statistical tests including t test, Mann-Whitney, one-way ANOVA.

Results: The mean score of socio-cultural beliefs in recipients and non-recipients women was 70.2 ± 9.5 and 66.9 ± 12.0 , and in

recipients and non-recipients men was 73.7 ± 12.2 and 65.9 ± 12.0 , respectively (percentage). There was a significant difference between socio-cultural beliefs of recipients and non-recipients men ($P=0.006$), that is, men recipients had more positive socio-cultural beliefs compared with non-recipients men towards donation procedures. The mean score of knowledge in recipients and non-recipients women was 11.1 ± 4.1 and 9.0 ± 4.2 and in recipients and non-recipients men was 11.8 ± 3.6 and 9.7 ± 3.9 , respectively. There was also a significant difference between two groups in terms of their knowledge about donation procedures in recipients and non-recipients men ($p=0.020$) and recipients and non-recipients women ($p=0.032$).

Conclusion: Considering the results, promotion of knowledge and socio-cultural beliefs at the community level may endorse positive attitudes in infertile couples which could provide appropriate bases for the decision made by infertile couples in an environment free from tension and fear of the wrong beliefs.

Keywords: Socio-Cultural Beliefs, Knowledge, Donation Procedures

P-165: The Relationship of Sexual Function and Marital Adjustment with Menstrual Pattern and Factors Affecting on Infertile Women

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Background: Infertility is one of the important components of reproductive Health that has some effects on physical and psychological aspects of couple's life. Infertile women are concerned about their sexual and physical health. It is even possible that using and evaluation of assisted reproductive techniques have negative effects on women feelings about the sexual value and marital relationships. This study aimed to investigate the relationship of sexual function and marital adjustment with infertile women menstrual pattern and factors affecting on infertile women in 2011 in Mashhad, Iran.

Materials and Methods: This correlational study was carried out on 130 infertile women referred to Montaserie Infertility Research Center in Mashhad, who were selected by using convenient sampling. Research tools were consisted of valid and reliable demographic questionnaires including personal and infertility-related information, menstrual pattern, ROSEN Female Sexual Function Index (FSFI) and Spanier Marital Adjustment Scale (DAS), which were completed by the subjects. Data analysis was performed by SPSS software using t test, one way ANOVA, Spearman and Pearson correlation tests.

Results: The mean score of duration of information and treatment of infertility were 5.24 ± 4.12 and 4 ± 3.95 years respectively, and causes of infertility were 46.9% in female, 38.7% in male, and common and unknown factors 45.4% of the women had high sexual function and 76.9% had high marital adjustment. 71.7 and 65.4% of the women had regular menstrual and premenstrual distress respectively. 66.9% of the women had dysmenorrhea. There was not significant relationship between sexual function with menstrual cycle, but there was significant relationship between marital adjustment with regular menstrual periods in infertile women ($p=0.001$). 87.7% of the women with high marital adjustment have regular menstrual pattern.

Conclusion: The findings showed that there is better regular menstrual pattern in infertile women with improved marital adjust-

ment, that will help to improve infertility. Therefore we can use these results in implement training programs and consulting, specially in infertile women who have marital disorders.

Keywords: Sexual Function, Marital Adjustment, Menstrual Pattern, Infertility

P-166: Knowledge and Attitudes towards Long Term Usefulness of Reversible Contraceptives among Females in Reproductive Age in Yazd, Iran

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Background: Yazd has important potential role in treatment of infertility and promotion of sexual health in south of Iran. Long term usefulness of reversible contraceptives (LTURC) is the most cost-effective contraceptives between women. This study aimed to assess the reproductive aged women's knowledge, attitudes, and factors associated with use of LTURC.

Materials and Methods: This cross sectional study was performed on 300 women in city of Yazd. Semi-structured questionnaires were used to measure knowledge, attitudes and factors associated with use of LTURC; Intra-Uterine Devices, injectable and Implants. The outcome variable was current use of LTURC. A linear regression model was analyzed in STATA. Prevalence Risk Ratios for associations between these parameters were regarded significant at 95% CI with $p<0.05$.

Results: Mean age (and current use of LTURC was 31.80 and 58.3% respectively. Factors associated with current use of LTURC were; previous use RR 3.91; (95% CI 2.86, 4.02), knowledge of implant administration site RR 2.72; (95% CI 2.34, 3.51), and perception that; male partner decisions positively influence their contraceptive choices RR 2.38; (95% CI 2.12, 2.64).

Conclusion: Knowledge about site of administration, previous use of LTURC and women's attitude that male partners' choice influence their contraceptive decisions were positively associated with current use of LTURC.

Keywords: Knowledge, Attitudes, Reversible Contraceptives

P-167: Overview on Sexual Health in Male Patients Undergoing Major Cardiac Surgery

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Background: Sexual dysfunction is one of the most common problems in men after coronary artery bypass graft (CABG). Etiology of sexual dysfunction in these patients may be psychological or organic due to hormonal changes. The purpose of this study was to evaluate the incidence and type of sexual dysfunction and changes in serum concentration of sex hormones in male patients undergoing on-pump CABG.

Materials and Methods: In this before and after study, we enrolled 40 men aged less than 70 years who were candidate for on-pump CABG. Interviews were done by a physician before and 12

weeks after the operation in regard to the impact of surgery on their sexual activities. The serum levels of 6 sex hormones were also determined. The statistical tests used for data analysis included analysis of variance, McNemar's test and chi-square analysis.

Results: The mean \pm SD age of the patients was 51.27 ± 7.86 years. Incidence of sexual dysfunction was 22.5% (9 cases) before and 47.7% (19 cases) after operation. Types of sexual dysfunction were premature ejaculation (5% before, 2.5% after), impotence (7.5% before, 12.5% after) and loss of libido (10% before, 32% after). The level of sex hormones were generally decreased after operation but it was statistically significant only for estrogen ($p=0.02$).

Conclusion: Sexual dysfunction and reduction in serum level of sex hormones are common in patients before on-pump CABG and they mostly get worse after surgery. Complementary studies are suggested for prevention and treatment of sexual dysfunction.

Keywords: Coronary Artery Bypass, Sexual Dysfunction, Sex Hormones, Ejaculation, Impotence

P-168: Attitude toward Governmental Incentives on Childbearing and Its Relationship on Fertility Preferences in Couples Referred to Premarital Counseling Clinic of Health Centers in Mashhad

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Background: Considering a rapid reduction in the total fertility rate in Iran compared to past three decades and changing population policies in 2012 and suggestion of the governmental incentives on childbearing, this study was conducted to determine the attitude toward Governmental incentives on childbearing and its relationship to fertility preferences in couples referred to premarital counseling clinic of health centers in Mashhad in 2013.

Materials and Methods: In this descripto-analytical study, 450 couples referred to the health centers providing premarital counseling were sampled conveniently. Data was gathered using Miller Fertility preferences Questionnaire and Governmental incentives on childbearing in Iran and was analyzed using pearson correlation and independent t test.

Results: Average couple's desire for childbearing calculated based on a numerical rating scale (1 to 10) was 5.58 ± 2.88 . The average number of desired children was 2.37 ± 1.11 , desired daughters 1.14 ± 0.64 and desired sons was 1.22 ± 0.68 . The mean desired interval between marriage and the birth of the first child was 31.01 ± 20.64 months. Attitude toward Governmental incentives on childbearing was in significant reverse relationship with childbearing desires and child-number desires ($p<0.001$) and in positive relationship with child-timing desires ($p<0.001$).

Conclusion: The results indicate the desire for having two children in most of couples and increasing the distance between marriage and the first childbirth. Results showed a positive attitude to the impact of governmental incentives for childbearing in couples who tend to have lower fertility and delayed onset of childbearing.

Keywords: Fertility Preferences, Attitude, Governmental Incentives on Childbearing, Couples

P-169: Relationship on Fertility Motivation and Fertility Preference in Couples Referred to Premarital Counseling Clinic

of Health Centers in Mashhad

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Background: Reproductive is voluntary and motivational work. Given the lack of information about the motivations and preferences of young couples in the country, this study was conducted to determine fertility motivation and relationship fertility preferences in couples referred to premarital counseling clinic of health centers in Mashhad in 2013

Materials and Methods: In this descripto-analytical study, 450 couples referred to the health centers providing premarital counseling were sampled conveniently. Data was gathered using Miller Fertility preferences and fertility motivation. Questionnaire and analyzed using pearson and independent t test.

Results: Positive childbearing motivation was in significant positive relationship with childbearing desires and child-number desires ($p<0.001$) and in reverse relationship with child-timing desires ($p<0.001$). Negative childbearing motivation is no statistical relationship with fertility preference.

Conclusion: The findings of this study indicate that positive childbearing motivation are stronger in couples, childbearing desired and childbearing number is higher and childbearing distance is lower.

Keywords: Fertility Preferences, Fertility Motivation, Couples, Premarital Counseling

P-170: The Influence of Education on Health Beliefs and Practice of Women Eligible for Breast Cancer Screening by Health Belief Model in Shahid Behtash Lavizan

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Background: The systematic application of screening helps early diagnosis and effective treatment of breast cancer. This study was conducted to assess the effects of education on health beliefs and practice of women eligible for breast cancer screening using Health Belief Model (HBM).

Materials and Methods: This quasi-experimental study was conducted in Tehran, the north of Iran, using before after design. In this study, 144 women aged 20 to 62 years participated voluntarily. The volunteers divided into 2 groups, case and control. 3 sessions were held for case group and every session contained 1 hour training. The data collection tool was a self-administered multi-choice questionnaire that was developed based on Health Belief Model. Health beliefs and practice of the target group was evaluated pre-intervention and four months later.

Results: Our findings indicated that education based on HBM was effective and could enhance the participants' knowledge significantly and improve the HBM constructs including perceived susceptibility, severity, benefits and barriers. The training program enhanced

the practice from 0.6 to 3.5. The result of the present study relieved that increase in knowledge had effect on the HBM constructs. Furthermore, there was a significant relationship between knowledge and educational level.

Conclusion: We concluded that health education based on HBM can enhance women's knowledge of breast cancer, change their health beliefs and improve their behavior regarding screening programs like self examination.

Keywords: Health Beliefs Model, Knowledge, Practice, Screen

P-171: A Review of The Patient Consent (Satisfaction) in Medical Procedures and The Legal Responsibility of Physicians Toward Patients

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Background: Consent (satisfaction) of patient is effective in criminal responsibility of physician and his/her compurgation. Consent in word means agreement or permission. Before discussion about the effect of patient consent or his/her parents in medical responsibility, there is a question that "if victim (patient) accepted crime against himself with free will all its consequences, is this a reason for the legitimacy of the crime and staying the proceeding in jurisdictions or not?". According to the views of legal experts, since the criminal law is one of the peremptory rules and related to the public order, the victim's will, cannot disrupt the law enforcement, so the consent of victim is ineffective in the crimes which are harmful for his/her life, health or physical integrity such as murder, assault and battery. Thus the default is "The consent of victim has no effect on the nature of crime". One of the main conditions for modifying or manipulating one's body is not only necessity of treatment but the permission of patient or his/her parents to the surgeon or physician for surgery operation. Now it is the question that "How the consent of patient can affect the responsibilities of physician?" Criminal legislator of Iran in the articles 495, 496 and 497 of Islamic Penal Code (adopted on 21.04.2013) stipulates that the physician responsibility in the event of abuse, wastage and lack of technical standards, despite the patient's consent or knowledge, is established. So if the doctor proceeds to treat the patient by permission of him or his legal guardian if has no aggression and no-fault, then he would have no liability, because he himself is a legislator. Another issue is the separation surgery into general surgery that is important or necessary and cosmetic surgery. Such question is discussed. What is known as Surgery and located against the general or necessary surgery, and only be legitimated with the permission of the patient, in this kind of surgery how patient satisfaction is effective in the doctor's responsibility? Current practices did not see such surgeries as illegal but the doctor's responsibility in such a case is heavier than normal operation. Because the necessity factor makes many risks lawful and such practices (which is necessary for the progress and well-being) may be traditionally given by common sense. But "fun and whimsy" has not the power of "necessity". Thus, some people who consider commitment of the physician in patient treatment as a care commitment, consider the commitment of physician in unnecessary plastic surgery as a obligation of result, and they consider the physician as responsible for injury or death of patient. The patient's consent is either implicitly or explicitly, and nowadays explicit consent is written. To announce the patient consent it is expected the following conditions, which consent is ineffective in the absence of them. The first condi-

tion is the existence of the intellect, maturity and capacity in consent provider. The second condition of the consent of the patient or his legal representative is that it should be provided before surgery or in contemporaneous with it, because consent of patient or his legal representatives after the offense cannot remove the criminal fault of the offender and it is only a forgiveness. The third requirement for consent is that it is free from defects and freely expressed. The main purpose of this subject is that many people believe that can not ask the doctor to explain his activates. So discussing about this theme in a professional and scientific way can help to fix this misconception. Because this vision not only cause to violate the patient's rights but make some doctors, who think only for their own profits rather than patients recovery, to be more insolent.

Materials and Methods: Regarding the library nature of topic, the research method in this article is to refer the sources in libraries and National Documents Center of Iran by taking note. Of course we should consider the role of internet and its sources in this research.

Results: Since the injured patient is incapable in proving fault or negligence of a doctor, legislator supports the weak party in this contract and for establishing responsibility of a doctor, suffered person is deferred from the proof of guilt, unless the patient or his parents give a written permission to doctor in the contract, which in this case should prove the violation suffered by the doctor. Meanwhile in cosmetic surgery discussion, the unnecessary surgery, the doctor is obliged to conclude. Therefore, if any injury or death is applied by his actions, he has responsibility. Consent, cannot negate the responsibility of the doctor because it is so original that no one has a lien on the property and lives of others, unless it is sentenced by the religious or law. Consent is only valid to permit the doctor for examination and treatment of his body. In other words, Patient or his parents are not satisfied for crimes against themselves. Innocence does not means that the doctor citing to the contract's condition, release him from his legal responsibility against his fault. But, given the doctor take acquittance from the patient or his legal parents, the physicians are obliged to observe caution and scientific aspects and legal regulations. If the violation of these things is led to patient death or injury, the doctor is responsible for it.

Conclusion: One of the important discussion in this context and focuses on the surgeries in hospitals, is the issue of consent(satisfaction)and acquittal, which due to unawareness of patient to the provisions and their rights, and above all, since patients and their companions are in the passivity and the need position and thus cannot explain their actual demands clearly, so it is necessary that a committee including representatives of judiciary, medical council and public associations be formed and undertake the obtain of these consents and acquittals and explain the perspective of the problem clearly to the patients and their family, in order to perform the consent and acquittal perfectly. On the other hand, in the private clinics, e.g. a dentist's clinic, there is no comment or word about consent and acquittal or the medical responsibility domain, and always physician has significantly more chance. First of all the physician gets his fee, and if the patient has any claim, he should be confused between the clinic, the medical council and the court. So it is better that the quality of the relation between patient and physician and the responsibilities of each be written on a board and install in a place in sight of patients, in private and public clinics, hospitals, and authorized medical centers to make contract consciously.

Keywords: Legal Responsibility, Victim, Physician, Patient, Consent (Satisfaction)

P-172: Viewpoint of Infertile Women about Embryo or Egg Donation

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Background: Applying modern method of infertility treatment must be necessarily based on the cultural and social norms of the community. The purpose of this study, is based on the deeper understanding of the perceptions and attitudes of infertile couples about the socio-cultural aspects of using modern technologies of embryo or egg donation.

Materials and Methods: The data were collected from 110 anonymous visitors of infertility IVF Isfahan and Dr Rasekh clinic in Jahrom, and in Isfahan IVF Center and by completing Questionnaires that were randomly distributed among them. questionnaire consists of demographic and 12 other questions survey. The data was collected during summer 2013. Data were reported as frequency and percentage.

Results: The mean age of the responders was 29.5. The level of education range is from illiterate to graduate. 81% of them were housewives. The results of this study indicate: 57% responders don't pay attention to their child gender, 15% prefer adoption to donation. 11% of people were fully aware of the laws of donations. 32% of the people insist that their children be not informed of his(her) birth with egg donation. 42% of people tend to correct genetic abnormalities in embryos or egg donation. 76% of people are requested to keep the treatment with eggs donation secret. 70% of people express that they can love the offspring of egg donation as much as their own genetic offspring.

Conclusion: The egg donation process, should provide accurate medical information, counseling sessions, the exact dimensions of legal, religious orders, medical complications prior to treatment. In addition, increasing public awareness about medical complications and laws related to new treatment methods affected on the general change of attitude of individuals about infertility and on the decision of infertile couples for the use of egg or embryo donation in infertility treatment.

Keywords: Egg, Embryo, Donation, Infertility, Viewpoint

P-173: Viewpoints of Women about Spiritual Resistance of Men against Performing Semen Analysis Test

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Background: Viewpoints of women about spiritual resistance of men against performing semen analysis test referred to infertility Jahrom and Isfahan clinics affiliated to University of Medical Sciences.

Materials and Methods: In this cross-sectional study, 430 women were selected randomly who referred to Dr. Rasekh's clinic in Jahrom city, medical Clinic in Isfahan city. Women responded to a questionnaire containing 7 questions and variables such as age, education, occupation, number of children, place of residence, with five options: strongly agree, agree, neutral, disagree, strongly disagree. The data were analyzed by SPSS 16.

Results: This study was performed on 430 women. Mean age is 29.7 % ± 6.7. Education Level; 12.4%(49) were under 12 years,

44.4% (176) 12 years and 43.2% (171) were More than 12 years of education. Job: 9.1%(36) non-government Occupation, 56.8%(225) employees, Housekeeper 34.1%(135) person. The results are as follows: Men's fear of disclosing secrets that are not normal conditions sexually 68.8%, Men shy 66.5%, men pride 60.6%, fear of humiliation 59.4%, Men believe that the main cause of infertility is women 58.8%, Influenced by public opinion (Interventions of people) 51.7%, fear of life disrupting 37%.

Conclusion: Most of their opposition to the sperm analysis test is men's fear of disclosing secrets that are not normal conditions sexually and the next men pride. Men should be recommended that if they perform this test doesn't reduce the character, status and manhood. comment: Interview by professional psychologists and relatives influencing the men to perform this test. Men should be informed that Without performing this test, there is no possibility to follow up for Infertility treatment, should not reveal their secrets. If the test of semen analysis is abnormal, it does not have any effect on the lives and marital relationships. If the man refuses semen analysis, use of PCT technique for the presence of sperm.

Keywords: Semen Analysis, Spiritual Resistance, Men, Infertility

P-174: Right to Treatment with Assisted Reproductive Technology

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Background: Infertility leads to profound human suffering, particularly on the women. Women's lack of pregnancy is both physically and socially visible, especially in high fertility societies where women are typically blamed for reproductive failures, even in cases of male infertility. Childless women are more likely to be the victims of domestic violence and may also endure various forms of verbal and emotional abuse perpetrated by their husband and husband's family members. Assisted reproductive technology (ART) is used for infertility treatments and is also known as fertility treatment.

Materials and Methods: For this study, original and review articles from data bases such as Pub Med and Science Direct were used.

Results: Infertility is a topical problem in gynecology, which requires delicate approach, analysis and treatment. Accessing to assisted reproductive technologies is right for any infertile couple. Reproductive rights must encompass the right to facilitate fertility when fertility is threatened. For millions of couples in low-resource countries, particularly those facing tubal or male infertility, facilitation of fertility may require resort to ARTs. The High costs of ART is prevents people with disabilities from using ART as a means to parenthood. Most assisted reproductive technologies (ART) are not covered by insurance and the patient has to pay "out-of-pocket," often leading to increased stress as well as long-term financial burdens.

Conclusion: Effective infertility treatments and ARTs must be accessible in the people especially resource-poor and mostly rural nations of the low income world.

Keywords: ART, Infertility, Treatment

P-175: Examine The Role of Social-Legal Trainings To Improve Reproductive Health of Women in Traditional Societies

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Reproductive health is only a small part of a series of interconnected it is considered necessary for the subject to come around or to take account of women arises. Reproductive health from the perspective of the International Conference on Population and Development in Cairo, was defined solely from the perspective of biology. Reproductive health was based on the view of "health for all activities related to the reduction of maternal and infant mortality, or all deaths due to pregnancy and childbearing seems to appeal. But the reality is that only relies on reproductive health, health education, access to trained professionals, the availability of resources and tools not. Although no substitute for such facilities are necessary, but sometimes so powerful that social _ cultural factors have high availability and make a meaningless and ineffective. In the absence of some ethnic groups in traditional societies or underdeveloped pregnant woman should be at least every two years and if she did not do, her husband the right to divorce her wife or remarriage, or does not pay alimony..... How can the health of women and reproductive health will was ? This paper aims to illustrate the fact that although health care and universal access to essential health services are no doubt, but if social factors, cultural and educational legal overlooked, consequence plan resulting what expected distance would be unlikely. In this paper an attempt has been introduced to the conceptual framework of reproductive health, and briefly, the women's liberation movement and the impact of feminist though on the adoption Holistic approach in all of components legal and social structure i.e the economy, culture and politics and and therefore their role in the development of reproductive health will be discussed.

Keywords: Reproductive Health, Women, Feminist, Social-legal Training

P-176: Characteristics of Premenstrual Symptoms in Medical Science Students in Tehran

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Background: The nature of the premenstrual condition is based upon premenstrual symptoms, which women experience them and only in those cases where symptoms have negative influence on the physical, psychological or social functioning; we speak of a premenstrual syndrome, which reduce quality of life. The objective of this study is to assess frequency and severity of PMS in Medical Science University Students In Tehran.

Materials and Methods: In this cross sectional study, 571 unmarried volunteer medical students, with age 17-34 years, who accommodated in dorms of one of Medical Sciences University in Tehran, were involved after filling in informed consent. Tools of study had three main parts of socio- demographic, 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), and

visual Analog Scale (VAS) for assessing severity of premenstrual symptoms. All ethical points were considered and approved by Research Ethics Committee Of Tehran University of Medical Sciences.

Results: Average of age was (21.63 ± 2.63) years. 89.2% had experience of PMS. Most prevalent symptoms were, decreased interest in usual activities (85.4%), affective liability (83.8%), irritability (81.4%), lack of energy (76.3%), depressed mood or dysphoria (71.7%), concentration difficulties (68.4%), physical symptoms i.e. breast tenderness, bloating (62.3%), anxiety or tension (60.7%), marked change in appetite, overeating, or food cravings (57.5%), feeling overwhelmed (53.1%), hypersomnia (58.6%) or insomnia (39.9%).

Conclusion: Due to high rate of PMS in Medical Science students, who lives in dorms, it is necessary to do a study for finding its associated factors, and also finding safe method for prevention and solving this problem as well.

Keywords: Premenstrual Symptoms, Medical Sciences Students

P-177: The Meaning of “Abawain” and “Waledain” and Their Implementation on Cultured Embryos

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Background: The increase in cases of assisted reproductive Technologies (ART) in the modern world rises concerns about the rights and responsibilities of the offspring born via these methods. In this research, we are trying to distinguish between the donors' rights and parents' rights with regard to the holy Quran verses about parents and children, and their rights and duties.

Materials and Methods: In order to achieve this goal, we looked into the roots and meanings of the words used in Quran versus to address parents such as “Omm”, “Abb”, “Waledah” and “Waled”. Then we categorized the versus that contained these words and tried to conceive new meanings according to the holy versus We used reliable interpretations to verify our understanding of the words in different verses. Afterwards, we developed a framework of rights and duties, by benefiting from the differences in the meanings of the various words Quran uses to address parents, in order to distinguish between the roles of donor and birth parents versus parents growing the child.

Results: Since Muslims believe that every single word of the holy Quran is a revelation from Allah, we can determine the rights and duties of the people involved in an assisted reproductive process according to the uses of these words in the Quran.

Conclusion: We conclude that the word “Waledain” refers to the biological parents and we can determine their duties based on how this word is used in the Quran. On the other hand, the concept of “Abawain” is more inclusive and can be used to refer to parents nurturing a child despite having no role in his or her birth. Accordingly, rights and responsibilities can be defined for parents who have not participated in the reproduction process. Moreover, the relationship between biological parents and the child is not terminated and has to be considered at all times.

Keywords: Parental Rights and Duties, Biological Parents, Donors Rights, Quran Framework

P-178: Female Genital Mutilation: The Jewish, Christian and Islamic Views

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Background: Female genital mutilation (FGM) is a practice involving the removal of all or parts of the female external genitalia. It has been documented in 28 African countries and in some countries in Asia and the Middle East, but due to increasing immigration from these countries to the western world, FGM has become a worldwide human rights and health issue.

Materials and Methods: This paper is written with an overview of related articles in Scienedirect, and WHO fact sheets about FGM.

Results: While, according to the Bible, circumcision is required for all male Jewish children in observance of God's commandment to Abraham, female circumcision was never allowed in Judaism. Literature dealing with the Christian view on FGM is very scarce, however, Christian authorities unanimously agree that FGM has no foundation in the religious texts of Christianity. One of the highest values of Islamic law is the Prophetic command to neither inflict nor accept harm. This imperative will be familiar to non-Muslims as the golden rule. Islam is a religion of knowledge, learning and research. While FGM was previously practised as a social custom, the state of today's knowledge makes clear the serious negative effects of such practices on women. As such, it becomes a religious obligation to say unequivocally that the practice of FGM is today forbidden in Islam. As described above, FGM cannot be justified by any of the three monotheistic religions. The reasons for FGM are various and are clearly a mixture of cultural, social and religious factors. In societies, where FGM is practiced, the social pressure on the families is very high and the necessity to conform to what is considered right may be reason enough to continue the practice.

Conclusion: Contrary to the belief that it is a practice carried out only by Muslims, it is also practiced by Christians and a minority group of Ethiopian Jews. However, FGM is neither mentioned in the Torah, nor in the Gospels, and - like in Islam - bodily mutilation is condemned by both religions.

Keywords: Female Genital Mutilation, Islam, Christian

P-179: Predisposing Factors of Postpartum Depression

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Background: In the postpartum period, the highest risks for mood disorders include distress, depression and psychosis. Postpartum depression (PPD) is a syndrome which is more severe than postpartum distress with great influence on family. The aim was to determine the predisposing risk factors of PPD among fertile women.

Materials and Methods: It was a cross-sectional study with multi stage samplings of 233 women referred to Larestan health centers in Iran during 2 months post delivery. The data collection

tools consisted of two questionnaires. The first questionnaire included demographic characteristics such as age, education, employment status, income, age at the time of marriage, age difference with spouse, periods of relationship before marriage, type of marriage, number of children, newborn's gender, accommodation status and residency. The second questionnaire was Beck depression inventory. Data was analyzed using one way ANOVA, independent T test, Spearman's correlation coefficient and linear regression.

Results: A significant relationship was noted between PPD and husband's working status ($p=0.04$). It means that women whose husbands worked in the Persian Gulf states showed the highest rate of depression. Whereas, there was no significant association between PPD and age ($p=0.49$), age by time of marriage ($p=0.74$), women education ($p=0.24$), women working ($p=0.78$), man education ($p=0.46$), age difference with spouse ($p=0.76$), period of dating before marriage ($p=0.72$), duration of marriage ($p=0.27$), contraception methods ($p=0.90$), husband's addiction ($p=0.16$), accommodation ($p=0.29$), residence ($p=0.50$), number of children ($p=0.861$), number of female children ($p=0.16$), and gender of newborns ($p=0.82$).

Conclusion: Poor social supports, especially lack of supportive spouse, is a major risk factor for PPD among fertile women in Iran.

Keywords: Postpartum Depression, Predisposing Factors, Supportive Spouse

P-180: Prevalence of Premenstrual Syndrome in Female Medical and Dental Students of Guilan University of Medical Sciences

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Background: Majority of women experience PMS syndrome, but they may not be aware of the condition. The number of studies on prevalence, origin and management of PMS has increased dramatically. In the last two decades these studies have indicated that PMS is a disabling disease in women. This study was performed to estimate prevalence of PMS in medical and dental students and their own awareness of the suffering.

Materials and Methods: In this cross-sectional study, 320 students were selected by convenient sampling. APA (American Psychological Academia) used for diagnosis of PMS. Questionnaires were given to the selected students to be completed for 3 consecutive months. Each questionnaire included academic year, age and awareness of their own suffering from PMS. The questionnaires were collected after 3 months and data were analyzed by using SPSS.14.

Results: From total number of students, 280 (87.5%) had some degree of PMS. From 252 medical students 222 (88.1%) and from 68 dental students, 58 (85.3%) were suffering from PMS. 39.3% of cases had minor, 53.6% moderate and 7.1% severe PMS. Most common symptoms included irritability, cramp and nervousness. Students who had PMS reported at least 3 symptoms in 3 cycles of menses. The mean time and standard deviation of the PMS course was 10 ± 2.72 which started for 2 weeks prior to menses period and finished in 3 days after menses. Only 29 out of 280 students who had PMS (10.35%) were aware of their own suffering.

Conclusion: In spite of high prevalence of PMS among medical and dental students of Guilan university of medical sciences, their awareness of the condition was very low, indicating special need for providing information to all women in general and emphasizing on PMS in medical education.

Keywords: Menstruation, Premenstrual Syndrome, Irritability, Gramp, Nervous Tension

P-181: Correlation between Maternal Complications with Age

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Background: It seems pregnant women who are young or those who are near menopause have risk for maternal complications of pregnancy. In this study, it was decided to investigate the correlation between the complications of pregnancy and age in Alzahra hospital in Rasht.

Materials and Methods: In this survey the maternal complications of pregnancy (cesarian section, pregnancy induced Hypertension, preterm labor, placenta, placenta previa, Gestational DDM, Abnormal presentation and PROM) were studied in patients who referred to Alzahra hospital in Rasht for labor. The patients were divided in 3 subgroups according to their age: A) Patients were between 16-36 years. In this survey only patients were studied whose gestational age was 20 weeks according to LMP.

Results: In this survey, 6045 studied files were referred to Alzahra hospital in 1378. Among them, 52 (0.8%) who were under 16, 5406 (89.4%) were between 16 to 35, and 587 (9.8%) were above 35. Distribution of various complications in this population, without considering the age, is as follows: 6.8% was Hypertension, 0.72% was Geatationaldibetes, 0.56% was placental abruption, 37.4% C/S, 0.46% was lacentaprevia, 3.9% was preterm labor, 3.7% was PROM and 3.6% was abnormal presentation we used X² test in order to indicate the relationship between age and above mentioned complication increases as follows. Hypertension (16.01%) gestational diabetes (2.04%), placental abruption (2.04%), C/S (43.4%), placenta previa (1.19%), preterm labor (7.6%) X² test indication a significant relationship between each of these complications and age of above 35 (p<0.01). Most of the cases of PROM (5.71%) were in age group of under 16.

Conclusion: This survey revealed that most of the complications occur in age group 35 and under 16 respectively. we can conclude that the majority of complication, occur in the upper and lower limit of fertility course.

Keywords: Advanced Maternal Age, Gestational Diabete Mellitus, Maternal Complication

Female Infertility

P-182: Evaluation Type of Complementary and Alternative Medicine and Its Impact in The Treatment of Infertile Couples

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Background: Infertility as a crisis in the life of infertile couples led to difficulty in accepting and using treatment. This review aims at studying the forms of complementary medicine and its impact on infertile.

Materials and Methods: Study overview, the present internet search at time scales from 1999 to 2013 databases iranian and international iranmedex, EMBASE, Uptodate, Scopus, Science-Direct, PubMed, Cochran Library, ISI, magiran, Springer, Elsevier, SID with a collection of 126 articles that at least 42 articles with keyword: infertility, assisted reproductive techniques, alternative medicine, effects, quality of life was evaluated.

Results: Given that drug use is associated with complications. use of Complementary and Alternative Medicine (CAM) including homeopathic, massage therapy, reflex therapy, aroma therapy, yoga, acupuncture, spiritual therapy, praying therapy, herbal medicine, traditional medicine, light therapy, meditation, psycho therapy, touch therapy, special diets, vitamin therapy, magnet therapy, music therapy, relaxation alongside conventional methods of infertility treatment, the use of CAM with respect to all issues and aspects different each application is helpful. Because the physiological and psychological homeostasis and potential direct effect on quality of life.

Conclusion: The main purpose of using alternative medicine techniques is to reduce stress and anxiety. It can affect the outcome of assisted reproductive techniques, sperm count, motility and sperm concentration, balance hormones, improvement relationships between married couples, relax the body, improvement menstrual cycle, the increase of ovulation, improvement quality of life.

Keywords: Infertility, Assisted Reproductive Techniques, Alternative Medicine, Effects, Quality of Life

P-183: Evaluation of Endometrial Thickness on The Day of HCG Administration on IVF Outcome

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Background: Despite recent technical improvement in assisted reproductive techniques (ART), the implantation rate per embryo still remains low (15%). The aim of the study is to determine whether the failure of IVF cycle is associated with endometrial thickness on the day of HCG (human chorionic gonadotropin) administration.

Materials and Methods: Endometrial thickness of two hundred and seven patients was evaluated in this prospective study. Transvaginal ultrasound assessment of endometrial thickness was performed on the day of HCG administration. The endometrial thickness was divided into 3 groups: ≤7 mm (group 1), 8-14 mm (group 2), >14 mm (group 3).

Results: Comparing 3 groups, patients with endometrial thickness of >14 mm (group 3) has shown the better pregnancy rate (46.2%), but here is no statistical significance in sagittal thickness of endometrium and pregnancy rate by using CHI square test (p=0.358)

Conclusion: Endometrial thickness in predicting pregnancy could not reach statistical significance.

Keywords: Real Time 3 DUS, IVF Outcome, Endometrial Thickness

P-184: Effects of Licorice Hydroalcoholic Root Extract on Letrozole-Induced Polycystic Ovary Syndrome in Mouse

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Background: Polycystic ovary syndrome (PCOS) is multifaceted and includes reproductive, metabolic, and general health disorders, so employing different animal models is needed to study the syndrome. Use of an aromatase inhibitor, letrozole, on adult female rats produces a phenotype remarkably similar to that of PCOS. Licorice has been used in treating women infertility for thousands of years. Licorice is able to affect androgen metabolism. Various studies have demonstrated that licorice blocks the activity of 3- β -hydroxysteroid dehydrogenase, 17-hydroxysteroid dehydrogenase and 17-20 lyase and stimulates the activity of aromatase. All these enzymes are involved in the synthesis and/or metabolism of androgens and estrogens. It also affects the *in vitro* conversion of androstenedione to testosterone and stimulates aromatase activity, promoting the production of estrone and estradiol.

Materials and Methods: Eighty four mice were divided into six groups to study different fertility parameters like ovary and uterus histological morphology, hormonal level, serum antioxidant capacity and *in vitro* fertilization. Letrozole dose was 2 mg/kg which sonicate and gavaged daily for twenty four consecutive days. Licorice hydroalcoholic root extract doses were 150, 300 and 450 mg/kg which were also administered orally two hours after the letrozole gavage. The latest dose was used alone as control-sham group.

Results: Putting together all the results, we obtained that 300mg/kg dose had better improvement in this PCOS model. Although administering hydroalcoholic root extract alone, it had no significant effect in fertility parameters.

Conclusion: Licorice and its derivatives, with suitable dose, can be beneficial for PCOS studies to make a herbal based drug.

Keywords: Licorice, Mouse, PCOS, Ovary, Fertilization

P-185: Hawthorn Berries Ethanolic Extract Lowered Polycystic Ovarian Syndrome-Elevated RNA Damage in Follicular Cells

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Background: Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder. Various constituents including flavonoids with biological activities including free radical scavenging, anti-lipoperoxidation and anti-inflammatory have been isolated from hawthorn. Due to adverse effect of PCOS on ovarian vascular remodeling as well as PCOS-induced oxidative stress, we aimed to study the hawthorn's berries hydroalcoholic extract potential effects on estradiol valerate-induced PCOS in rats.

Materials and Methods: PCOS was induced in 3 groups of rats (n = 6) by intramuscular injection of estradiol valerate (EV, 4

mg/ratin 0.2 ml sesame oil). The control group only received the vehicle (0.2 ml sesame oil). A 60-day interval was chosen to let the cysts become established. Following the interval time, effects of hawthorn berries extract (HBE, 100 mg/kg, orally) and oral contraceptive pills (OCP, containing 60 μ g/kg estradiol, orally) on PCO-induced RNA damage and Angiogenesis were evaluated. The RNA damage and angiogenesis were evaluated by using Bicki's method and CD31+ immuno-fluorescent analyses, respectively.

Results: Observations demonstrated that HBE significantly (p<0.05) reduced PCO-induced RNA damage and enhanced the micro and macro vessels distribution per one mm² of the ovarian tissue.

Conclusion: Our data suggest that HBE partly reduce the PCOS-induced damages on the ovary by up-regulating the angiogenesis process and down-regulating the RNA damage.

Keywords: PCOS, Hawthorn, RNA Damage, Angiogenesis

P-186: The Relationship between Spiritual Health and Quality of Life in Infertile Women

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Background: Spiritual is the most important dimension of existence in more than a third of the people and it has been at the center of their lives as strong force and so may have an influence on a person's quality of life. Infertile women are at risk of so much stress and anxiety. The purpose of this study was to investigate the relationship between spiritual health and quality of life in infertile women who referred to Isfahan infertility.

Materials and Methods: In this cross-sectional study, 210 infertile women 20 to 45 years who referred to Isfahan infertility centers were selected through convenience sampling. To assess the spiritual health and quality of life spiritual well- being scale (SWBS) and World Health Organization WHOQOL- BREF Quality of Life Questionnaire were used.

Results: The results of this study showed that the mean of total score of quality of life was (87.9), the psychological dimension of quality of infertile women (20.2), social dimension (11.1) and spiritual health (97.7). There is a direct relationship between Spiritual health and the total score of quality of life (r=0.519 p<0.001), psychological dimension (r=0.554, p<0.001) and social (r=0.419, p<0.001) quality of life.

Conclusion: Infertility is a multi-faceted problem and can cause multi damage to women and influence the quality of their lives which can improve quality of life in infertile women with promotion of the spiritual health.

Keywords: Quality of Life, Spiritual Health, Infertility

P-187: Poor Endometrium and Repeated IVF Failure: Is That Any Place for Modified Natural Cycle Frozen-Thawed Embryo Transfer?

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Background: The objective of this study was to compare the pregnancy rate of Frozen-thawed Embryo Transfer (FET) on natural versus hormone replacement therapy (HRT) cycle endometrium, in patients with repeated IVF failure (RIF).

Materials and Methods: In this observational study, the pregnancy rate after FET on natural endometrium in patients with RIF and previously poor endometrium (<7mm) on HRT (group 1, n=14) and patients with RIF with previously normal endometrium (group 2, n=42), comparing with patients with RIF and normal endometrium, in whom frozen-thawed embryos on HRT cycle were transferred (group 3, n=52), was analyzed.

Results: The pregnancy rate in group 1 and group 2 was 35.7% (5 out of 14) and 42.2% (18 out of 42) respectively, in comparison with the pregnancy rate of group 3; 36.5% (19 out of 52). The abortion rate was not significantly different between groups.

Conclusion: To sum-up, in this study it was concluded that modified natural cycle is comparable with HRT cycle under certain conditions.

Keywords: Frozen-thawed Embryo Transfer (FET), Modified Natural Cycle Endometrium, HRT, Poor Endometrium, Repeated IVF Failure

P-188: Vaginal Progesterone Effects for The Prevention of Preterm Birth and Neonatal Complications in Women at Increased Risk: A Randomized Placebo-Controlled Double-Blind Study

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Background: The purpose of this study was to evaluate the effect of prophylactic vaginal progesterone in decreasing preterm birth rate and neonatal complications in a high-risk population.

Materials and Methods: A randomized, double-blind, placebo-controlled study included 100 high-risk singleton pregnancies (prior preterm birth, short cervix, uterine malformations and large intramural uterine myomas). Vaginal suppository progesterone (400 mg) or placebo was administered daily between 16-22 weeks to 36 weeks of gestation. Progesterone (n=50) and placebo (n=50) groups were compared to incidence of preterm delivery and neonatal complications.

Results: The preterm birth rate was 52% (52/100). There was a statistically significant difference in the rate of preterm labor between progesterone and placebo groups (36 vs. 68%, respectively; p=0.001). Preterm birth before 34 and 32 weeks of gestation was significantly more in the placebo group (42 and 34%, respectively; p=0.009) than in the progesterone group (18 and 8% respectively; p=0.001). Our study also showed that administration of vaginal progesterone suppositories was associated with a significant decrease in low birth weight (<2500 g), duration of hospitalization of neonates in neonatal care unit, and respiratory distress syndrome. However, we did not observe significant

decrease in the other neonatal complications including neonatal death, intraventricular hemorrhage and necrotizing enterocolitis.

Conclusion: Prophylactic vaginal progesterone reduced the rate of preterm delivery in women who were subject to high risk for preterm birth. It was also associated with a significant decrease in low birth weight (<2500 g), duration of hospitalization of neonates in neonatal care unit, and respiratory distress syndrome.

Keywords: Preterm Delivery, Preterm Birth, Prevention, Progesterone

P-189: Gene Variations of Toll-Like Receptor 2 in Endometriosis

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Background: Endometriosis, a common, benign, estrogen-dependent and chronic inflammatory condition, is characterized by the ectopic growth of endometrial tissue that is found primarily in the peritoneum, ovaries and rectovaginal septum. In addition to its usual description as a hormonal disorder, some genetic, immunological and environmental factors can affect this disease. Toll-like receptors (TLRs) constitute a family of pattern recognition receptors, recognizing a variety of exogenous and endogenous ligands playing the key role in innate immune response. Also they would be involved in cell proliferation processes, apoptosis, angiogenesis, tissue remodeling and repair. Since TLR2 expression decline has been observed in endometriosis in our group, we decided to study the genetic variations of TLR2, as well.

Materials and Methods: In this case-control study, 70 DNA samples were recruited from endometriosis patients who had been confirmed by laparoscopic surgery and 55 fertile women as control group that no history of inflammatory disorders or using any related drugs. All DNA samples were obtained from Royan DNA bank that had been extracted from peripheral blood along 2012-14. The LRR regions of TLR2 in exon3 divided into 2 fragments were amplified by polymerase chain reaction (PCR) and products were analyzed by sequencing.

Results: In the first fragment, Synonymous Single Nucleotide Polymorphism (SNP) (rs3804099) C/T was observed in 48 out of 70 patients (68.5%), and 29 out of 55 controls (52.7%), while 12 patients (17.1%) and 9 controls (16.3%), had shown Synonymous SNP (rs3804100) C/T in the second fragment. Observed SNPs' locations are in LRR6 and LRR11, respectively. The present study showed that the SNP frequencies between patients and controls, were not significantly different (p=0.07).

Conclusion: Although both of these SNPs are very common in inflammatory disorders, only SNP (rs3804099) was observed more in both groups of our studied population.

Keywords: TLR2, Endometriosis, Polymorphism

P-190: Expression Changes of GDF- 9 and

BMP-15 in The Oocytes of PCOS Patients Undergoing Treatment with N-acetylcysteine

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Background: Polycystic ovary syndrome (PCOS) is a frustrating experience for women, considered as a complex challenge for researchers and clinicians. Despite the enormous effort to define the cause of PCOS, the etiology and pathogenesis still remain unclear. Oocyte quality is reflected in the oocyte's intrinsic developmental potential. Studies have shown that the follicular development is disrupted in the PCOS ovaries, manifesting a large number of growing follicles arrested at a very early stage. Furthermore, data has demonstrated lower fertilization rate in PCOS patients indicating impaired oocyte and embryo development. BMP-15 and GDF-9, major oocyte secreted factors (OSFs), play a crucial role in follicular development, ovulation, oocyte maturation, and embryo development. GDF-9 and BMP-15 are members of the transforming growth factor b super family which are secreted from oocytes during folliculogenesis. They are essential for folliculogenesis and female fertility. The aim of this study was to investigate the effects of N-acetylcysteine (NAC) on the expression of GDF-9 and BMP-15, markers of oocyte quality in human ICSI cycles.

Materials and Methods: A prospective randomized, placebo controlled experimental study was conducted in the IVF/ICSI Unit of infertility treatment center of the Qom. Total 40 cases between the age of 25 and 35 years with polycystic ovarian syndrome were selected and divided into two groups as follows (n=20): N-acetyl cysteine (treated with 600 mg NAC three times daily for six weeks) and placebo (group control). 40 oocytes (12 at the GV stage, 10 at the MI stage and 18 at the Unfertilized-MII stage) were obtained in control group and 30 oocytes (12 at the GV stage, 8 at the MI stage and 10 at the Unfertilized-MII stage) in NAC group. Oocytes were washed in PBS and transferred into RNase-free microcentrifuge tubes, followed by the addition of buffer RLT. Isolation of total RNA was performed by using the RNA mini-preps kit and the RNase-free DNase I set from the oocytes by using RT-PCR.

Results: The expression level of GDF9 mRNA at the immature (GV+MI) and unfertilized-MII oocytes significantly increased in the NAC group in comparison with the control group. However, the expression level of BMP15 mRNA at the immature (GV+MI) and unfertilized-MII oocytes in the NAC group showed no significant difference in comparison with the control group.

Conclusion: The low expression of oocyte secreted factors in the oocytes of PCOS patients might be associated with impaired oocyte quality; therefore NAC supplements can greatly improve the general quality of oocytes in PCOS patients.

Keywords: GDF-9, BMP-15, Polycystic Ovary Syndrome, Oocyte Quality, N-acetylcysteine

P-192: A Comparison of The Effects of

Transdermal Estradiol and Estradiol Valerate on Endometrial Receptivity in Frozen-Thawed Embryo Transfer Cycles: A Randomized Clinical Trial

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Background: The aim of this prospective randomized clinical trial was to determine the optimal endometrial preparation protocol by comparing the clinical outcome of two methods of endometrial preparation in FET cycles, that is, oral estradiol and 17 β -estradiol transdermal patch.

Materials and Methods: A total number of 90 patients were scheduled for FET. In the study group (n=45), 17-B estradiol transdermal patches 100 μ g were applied from the second day of the cycle and continued every other day. Then each patch was removed after four days. In the control group (n=45), oral estradiol valerate 6 mg started at the same time above and continued daily.

Results: There was a significant difference in estradiol level on the day of progesterone administration and the day of embryo transfer between the two groups (p=0.001 in both), but no significant difference was observed between them in biochemical and clinical pregnancy rates (32.6 vs. 33.3%, p=1, 30.2 vs. 33.3%, p=0.81 respectively). The implantation rate tended to be higher in the study group, but it did not achieve a statistical significance. (20.45 vs. 11.7% respectively, p>0.05).

Conclusion: In spite of no significant differences in implantation, biochemical and clinical pregnancy rates between the two groups; we can use estradiol transdermal patches instead of oral estradiol in FET cycles. This is due to the reduced costs, drug dose, and emotional stress as well as the simplicity of the protocol for patient.

Keywords: Frozen-Thawed Embryo Transfer, Transdermal Estradiol Patches, Endometrial Preparation, Pregnancy Rate

P-192: Evaluation The Effect of Intrauterine Human Chorionic Gonadotropin Injection before Embryo Transfer in Infertile Patients and Comparison with Conventional Embryo Transfer in IVF/ICSI/ Embryo Transfer Cycles: A Randomized Clinical Trial

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Background: Implantation is one of the key stages for the success of assisted reproductive techniques (ART). The complex process of Implantation is regulated by various factors which one

the most important of them is human chorionic gonadotropin (HCG). This prospective, randomized clinical study aimed to investigate the effect of intrauterine HCG injection before Embryo Transfer (ET) on pregnancy outcome in infertile couples.

Materials and Methods: We evaluated 159 patients undergoing In vitro Fertilization/Intracytoplasmic Sperm Injection (IVF/ICSI) with antagonist protocol from 317 patients candidates for IVF/ICSI. The patients divided into three groups by a computer-based program. Case group I (n=53) received 500 IU of HCG and case group II, (n=53) received 1000 IU of HCG intrauterine injection before ET, and control group undergoing ET without a preceding intrauterine injection of HCG.

Results: There was no significant difference between case group I, II and control group. The implantation rates were 18.86, 13.52, 14.37% respectively, chemical pregnancy rates were 34, 32.15, 35.3% respectively, and clinical pregnancy rates were 32.1, 32.1, 31.4% respectively.

Conclusion: Intrauterine injection of HCG before ET does not improved the pregnancy outcomes in IVF/ICSI /ET cycles.

Keywords: Intrauterine HCG Injection, IVF/ICSI, Embryo Transfer, Pregnancy Outcome

P-193: Effect of Calcium Ionophore on Fertilization and Pregnancy Rate in Infertile Patients with Teratospermia Candidate for Intracytoplasmic Sperm Injection

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Background: Chemical activation is the most frequently method for artificial oocyte activation, resultant in high fertilization rates. The aim of this prospective randomized controlled study is evaluating the effect of calcium ionophore on fertilization and pregnancy rate in infertile men suffering from teratoospermia.

Materials and Methods: 38 women with teratoospermic partner were divided into two groups and underwent Intracytoplasmic sperm injection (ICSI) with antagonist protocol. 313 metaphase II oocytes selected for ICSI. In control group (n=145) routine ICSI was performed. In study group (n=168) immediately after ICSI, oocytes were entered to culture media plus 5 μ M calcium ionophore (A23187) for 5 minutes, then washed at least 5 time by MOPS solution. In both groups, the oocytes were evaluated about 16-18 hours later for fertilization.

Results: Thirty-eight ICSI cycles were included in this study. The number of embryos obtained and fertilized oocytes were significantly different in two groups (p value=0.04 in both). The fertilization and cleavage rate was not significantly different between study and control groups respectively (84.4, 87.74 vs. 95.33, 89.56%). Implantation rate was higher in study group in comparison with that of control group but was not significant (7.4 vs. 17.64%, p=0.14). No significant differences were observed between them in chemical and clinical pregnancy rate respectively (16.7, 16.7 vs. 47.1, 41.2%, p=0.07, p=0.14).

Conclusion: Although in this study there were no significant difference in implantation and pregnancy rate, oocyte activation with calcium ionophore could increase fertilization rate in patients with teratoospermia. If the sample size was larger, statistical results may be significant.

Keywords: ICSI, Calcium Ionophore, Oocyte Activation, Fertilization Rate

P-194: Follicular Fluid Adiponectin Concentrations in Follicles in The Luteal Phase of The Estrous Cycle in Dairy Cows

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Background: Adiponectin is an adipocytokines which is secreted from the adipose tissue of human and domestic animals. No information is available regarding the follicular fluid adiponectin concentrations in dairy cows. Therefore, the aim of the present study was to determine the follicular fluid adiponectin concentrations in different-sized follicles in the luteal phase of the estrous cycle in dairy cows.

Materials and Methods: Ovaries and blood of 18 Holstein dairy cows in the luteal phase were collected from the Shiraz slaughterhouse. The clear follicles divided into three diameter groups (small, 3-5 mm; medium, 6-9 mm and large, \geq 10 mm) and their fluid was aspirated using a needle. Concentrations of follicular fluid adiponectin (using ELISA Cusabio Biotech kit, China) and serum progesterone (using radioimmunoassay Immunotech kit, France) were determined. Differences of means and standard deviations of follicular fluid adiponectin concentrations in three groups were analyzed using one way ANOVA. Correlation coefficient of adiponectin concentration between the follicular fluid of different sizes of follicles and concentrations of serum progesterone was also analyzed using the Spearman's test (SPSS 11.5, p<0.05).

Results: Progesterone assay showed that all cows were in the luteal phase of their estrous cycle. Follicular fluid adiponectin concentrations of small-sized (5.05 ± 0.78 μ g/mL), medium-sized (4.89 ± 0.79 μ g/mL) and large-sized (5.04 ± 1.27 μ g/mL) follicles in luteal phase were not different (p>0.05). In the luteal phase of the estrous cycle, a positive correlation was observed between the adiponectin concentrations in large-sized and medium-sized (r=0.72, p=0.01), large-sized and small-sized (r=0.53, p=0.01), medium-sized and small-sized (r=0.86, p=0.01) follicles. Further, There was a positive correlation between the serum progesterone and follicular fluid adiponectin in the medium-sized follicles (r=0.50, p=0.03).

Conclusion: Results of the present study showed that follicular fluid adiponectin concentration does not alter as the follicle development occurs during the luteal phase of the estrous cycle in dairy cows.

Keywords: Adiponectin, Follicle, Follicular Fluid, Luteal Phase, Cow

P-195: Thymoquinone Increases Efficacy of Tamoxifen Induced Apoptosis in Human Breast Cancer MCF-7 Cells: In Vitro

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Background: The objective of this study is to evaluate combined effect of Thymoquinone (The main active component of black seeds) with Tamoxifen drug on apoptosis of human breast cancer

MCF-7 cells (Noninvasive human breast cancer cell line, estrogen receptor positive).

Materials and Methods: The human breast cancer MCF-7 cells were treated with Tamoxifen (2 µM) alone or in combination with Thymoquinone (150 µM). Morphological conformation of cell death in MCF-7 cells treated by Tamoxifen, Thymoquinone and combination after 48h were studied by Acridine orange/ethidium bromide staining and TUNEL assay. Statistical analysis was conducted using One-Way ANOVA and Tukey's test. All statistical analysis was done by using SPSS software (version 19.0). In all cases, p values < 0.05 was significantly considered.

Results: The data of TUNEL assay and Acridine orange/ethidium bromide staining in MCF-7 cells after 48h treatment were indicated that Tamoxifen and the Thymoquinone alone or in combination significantly increased apoptotic index (p < 0.001). Also, TUNEL staining showed an increased number of apoptotic cells in synergic group. It is indicated that higher doses of Thymoquinone in combination with lower dose of Tamoxifen (2 µM) induces apoptosis in MCF-7 cells. Herein, we showed that high doses of Thymoquinone in normal cells could be free of side effects of this natural component.

Conclusion: This study indicates that high doses of Thymoquinone acts for lowering the dose of Tamoxifen and shortening the time course of Tamoxifen exposure in estrogen receptor positive breast cancer cells in a safe and non-hazardous manner. These data could bring a new light for the treatment of breast cancer and also for other types of cancer patients.

Keywords: Apoptosis, Breast Cancer, Tamoxifen, Thymoquinone

P-196: Atypical Presentations of Primary Amenorrhea with Multifactorial Etiologies, Hypothalamic Amenorrhea with Transverse Vaginal Septum No Hematocolpos: A Case Report

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Background: Patients with a transverse vaginal septum generally present at the age of menarche with complain of cyclic pelvic or abdominal pain due to retention of blood above the obstruction. In the absence of menstruation this women did not have typical hematocolpos.

Materials and Methods: A rare case of hypothalamic amenorrhea and transverse vaginal septum were reported. A 28-year-old woman presents with primary amenorrhea and no complaint of abdominal pain. Her laboratory testing showed FSH 0.2 mIU/ml and LH 0.3 mIU/ml. Laparoscopy revealing a small rudimentary uterus with streak ovaries and a vaginal pouch. The patient with diagnosis of Mayer-Rokitansky-Kuster-Hauser syndrome was subject to a vaginoplasty in another infertility center. When the patient referred to our center, she was suffering amenorrhea, osteopenia and infertility because of misdiagnosis.

Results: After two courses of estrogen and progesterone, sonography revealed that hematocolpos under anesthesia transverse vaginal septum was resected. Hysteroscopy revealed normal uterine cavity. She became pregnant five months postoperatively with controlled ovarian hyper stimulation (COH) in conjunction with intrauterine insemination.

Conclusion: This case highlights the importance of careful evaluation of all primary amenorrhea. The clinicians should be aware of presence of more than one etiology which cause atypical presentations and accomplish a systematic strategy for the evaluation of amenorrhea potential to avoid long-term effects of a misdiagnosis.

Keywords: Primary Amenorrhea, Transverse Vaginal Septum, Hypothalamic Amenorrhea

P-197: Study about Relation between Cancer, Chemotherapy and Chemical Drugs in Woman Infertility

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Background: Female infertility varies widely by geographic location around the world. In 2010, there was an estimated 48.5 million infertile couples worldwide, and from 1990 to 2010 there was slight change in levels of infertility in most of the world. In 2010, the countries with the lowest rates of female infertility included the South American countries of Peru, Bolivia, Ecuador and Bolivia, as well as in Poland, Kenya, and Republic of Korea. The highest rate regions included Eastern Europe, North Africa, the Middle East, Oceania, and Sub-Saharan Africa. The prevalence of primary infertility has increased since 1990, but secondary infertility has decreased generally. Rates decreased (although not prevalent) of female infertility in high-income, Central/Eastern Europe, and Central Asia regions.

Materials and Methods: This case-control study investigated the relationship of infertility with occupational handling of chemotherapy drugs by nurses and pharmacy personnel. Data were collected by questionnaire from 4659 staff at facilities participating in the National Surgical Adjuvant Breast and Bowel Project collaborative clinical trials network of the National Cancer Institute.

Results: Result for the total sample and for women showed a significantly elevated odds ratio (OR = 1.5; CI = 1.1 to 2.0) for self-reported infertility associated with occupational handling of chemotherapeutic drugs prior to onset of infertility.

Conclusion: Recently, cryopreservation procedures such as *in vitro* fertilization and embryo storage, or ovarian tissue cryopreservation have been used to preserve fertility in patients who are subjected to cancer treatments. Knowledge of the risks and probabilities of ovarian failure as well as the risks of the cryopreservation procedures is crucial for patients and physicians in order to make informed choices that will best serve to the patients interests.

Keywords: Cancer, Chemotherapy, Infertility, Psychology

P-198: Study Effects of EMF on Pathology of Ovary in Newborn Rats during Developmental Period

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Background: The effect of electromagnetic field (EMF) as an environmental factor on different organs including female reproductive system is of critical concern. In view of the fact that embryo fetuses and young, growing animals are more susceptible to any xenobiotic during gestational and lactation stages of gonadal development may it lead to permanent damage to the gonads. The early phases of human development have scarcely been studied with regard to their correlation with EMF; therefore, the aim of the present study is to evaluate the effect of EMF 50Hz on follicular development on neonatal rats.

Materials and Methods: Pregnant wistar rats in treatment group were exposed to 3mT, EMF for 21 days, 4 hours/day. The EMF was produced by a system using 50 Hz alternative current, pregnant rats under the same condition of treatment group, but off the field as a sham group intended and pregnant rats were used as control in the room. After delivery, ovaries were removed from female pups, fixed and prepared for light microscopic studies.

Results: Microscopy results revealed that in the treatment group in comparison to control group, some oocyte is partially surrounded by pre-granulosa cells, the oocyte nest appeared to be fewer in comparison to that of the control group. Few oocyte nests were binucleated.

Conclusion: The current study shows that EMF exposure increases degenerative changes and oocyte nest breakdown and follicular formation, undergo a series of incomplete cell division, resulting in clusters called cysts or nest. The germ cell nest is an important developmental stage in the formation of the germ line. It is concluded that exposure to EMF during the developmental period could affect on oocyte differentiation and may result in reduced fertility by decreasing ovarian reservoir.

Keywords: EMF, Newborn, Ovary, Prenatal

P-199: Comparison of Clomiphene Citrate and Letrozole Effect on Folliculogenesis in Polycystic Ovarian Syndrome Patients

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Background: Polycystic ovarian syndrome (PCOS) which is the most common form of anovulatory infertility affects 5-10% women of reproductive age. Clomiphene Citrate (C.C) is the first line

treatment in infertile women entering intrauterine insemination (IUI) cycle. For C.C resistant patients the second approach is aromatase inhibitors such as Letrozole. The aim of this study is to compare the effectiveness of C.C with Letrozole on folliculogenesis and pregnancy rate in PCOS women undergoing IUI cycles.

Materials and Methods: Total of 207 IUI candidate women including 101 PCOS patients and 106 women with male factor infertility (controls) were selected. All women were matched by Age (20-35 years) and they were divided into normal- and over-weight (BMI=18.5-25 and 25-30 kg/m², respectively) groups. Patients were given either C.C or Letrozole daily for 5 days followed by Gonadotropins. The number of follicles ≥ 15 mm, as a character for drug response, was monitored on day 10 of stimulation by trans-vaginal scans. Multiple linear regression analysis was performed.

Results: The mean of mature follicle numbers in controls was significantly higher in comparison with PCOS patients ($p=0.00$), however in both groups, Letrozole showed more effect on achievement of follicles ≥ 15 . Patients with normal weight showed better response to ovulation induction cycles compare to women with BMI=25-30 ($p=0.001$). Pregnancy rate was 31 and 20% in PCOS and control groups, respectively.

Conclusion: In PCOS patients the follicular response was significantly lower than that of control group. Aromatase inhibitors (Letrozole) can be more effective than C.C in PCOS patients since hyperandrogenism is one of the main features of disease and it affects on folliculogenesis. More study on molecular activity and pharmacogenetics of Letrozole is recommended.

Keywords: Clomiphene Citrate, Folliculogenesis, Letrozole, PCOS

P-200: Relation between BMI and Age on Laboratory Parameters in Infertile Women

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Background: Age is an important factor in the success of infertility treatment. Upper limit of the age that people can get into infertility treatments vary in different countries. This study investigated the relationship between age and BMI with laboratory parameters in women undergoing ART and oocytes extracted from the ICSI procedure in Omid Infertility Center in Babol.

Materials and Methods: In this cross-sectional study 367 cases from April 2009 until November 2013 infertile couples under treatment in Omid Infertility were selected using convenience sampling. A questionnaire including demographic, clinical Couple hormonal tests, number and type of eggs and embryos, and results of treatment for each patient was completed

Results: There is a relation between age, number of oocytes retrieved, MII oocyte, fertility success and FSH, $p<0/05$. 66/5% of embryo formation was related to MII oocyte. $R^2=0.6$. There is not a relation between BMI and the number of MI and MII oocytes, FSH, TSH, Estradiol, length of infertility and type of infertility $p>0/05$. The mean BMI level in people who had hirsutism and galaktore was more than others.

Conclusion: Age is an important factor in reproductive success. Age increasing cause decline the number of oocytes extracted, MII oocytes and pregnancy success.

Keywords: Infertile Women, BMI, Laboratory Parameters

P-201: Relation between AMH Levels and Ovarian Response in ART Cycles

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Background: Ovarian reserve and ovarian response both are of the most important key factors in infertility treatment. Anti mullerian hormone (AMH) is one of lab tests that can predict ovarian reserve. relation between this assay and ovarian response can guide physician to choose the best protocol for the worst reserve.

Materials and Methods: In a prospective cohort study, 110 infertile women (21-40 years old) who were candidate for ICSI attending assisted fertility unit, MAHDIEH hospital, TEHRAN, IRAN, from 2011-2012 were enrolled in this study. At first (day 2-4 of menses) serum level of AMH, FSH, ESTRADIOL were checked, then standard long protocol with GnRh agonist and then stimulation with HMG (merional 75 IU, IBSA) started. dose of HMG was based on patient age (standard protocol of our infertility ward). After ovum pickup, number of oocyte and embryo were recorded and 14 day after embryo transfer BHCG was cotroled. Analysis of data was done by using spss version 18 software.

Results: The mean of AMH level was 3.9 nmol/ml (0.1-22.1) and mean of oocyte and embryo was 11.29 (1-42) and 6.1(0-19) respectively. There was positive correlation between AMH levels and number of ovums and embryos. 9% of all cycles were cancelled because of ovarian unresponsiveness and in this group AMH was <0.67 nmol/ml. regression analysis of data shows that AMH <0.75 nmol/ml was compatible with lower ovarian response (<4 oocyte)

Conclusion: AMH level in day 2-4 of non stimulated cycle has positive correlation with ovarian response and AMH<0.75 nmol/ml was compatible with poor ovarian reserve.

Keywords: AMH, Ovarian Reserve, Ovarian Response

P-202: An Overview of The Influence of Trans Fats Compounds on Female Infertility, Pregnancy and Abortion

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Background: Lifestyle changes over the years and expanded fast foods and a generally unhealthy diet causes damage to the reproductive potential of women, and the rise of infertility among them as well. Trans fat is one of the unhealthy diet. A small quantity of Trans fat is found naturally in foods usually in animal products but the vast majority of trans fats are artificial and come from the partially

hydrogenated oil found in packaged foods.

Materials and Methods: We followed a cohort study of 544 women a history of infertility 2 years as they tried to become pregnant or became pregnant. Retrospective study comparing dietary data on TFAs and total calories from Block 98 quantitative food frequency questionnaire on these women. We evaluated evidence from TFA and CHD risk controlled feeding trials evaluating risk factors and long-term observational studies evaluating clinical outcomes. A dietary score based on these factors previously related to lower ovulatory disorder infertility and other lifestyle information was prospectively related to the incidence of infertility.

Results: Studies released show that foods with Trans fats increase 67 percent the risk of ovulatory infertility. Each 2 percent increase in calories from trans fat was correlated with a 73 percent increased risk of ovulatory infertility. 58 percent of babies whose mothers, dietary intake of Trans fatty acids 35/2 grams per day, especially in the second and third trimester of pregnancy were born with high birth weight and approximately 26 percent of these mothers were diagnosed with gestational diabetes.

Conclusion: Trans fat consumption, may lead to birth weight. In addition Trans fats cause the negative impact on mother and fetus health. Reports on the harmful action of Trans fats on humans persuasively reveal the need to limit their intake.

Keywords: Trans-fatty Acids, Epidemiology, Infertility, Pregnancy, Abortion

P-203: The Impact of Low-Dose Aspirin on Clinical Reproductive Outcomes in Frozen-Thawed Embryo Transfer Cycles; A Randomized, Placebo-Controlled Double-Blind Study

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Background: The objective of this study is to evaluate whether low-dose aspirin affects doppler sonographic parameters of the uterine arteries, endometrial thickness and pregnancy rate of women undergoing frozen-thawed embryo transfer cycles.

Materials and Methods: This randomized, double-blind, placebo-controlled trial study was conducted between April 2012, and March 2013. A total of 60 eligible patients (aged 18-40 years) were randomly assigned to the study and control groups by using computer-generated random numbers. Allocation concealment was performed by using sealed envelopes. With the onset of endometrial preparation and estrogen treatment, the study and control groups received 100 mg of oral aspirin or placebo respectively. Doppler ultrasonography also was performed to calculate resistive index (RI) and pulsatility index (PI). The results of treatment cycle were compared in both groups.

Results: There were no statistically significant differences between groups with regard to age and basal hormone levels. Both groups were comparable with respect to endometrial thickness on

ET day and impedance indices (PI, RI) for both uterine arteries. Compared with placebo controls, aspirin group had a significantly higher pregnancy ($p=0.045$) and implantation rates ($p=0.047$). No statistically significant difference was detected in miscarriage rate between two groups.

Conclusion: It seems that low-dose aspirin therapy positively affects pregnancy and implantation rates of women undergoing frozen-thawed embryo transfer cycles.

Keywords: Aspirin, Frozen-Thawed Embryo Transfer Cycle, Endometrial Thickness, Reproductive Outcome

P-204: Relative Frequency of Chlamydia Trachomatis Infection in Infertile Women

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Background: The majority of Chlamydia trachomatis infections in women are asymptomatic, but they may give rise to tubal infertility. Screening programmes aim at reducing morbidity in individual by early detect and treatment, and at decreasing the overall prevalence of infection in the population.

Materials and Methods: A total of 135 women presenting with a history of infertility ($n=113(83.7\%)$ primary infertility, $n=22(16.3\%)$ secondary infertility) between 20-40 years of age were consecutively induced into this descriptive-analytic study. Specimens were taken from endocervical canal. C.Trachomatis were detected with polymerase chain reaction (PCR). The data collected in check list and analyzed statically using spss (ver.16).

Results: C.Trachomatis was detected in 16(19.3%) of 135 asymptomatic infertile women. The over age of patients was 31.50 ± 5.62 near and the most common age range was 35-40 years. 91.9% of patients were housewives and average duration of marriage was 6.33 ± 4.55 near. 35 patients expressed a positive family history of infertility. 9women(18%) had adhesion in hysterosalpingography. There was no statistically difference between PCR positive results to age of patients, type of infertility, obstructed salpingography, family history and duration of infertility.

Conclusion: Genital C.trachomatis is the leading cause of tubal factor infertility. The present study shows that C.trachomatis infection could be present in 19.3% of asymptomatic infertile women. Therefore a screening test for C.trachomatis infection is recommended for all women who refer to infertility outpatient departments in Rasht and perhaps other parts of Iran.

Keywords: Chlamydia Trachomatis, Female, Infertility, PCR

P-205: Effects of Aerobic Exercise on Plasma Lipoproteins in Overweight and Obese Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine disorders. Obesity and anovulation in

the women affected by this syndrome leads to a type of obesity that is followed by cardiovascular disease, hypertension and cholesterol and lipoprotein improper profiles. Changing the life style, e.g. increasing physical activities is the first approach in controlling PCOS

Materials and Methods: 24 women affected by PCOS after medical screening were divided into two groups: Experimental group ($n=12$) and control group ($n=12$), with the average age, weight, height, BMI and WHR of 26.87 ± 4.43 years, 75.71 ± 10.65 kg, 159.29 ± 6.44 cm, 29.86 ± 3.22 kg/m² and 91.75 ± 5.86 , respectively, First, the body composition such as BMI, WHR, Percent of body fat, weight and body fat mass were measured. Then blood samples from the people under test and HDL, LDL, VLDL, triglyceride and cholesterol were measured. Then the experiment group underwent the effect of an aerobic exercise (a type of physical activity that increases the heart rate and promotes increased use of oxygen in order to improve the overall body condition) program. After 12 weeks all the measured variables before intervention the test were re-measured. Correlated t test was used for comparing the two groups before and after intervention the test and independent t test was used for comparing the two groups ($p<0.05$).

Results: The results showed that after 12 weeks of exercise, BMI, WHR, fat rate, weight and fat mass and triglyceride had significant reduction and HDL had significant increase. But no significant changes happened in LDL, VLDL, and cholesterol levels.

Conclusion: In addition to confirming the positive effect of aerobics on body compositions and lipoproteins of plasma, the findings showed that the risk reduction of cardiovascular diseases in obese and overweight people affected by polycystic ovary syndrome is possible by reducing the weight doing aerobic exercises.

Keywords: Polycystic Ovary Syndrome, Aerobic Sport, Plasma Lipoproteins, Body Compositions

P-206: Gestational Diabetes Increased the Astrocytes Density in Cerebellar Cortex of Rat Offspring

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Background: Gestational diabetes mellitus affects 3-5% of all human pregnancy. Studies reported the neurotoxic effect of gestational diabetes on cerebellar and spinal cord in rat offspring. This study was conducted to determine the effect of gestational diabetes on astrocyte density in the cerebellum of rat offspring.

Materials and Methods: In this experimental study, 30 Wistar rats dams were randomly allocated in control and diabetic groups. Dams in diabetic group received 40 mg/kg/BW of streptozotocin, intraperitoneally at the first day of gestation (GD) and control group received an equivalent volume normal saline. Six offspring of gestational diabetic (GDM) and control dams, at the 21, 28 postnatal days were randomly selected and were sacrificed quickly with anesthesia. The coronal sections of cerebellum (6 micrometer) serially collected. The astrocytes were stained with PTAH. The density of astrocytes was evaluated in 20000 μm^2 by OLYSIA Autobioreport software.

Results: The astrocyte density in apex and depth of cerebellum in 21 postnatal days, in the experimental group significantly increased (38.15 and 25%, respectively) in comparison with controls group (apex:

10.5 ± 1.89 vs. 7.6 ± 1.28, depth: 12.25 ± 1.54 vs. 9.8 ± 1.2, p<0.05). The astrocyte density in apex and depth of cerebellum in 28 postnatal days, in the experimental group significantly increased (25% and 13.5%, respectively) in comparison with controls group (apex: 11.25 ± 1.37 vs. 9.0 ± 1.95, depth: 12.25 ± 0.62 vs. 9.75 ± 1.0, p<0.05).

Conclusion: This study revealed that gestational diabetes increased the astrocytes density in cerebellar cortex of rat offspring.

Keywords: Gestational Diabetic, Cerebellum, Astrocyte, Rat Offspring

P-207: Deltamethrin-Induced Derangements Correlates with Estrogen Synthesis, Angiogenesis and Oxidative Stress

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Background: Deltamethrin (DTM) is a pyrethroid derivative, which is the most potent products of Type 2 insecticides and acts by delaying the closure of sodium channels. Estrogen as a vascular growth factor on ovarian tissue plays an essential impact further to its involvement in follicular growth. On the other hand, the role of angiogenesis on ovarian tissue in inhibiting the apoptotic provokers has been reported previously. Impaired angiogenesis results in severe oxidative stress, which in turn enhances the cellular DNA and RNA damages. Therefore, current study was performed in order to evaluate the effect of DTM on estrogen synthesis and uncover the correlation between estrogen-dependent impaired angiogenesis and follicular atresia.

Materials and Methods: Twenty four mature virgin female Wistar rats were assigned into four groups as; control-sham group (received 0.5mL corn oil), 1mg/kg-1 DTM-administrated, 3mg/kg-1 DTM-treated and 7mg/kg-1 DTM-received groups. All animals received the chemicals orally by gavages for 14 days. The angiogenesis and RNA damage were evaluated by using CD31+ immunohistochemical and Bickis epi-fluorescent techniques, respectively. The tissue levels of GSH-px, SOD, TTM and TAC, and serum level of estrogen were assessed. Moreover, the total and atretic follicles were counted per ovary.

Results: DTM significantly (p<0.05) reduced angiogenesis and declined tissue GSH-px, SOD, TTM and TAC levels. The DTM-treated animals exhibited severe RNA damage and considerably enhanced follicular atresia. Serum level of estrogen decreased dose dependently.

Conclusion: DTM by reducing estrogen synthesis resulted in impaired angiogenesis that partly enhanced oxidative stress via down-regulating antioxidant capacity. Produced oxidative stress promoted the RNA damage and ultimately resulted in severe follicular atresia.

Keywords: Deltamethrin, Estrogen, Angiogenesis, RNA Damage, Oxidative Stress

P-208: Report of A Rare Case of Endometrial Stroma Sarcoma with Sex Cord Element

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Background: Endometrial stroma sarcoma (ESS) is usually formed of neoplastic cells similar to proliferative endometrial cells and comprises less than 10% of all uterine sarcomas. Presence of concurrent sex cord element with these tumors is rare.

Materials and Methods: A 36 year-old woman, from Yazd complaining of abnormal vaginal bleeding and history of G4P3A1Ch3 referred. On both occasions after clinical diagnosis of cervical polyp, biopsy specimen was taken in the out patient department and diagnosed as adenofibroma on pathological examination. The patient referred 15 months later with irregular short menstrual cycles and more severe bleeding. After hospitalization and diagnosis of a huge myomatous uterine similar to the seventh week of pregnancy after clinical examination and sonography observed, after that hysterectomy was performed. On pathological examination, low grade ESS with sex cord elements was reported.

Results: ESS with sex cord elements is considered as one of the rare tumors that is from the mixed Mullerian group and clinical and pathological differential diagnosis includes polyp.

Conclusion: This important point should be taken into consideration by gynecologists and pathologists during exacerbation of polypoid lesions or cervical and endometrial polyps.

Keywords: Adenofibroma, Endometrial Stroma Sarcoma, Sex Cord Elements

P-209: Evaluation of Common Risk Factors in Patients with Endometrial Hyperplasia

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Background: One of the most common abnormality in female genital system, especially in premenopausal period, is abnormal uterine bleeding caused by endometrial hyperplasia. Prevalence of endometrial hyperplasia is 5-20% with different risk factors such as hypertension, diabetes mellitus (DM), multiparity, nulliparity, advanced age, and using estrogen consumption. This study try to determine risk factors in endometrial hyperplasia.

Materials and Methods: This case-control study has been done on 200 patients with endometrial hyperplasia who refer to Shahid Sadoughi and Mother Hospital with simple sampling and were compared to 200 control with similar conditions. Questionnaires were filled according to pathological records of 200 patients, which included age, parity, history of hypertension and DM. Finally data were analyzed by SPSS version 14 and ANOVA and Chi-square statistical test.

Results: The average age of cases was 47.88. Frequency distribution of hypertension, DM, nulliparity was significantly higher in cases more than controls. Cases had more pregnancy than control.

Conclusion: Determining the endometrial hyperplasia risk factors that can be the major etiological factor of endometrial carcinoma has especial importance and in other hand help the medical groups

for having superb diagnosing and presenting better medical services to high risk groups and significantly reduce side effects and unnecessary medical costs.

Keywords: Hyperplasia, Endometrial, Nulliparity

P-210: Evaluation of Anti-FSH Antibodies Levels as A Prognostic Marker in IVF Outcome

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Background: The objective of this study is to establish importance of anti-FSH antibodies in IVF outcome.

Materials and Methods: This research was designed based on evaluation of several articles from 2000 till 2012 about anti-FSH antibodies in IVF outcome.

Results: Serum concentrations of anti-FSH IgG and IgA were linearly associated with poorer ovarian stimulation outcome. For example 1.0 AU difference in anti-FSH IgG was associated with a 220.6 IU increase in FSH needed for one embryo while the mean amount of FSH used per embryo was only 443.8 ± 401.2 IU. In fact, serum anti-FSH antibodies are associated with poor ovarian response to FSH stimulation in IVF. Based on Luborsky J et al. researches women who became pregnant had a lower frequency of anti-ovarian antibodies (AOA) than women who did not become pregnant. AOA positive patients often have antibodies against FSH. The follicular puncture performed in IVF procedure can induce the production of AOA.

Conclusion: The success of attaining pregnancy following IVF depends on the effectiveness of controlled ovarian hyperstimulation (COH). Increased serum levels of anti-FSH IgG and IgA at the day of oocyte retrieval, were in linear association with poorer outcome of COH and with cut-off values > 1.0 AU predicting poor ovarian response (≤ 3 oocytes). For treatment before IVF procedures, Corticosteroid therapy was initiated in women who were positive for AOA by Western blot analysis or ELISA. So, anti-FSH antibodies could represent an important information about the matter of fertility assessment parameters such as age, ovarian volume, AFC and AMH to predict ovarian stimulation outcome.

Keywords: Anti-FSH Antibody, Anti Ovarian Antibody, IVF, Controlled Ovarian Hyperstimulation

P-211: Recurrence Rate of Ovarian Endometriomas after A Laparoscopic Excision

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Background: Endometrioma is a common cause of gynecologic morbidity and the most common benign ovarian cysts are found at surgery. One of the most troubling aspects of treating endometrioma with laparoscopic excision is disease recurrence after surgery. Present study was conducted to evaluate risk factors for the recurrence of endometrioma after first laparoscopic excision.

Materials and Methods: A total of 130 patients who had a minimum of one year post-operative follow-up after first laparoscopic ovarian endometrioma excision were studied. Recurrence was considered as the presence of endometrioma more than 2 cm in size, detected by ultrasonography within one year after the surgery. The variables including age at surgery, history of infertility, presence of uterine myoma, previous medicine therapy for endometriosis, the size of the largest cyst at laparoscopy, unilateral or bilateral cysts, serum CA125 level, revised American Society for Reproductive Medicine (ASRM) score and stage, post-operative medical treatment and post-operative treatment were evaluated to assess their independent effects on the recurrence using logistic regression analysis.

Results: The rate of recurrence was 11.5%. Multivariate regression analysis demonstrated that the following factors were significantly associated with increased risk of recurrence; the larger cyst [odds ratio (OR)=4.0, 95% confidence interval (95% CI)=1.6-10.4, p=0.002], a high rASRM score (OR=1.2, 95% CI=1.0-1.4, p=0.04) and the younger age at surgery (OR=0.6, 95% CI=0.4-0.9, p=0.01).

Conclusion: Detection of risk factors for recurrence of endometrioma is very important to clarify the related factors to the possible causes of recurrence. The present results suggested that recurrence of endometrioma be inversely related to age at surgery and directly with total rASRM score and the size of cyst.

Keywords: Ovarian Endometriomas, Recurrence Rate, Laparoscopic Surgery

P-212: Association between Polymorphisms of CTLA-4 Gene and Unexplained Recurrent Spontaneous Abortion in An Iranian Population

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Background: Unexplained recurrent spontaneous abortion (URSA) has been suggested being associated with the failure of fetal-maternal immunologic tolerance in which the regulatory T lymphocytes (Tregs) play a crucial role. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) is expressed transiently on activated T cells and constitutively on Tregs. CTLA-4 interacts with the B7 cell surface molecule on antigen-presenting cells and inhibits T-cell activation and proliferation. Recent Studies have reported that single-nucleotide polymorphisms (SNPs) in the CTLA-4 gene could contribute to susceptibility to some autoimmune disorders. However, the association between polymorphisms CTLA-4 of and URSA has not been defined well enough. This study evaluated the association between CTLA-4 gene SNPs and URSA in an Iranian population.

Materials and Methods: In a case-control study, 195 patients with histories of at least three consecutive miscarriages with unexplained etiology before 20th week of gestation and 102 healthy

women with at least two normal pregnancies were included as case and control groups, respectively. We genotyped two SNPs in the CTLA4 gene (a C/T transition at position -318 and A/G transition at position +49), using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

Results: The results showed that -318C/T polymorphism ($p < 0.001$) in CTLA4 gene promoter region differed significantly between URSA patients and controls, while no association was found between +49A/G in exon 1 polymorphism and URSA.

Conclusion: Results from this study suggest that -318 C/T polymorphism of CTLA-4 gene promoter be associated with immunopathogenesis URSA, and individuals carrying this allele may be more susceptible to URSA.

Keywords: Unexplained Recurrent Spontaneous Abortion, Regulatory T lymphocytes, CTLA4, Polymorphism

P-213: Report of Clinical Results from 189 Patients Undergoing Microinjection with Long Protocol Addition Prednisolone

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Background: Regarding the developments of treatment and management of infertility during recent three decades, success of these methods is limited. Therefore, this study tried to show how corticosteroid therapy improves the results of ICSI.

Materials and Methods: This semi clinical trial study without control group, including infertile women were candidate to ICSI of Kosar infertility center in urmia university of medical sciences from 2011 to 2013. Patients received prednisolone that started from one day before embryo transfer to 7 days. Then the process stopped. Women were followed by pregnancy test (B-hCG), then in the 6 weeks and 20 weeks by sonography.

Results: 181 patients entered to the study. Incidence rate of chemical pregnancy was 48.1 % (87), clinical pregnancy rate with appearance of fetal heart was 44/2% (80), incidence rate of abortion before appearance of fetal heart (6 weeks) in women with intra uterus pregnancy was 5.9 % (5), incidence rate of abortion before 20 weeks was 12.9% (11). Incidence rate of Ectopic pregnancy was 2.3% (2) and rate of twine, triples pregnancy was 32.5% (26).

Conclusion: For further study, undergoing prednisolone effect including randomized clinical trial is suggested.

Keywords: ICSI, Pregnancy Outcomes, Long Protocol, Prednisolone

P-214: Testosterone to Induce Mice Models for The Study of Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is one of the most common causes of anovulation, infertility and hyperandrogenism in women, affecting 5-10% of women of reproductive age. Due to the ethical limitations on human experimentation, appropriate animal models that mimic many or all PCOS characteristics would facilitate research leading to improved understanding of the pathogenesis of PCOS and potential for curative treatments for the PCOS syndrome. During the last years some various animal models used for the study of PCOS have allowed a focus on different aspects of the pathology. Numerous experimental models for PCOS have been developed in rodent. Rodents are clearly precious models to assess disruption of fertility. Rodent models are inexpensive, and provide well-characterised and stable genetic backgrounds readily accessible for targeted genetic manipulation, as well as shorter reproductive lifespan and generation time. Recent rodent models display both reproductive and metabolic disturbances associated with human PCOS. To date, various methods used to induce PCOS models. So, we made a PCO mouse model with testosterone injection and assessed ovarian morphological and hormonal aspects polycystic ovary.

Materials and Methods: In this study, fifteen female prepuberal (20 day-old) mice of the C57 strain were daily injected (sc) with 1mg/kg body weight testosterone for 30 consecutive days. FSH, LH and testosterone serum levels measured by ELISA kit. Ovaries were dissected and fixed in 10% buffered formalin during 24 hours at room temperature. After fixation and alcohol dehydration the paraffin sections were first examined following the hematoxylineosin (HandE) staining.

Results: Our results showed serum testosterone levels in PCO model group were significantly higher than that of control group (1.57 ± 0.7 vs. 0.25 ± 0.1 ng/ml). LH and FSH levels in two groups were not different. We assessed 4 sections of each ovaries and counted follicle number. Ovarian morphological features showed that early antral and antral follicle count in PCO model was 2.31 ± 0.36 and 3.62 ± 0.29 , it was significantly higher than that of control group (1 ± 0.2 and 0.78 ± 0.6 respectively). Granulosa and theca layers thicknesses were measured and compared. Our results demonstrated granulosa/theca ratio was significantly higher in control than that of PCO model (4.06 ± 0.4 vs. 2.67 ± 0.1 respectively). In control group ovaries was observed corpus luteum (CL) but in PCO model we did not detect any CL.

Conclusion: We could make a PCO model that ovarian morphology would be similar to polycystic ovary without ovulation. In addition, the increase of androgen level similar to polycystic ovarian syndrome was observed. Nevertheless, decreasing of LH level in our PCO model was different from polycystic ovarian syndrome characteristics. Granulosa thickness reduction in our model determined decreasing of estrogen production made of androgenic environment of follicles. So, with this method, a PCO mouse model can be made. It has some polycystic ovarian syndrome characteristics.

Keywords: Testosterone, Polycystic Ovary Syndrome Model, PCOS Induction

P-215: Combination of Antioxidant and Ovulation Induction Drugs and Evaluation of Pregnancy Rate in Infertile Women

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Background: Many studies reported that antioxidants such as vitamin E, vitamin C help to scavenge the oxygen radicals throughout the female reproductive tract that might improve the infertility treatment. The aim of this research is to adjust a regimen that is a combination of antioxidant and ovulation induction drugs and evaluation of pregnancy rate.

Materials and Methods: This is a clinical study which was done on 202 infertile women who referred to Dr. Rasekh clinic in Jahrom city during 24 months. Results are reported as number and percentage and the Chi-square test. All these patients were treated with regimen including letrozole + tamoxifen + estrogen + vitamin E. Most of the women in this study were in range of 20-30 years old (63.4%). 24.8% of patients were case of PCO syndrome. Frequency of pregnancy was reported based on endometrial quality, endometrial thickness and follicular size. Data was analyzed by SPSS 21.

Results: At the end of the study 25.7% of patients who were treated with this regimen was pregnant. 44.6% of follicular sizes were more than 18 mm. 68.7% of patients had high quality of endometrium (triple layer and lucid). 71.6% of endometrial thickness was more than 8 mm.

Conclusion: Based on the results of this study, this regimen can improve the endometrial quality and thickness which are required for successful implantation of fetus in uterus. Also this regimen had acceptable effects on follicular size which is one of the basic steps in success of ovulation induction. Pregnancy occurred in 1 woman among 4 infertile women. For evaluation of the results, this study must be done on numerous infertile patients to achieve meaningful results. Other important factor are availability of drugs and low cost.

Keywords: Infertility, Antioxidants, Letrozole, Tamoxifen, Estrogen

P-216: Dual Targeting of TNF- α and Free Radicals Toxic Stress as A Promising Strategy to Manage Experimental Polycystic Ovary

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Background: It is now clear that oxidative stress (OS) and chronic low-grade inflammation are two main pathways involved in polycystic ovary syndrome (PCOS) pathogenesis. In this regard, simultaneous targeting of these pathways by means of agents with dual anti-cytokine and anti-oxidant potential may be a therapeutic alternative approach to the current treatments.

Materials and Methods: In this experiment we aimed to study the inflammatory and oxidative response and their association with ovarian functionality in hyperandrogenism-induced polycystic ovary (PCO) and protective effects of Carvedilol and Angipars, the medicines with strong antioxidant and anti-tumor necrosis factor alpha (TNF- α) properties in PCO rats via assessment of cellular, biochemical, molecular and hormonal parameters. Murine model of PCO was induced by daily oral administration of letrozol (1 mg/kg body weight) for 21 consecutive days and effective doses of Carvedilol (10 mg/kg/day; orally) and Angipars (2.1 mg/kg/day; orally) were administered for 21 days in PCO and non-PCO healthy rats.

Results: Hyperandrogenism caused an increase in lipid peroxidation (LPO) and reactive oxygen species (ROS) of serum and ovary while decreased total antioxidant power (TAP) in PCO rats. Moreo-

ver, serum testosterone, insulin and TNF- α were increased in PCO rats while estradiol and progesterone diminished. Comparing healthy controls, hyperandrogenization induced irregular cycles, high incidence of sub-capsular ovarian cyst, increased number of atretic pre-antral and antral follicles and absence of corpus luteum (CL). All mentioned parameters were preserved by application of Carvedilol and Angipars and maintained close to normal levels.

Conclusion: For the first time we evidenced the beneficial effects of Carvedilol and Angipars, the potent antioxidant and TNF- α blockers in hyperandrogenism-induced murine model of PCO which underpin the new alternative approach in using these kinds of drugs in female reproductive disorders.

Keywords: Polycystic Ovary Syndrome, Oxidative Stress, TNF- α , Carvedilol, Angipars

P-217: The vascular Endothelial Growth factor VEGF+405 G/G Genotype May Influence Embryo Implantation in Assisted Reproductive Techniques

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Background: Repeated implantation failure (RIF) is the main problem after using assisted reproductive techniques (ART). The main causes of RIF as a multifactorial problem including decrease in endometrial receptivity, defects of embryo or combinational. Successful embryo implantation depends on trophoblast proliferation, migration and invasion to the endometrium, all of which are associated with vascular endothelial growth factor (VEGF) as the major protein in stimulating of angiogenesis. This study aimed to determine the association between VEGF+405G/C polymorphism and RIF in infertile women.

Materials and Methods: The patient group included 74 women with >3 RIF and the control group consisted of 149 healthy fertile women. Genotypes and allele frequencies of VEGF+405G/C polymorphism were determined by PCR-RFLP method and verified by Sanger sequencing

Results: The frequencies of GG, GC and CC genotypes in patient group were 31.1, 48.6 and 20.3%, respectively while those frequencies in controls were 2.0, 47.0 and 51.0% respectively. The frequency of GG genotype was significantly higher in patients than that of controls (p<0.001). CC genotype frequency was higher in controls than that of patients (p<0.001). The frequency of GC genotype did not show any difference between groups. C as the wild allele was more frequent in controls while frequency of G as the mutant allele was higher in patients (p<0.001).

Conclusion: The VEGF+405 G/G genotype may influence embryo implantation and lead to RIF in ART candidates. Since this is the second report on association of this polymorphism with RIF, further studies in different ethnic population require determining this association.

Keywords: Recurrent Implantation Failure (RIF), Vascular Endothelial Growth Factor (VEGF), Angiogenesis

P-218: The Prevalence of Infertility Reasons among Referred Infertile Couples to IVF Ward of Ahvaz Imam Khomeini Hospital

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Background: Infertility has complex implications for individual and social well-being of those involved. The medical definition of infertility used by the World Health Organization is a disease of the reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. The aim of this review was to assess the prevalence of infertility reasons.

Materials and Methods: A retrospective cross sectional study. Setting was *in vitro* fertilization (IVF) ward. Population study was all (655) of couples had been admitted to infertility center of Ahvaz Imam Khomeini Hospital in 2013 year (12 months period). Intervention was analysis of data on patients' demographic and medicine characteristics according to infertility reasons in infertile couple's files at this period. The data was collected through completion of the questionnaire. Main outcome measure was prevalence infertility reasons among infertile couples referring to the center.

Results: Among the 655 were couples enrolled, the age range of men was 21 to 62 years (mean=34.91 ± 7.58) and in women 16 to 52 years (mean=30.52 ± 6.32). Infertility reasons were: 27% ovarian dysfunction, 16.3% fallopian tube difficulty, 8.2% uterus problems, 30.7% abnormal seminal fluid, 11.6% Varicocele, 5.1% Unknown reasons.

Conclusion: This study indicates that causes of infertility should be evaluated carefully. Therefore, Despite the presence of such known factors which are related to infertility, for reasons that are unknown profoundly extensive review is conducted. The problem of infertility -differs considerably in its causes, use of different effective prevention- training -management- strategy in future studies should be important for infertile patients.

Keywords: Infertility Reasons, Infertile Couples, Ahvaz

P-219: Premature Progesterone Rise (PPR) At HCG Triggering Day Has No Correlation with ICSI Outcome: A Prospective Cohort Study

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Background: The objective of this study is to evaluate the progesterone level (P Level) on the day of HCG triggering in GnRh agonist and antagonist protocols, and its correlation with clinical pregnancy rate and miscarriage rate. Premature luteinization during *in vitro* fertilization (IVF) was commonly happened before the introduction of GnRh analogues. High level of unwanted progesterone that is associated with adverse pregnancy outcome is thought to be induced by inappropriate LH elevation.

Materials and Methods: One hundred and seven women underwent intracytoplasmic sperm injection with long agonist protocol (n=46) or antagonist protocol (n=61). Blood sample was obtained from each patient for progesterone level measurement in HCG administration day. Clinical pregnancy and miscarriage rates were

evaluated as main outcomes and biochemical pregnancy rate and implantation rate were considered as secondary outcomes.

Results: The increased prevalence rate of premature progesterone ($P \geq 1.2$ ng/ml) in total patients was 13.1% (14/107) and in long agonist protocol group and antagonist protocol group was 15.2% (7/46) and 11.5% (7/61) respectively. premature progesterone rise (PPR) had no significant correlation with clinical pregnancy rate in total patients ($p=0.174$), agonist protocol ($p=0.545$), and antagonist protocol ($p=0.129$). Also premature progesterone rise had no significant association with miscarriage rate in all of the understudied patients.

Conclusion: A significant rise in progesterone levels at the time of HCG triggering doesn't lead to a decrease in pregnancy rate and implantation rate and an increase in miscarriage rate.

Keywords: Progesterone Level, HCG Triggering, Long Agonist Protocol, Antagonist Protocol, Intracytoplasmic Sperm Injection

P-220: A Two Purpose Use of Orlistat in Obese Women with Polycystic Ovary Syndrome: Weight Loss and Androgen Reduction

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in young women. PCOS affects 6% and 8% of women in reproductive age. Hyperandrogenism is the hallmark of PCOS. Clinically, it is possible to observe hirsutism, acne, androgenic alopecia, and signs of virilization. Laboratory examination reveals increased androgen levels. Excessive androgen has an important role in the pathophysiology of PCOS. The aim of this study was to appoint evidence based and clinically applicable advice effect of Orlistat on weight loss and serum androgen level reduction in women with PCOS.

Materials and Methods: The present study was performed in the clinic of Infertility and Reproductive Health Research Center, Shahid Beheshti University of Medical sciences, Tehran, Iran. 32 PCOS patients were enrolled randomly. The Sera of androgens (Testosterone, 17(OH) P, DHEAS, SHBG) were measured before and after treatment of 12 weeks with orlistat. For all patients performed Rotterdam Criteria and transvaginal sonography for evaluation of ovarian patterns. In this study, $p < 0.05$ was considered to indicate statistical significance.

Results: The study included 32 patients. The mean age was 27.75 ± 6.22 and the mean body mass index was 32.69 ± 0.94 kg/m². Comparing with baseline, treatment with orlistat resulted in a significant reduction in weight, BMI, and waist circumference ($p=0.001$). We found a remarkable reduction in total Testosterone levels ($p < 0.001$). Treatment improved the SHBG plasma levels, but the difference was not significant. There was no reduction in other androgen levels. As an incidental findings we encountered with a 30 mm decreasing size of uterine leiomyoma.

Conclusion: This study showed a significant reduction on weight and total Testosterone levels, as the most important androgens in PCOS patients. Therefore, controlling of weight and treat-

ment by a short course of Orlistat can be useful in management of PCOS cases.

Keywords: PCOS, Orlistat, Androgen, Weight Loss

P-221: Toxoplasmosis and Female Infertility

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Background: *Toxoplasma gondii* is an obligate intracellular parasite, which affects a wide-range of mammals including human. Based on serological studies, *T. gondii* is one of the most prevalent protozoan parasites. Most cases of toxoplasmosis are asymptomatic or mild and influenza-like, but immunocompromised patients often develop fulminating life-threatening symptoms as pneumonia and encephalitis. Recently recorded a relation between female infertility and toxoplasmosis in China, which raises the concern about the importance of toxoplasmosis in female fertility problems.

Materials and Methods: This study reviews the use of internal and external sources and is made available on the Internet

Results: It is well documented that toxoplasmosis is of crucial importance owing to cosmopolitan distribution and wide range of diseases which cause. It is known that toxoplasmosis has some unfavorable effects on the reproductive capacity of both men and women. The overall detection rate of toxoplasmosis among Egyptian females in childbearing age was 57.52%, distribution especially in the third world countries easier and cheaper to be carried out particularly in the time of high incidence of the disease. However, it was shown that the high number of toxoplasmosis cases were recorded among females seen among families with one baby being already delivered the seroprevalence of *T. gondii* IgG and IgM among non-pregnant women was higher than pregnant group. In contrast, pregnant women were mostly under risk of catching toxoplasmosis as concluded elsewhere. However, *T. gondii* infection in human may occur vertically that are passed to the foetus via the placenta, or horizontal transmission which may involve three life cycle stages i.e. the women examined might be already infected with disease before marriage and she might be chronic carrier of the parasite concerned distribution of toxoplasmosis is due to many risk factors including age, number of deliveries, contact with host animals (small ruminants), contact with uncooked meat, drinking raw sheep or goat milk

Conclusion: Accordingly, the implementation of health education programs aimed to primary prevention of toxoplasmosis is mandatory. Also, the infertile females had a significant higher prevalence of *T. gondii* infection than the controls especially in older ages. These data highlight the possible correlation between *Toxoplasma* infection and infertility

Keywords: Toxoplasmosis, Infertility, Female

P-222: Ovarian Stimulation for Fertility Preservation in Cancer Patients: A Prospective Study

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Background: To determine whether random-start controlled ovarian stimulation (COS), in which a stimulation is performed regardless to day of patient's menstrual cycle, has similar outcomes in comparison to conventional early follicular phase-start COS for cryopreservation of fertilized oocytes in cancer patients.

Materials and Methods: This prospective study was performed at two infertility centers; Vali-e-Asr reproductive health research center (a tertiary referral university hospital) and Shayanmehr private clinic from January 2011 to February 2014. The Institutional Review Board and Ethical Committee of Tehran medical sciences university approved this study. All patients were recently diagnosed with cancer and were chosen for chemotherapy/radiotherapy or bilateral oophorectomy. All the patients were evaluated for fertility preservation within 48 hours. Time frame until the initiation of cancer treatment was at least 2 weeks. All participants received COS cycles using GnRh antagonist for pituitary suppression. The decision to perform with a conventional- versus a random-start COS was elected by the patients' menstrual cycle on presentation. Primary outcome was total number of Metaphase II (MII) oocytes. Secondary outcomes were dosage of gonadotropins, number of days for ovarian stimulation, oocyte maturity rate (MII oocytes/total oocytes), and fertilization rate.

Results: During the study, 10 patients with ovarian tumor, 3 patients with uterine and one with breast cancer referred to study centers for fertility preservation. Seven patients underwent conventional-start and seven with random-start COS. The number of total and MII oocytes retrieved, and fertilization rates were similar between groups. No differences were observed in total dose of gonadotropins ($P=0.9$) and the duration of stimulation ($p=0.3$) between two groups

Conclusion: This pilot study presented that oocytes can be obtained before cancer therapy efficiently by random-start COS and this method could reduce delays and provide this opportunity for more patients to perform fertility preservation and still continue with cancer treatment within 2-3.

Keywords: Cancer, Fertility Preservation, Random Start, Controlled Ovarian Stimulation

P-223: Comparing Dietary Intake in Women with and without Polycystic Ovary Syndrome

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Background: Adequate nutritional status is a critical determinant of the onset and maintenance of normal reproductive function. More than 50% of women with PCOS are obese and central obesity plays a central role in the pathogenesis of PCOS by generating an insulin-resistant state. It has been proposed that recurrent hypoglycaemia due to hyperinsulinaemia could lead to decreased post-prandial satiety or increasing craving for or consumption of high carbohydrate foods. There is some evidence indicating that food choices may be altered in PCOS and a number of investigators showed that women with PCOS selectively choose foods that could worsen their presentation of PCOS, ie either foods that would predispose to obesity through their high energy density or foods that could have effects on metabolic parameters. The aim of this review article is to study the routine dietary intake among women with and without PCOS.

Materials and Methods: This review article prepared by studying of articles obtained from Google, pub med sites with key words such as polycystic ovary syndrome; insulin resistance; diet, glycemic index.

Results: Carmina et al indicated that American women with PCOS have a higher BMI and a greater use of saturated fat compared Italian women with PCOS. Further studies in patients with PCOS compared with healthy subjects indicated that diet does not differ between the two groups as regards energy, macronutrient with the exception of a lower percentage of energy from lipids and a higher intake of fibres by PCOS women. Altieri et al. (2012) indicated PCOS women were characterized by a higher consumption of cheese and high-glycaemic index starchy sweets and a preference for raw oil rather than other cooked fats, compared to controls. Similarly, Douglas et al. (2006) reported similar total energy, macronutrient, micronutrient and high GI food intake in overweight age and BMI-matched women with and without PCOS, but women with PCOS consumed higher amounts of a number of specific high GI foods (white bread, fried potatoes) than the subjects without PCOS. Barr et al. (2011) showed that nearly half of overweight women with PCOS had low physical activity and mean dietary glycaemic index (GI) was higher in obese women with PCOS compared with healthy weight women with PCOS. They found that the percentage energy from carbohydrate intake was significantly lower and the percentage of energy from fat significantly higher in lean PCOS patients compared with lean control subjects, but there is similarity between overweight/obese PCOS patients and control subjects. Conversely, Moran et al. (2013) observed no differences in macronutrient intake between overweight subjects with or without PCOS. Women with PCOS had a better diet quality as indicated by a higher diet quality score and higher energy, fibre, folate, iron, calcium, magnesium, niacin, phosphorus, potassium, sodium, vitamin E and zinc intake and lower percentage energy from saturated fat intake, glycaemic index and retinol intake than women without PCOS. Finally, Graff et al. (2013) studied dietary intake of Sixty-one women with PCOS and 44 non hirsute women with ovulatory cycles. It was found that PCOS and control women in the highest tertile of GI had higher body mass index and waist circumference than those in the lowest tertile. Dietary GI was higher in the classic PCOS group. Obesity and more severe PCOS phenotype explained 28.3% of variance in dietary GI.

Conclusion: This review suggested that women with PCOS consume a larger quantity of specific foods with a high glycemic index and saturated fatty acids, despite sharing a diet that is similar in total energy, macronutrient, micronutrient, with age and weight-matched control women.

Keywords: Polycystic Ovary Syndrome, Insulin Resistance, Diet, Glycemic Index

P-224: Chlorpromazine-Induced Hyper-

prolactinemia on Rat's Uterus

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Background: Chlorpromazine (CPZ), an antipsychotic drug, is known to be associated with the increased risk of sexual dysfunction with increasing prolactin and the aim of this study was to conduct evaluation of the effect of CPZ on serum prolactin, sex hormone concentrations and histopathological changes in uterine tissue of rats.

Materials and Methods: Animals were divided into four groups. One group served as control sham. In the drug treated groups (3, 10 and 30 mg/kg/day) CPZ administered for 28 days by gavage. 1 day after the last administration of drug, animals were sacrificed. Histopathological and histomorphometrical analysis of the uterus was carried out. Serum levels of prolactin and sex hormones (LH, FSH, estrogen and progesterone) and potential fertility of animals were evaluated.

Results: Female rats exposed to CPZ had significant increase in serum prolactin and progesterone concentrations and decrease in LH, FSH and estrogen levels at 10 and 30 mg/kg dose-levels ($p < 0.05$). CPZ induced histopathological changes in the uterus with increasing in thickness of epithelium, myometrium and endometrium at 10 and 30 mg/kg dose-levels ($p < 0.05$). CPZ decreased potential fertility in female rats as well.

Conclusion: These results showed that oral CPZ treatment caused significant ($p \leq 0.05$) hyperprolactinemia with negative effects on sex hormonal concentrations and uterine morphology and changed the fertility parameters.

Keywords: Chlorpromazine, Prolactin, Hyperprolactinemia, Uterus, Rat

P-225: Gene Variations of Toll-Like Receptor 3 in Endometriosis

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Background: Endometriosis is a common gynecologic disorder that is characterized by the ectopic growth of endometrial tissue. Recently, endometriosis has been alternatively described as an immune, genetic and hormonal disease caused by exposure to environmental factors. Toll-like receptor 3 (TLR3) comprises a family of receptors through directly recognizing exogenous and endogenous ligands plays the key role in realization of immune responses, and also involves in the processes of cell proliferation, survival, apoptosis, angiogenesis,

tissue remodeling, and repair. Polymorphisms in TLR3 may shift balance between pro- and anti-inflammatory cytokines, modulating the risk of infection, chronic inflammation and cancer.

Materials and Methods: We performed a case-control study of 70 endometriosis patients whom had been confirmed by laparoscopic surgery and 55 healthy controls that had no history of inflammatory disorders or using any related drugs. All DNA samples were obtained from Royan DNA bank that had been extracted from peripheral blood along 2012-14. Exon4 of TLR3 were amplified in two overlapped fragments by polymerase chain reaction (PCR) and products were analyzed by sequencing.

Results: In fragment1, Missense Single Nucleotide Polymorphism (SNP), rs3775291 (C/T), was observed in 22 out of 70 patients (31.4%) and 24 out of 55 controls (43.7%). Thirty six patients (51.4%) and 31 controls (56.4%) also had shown synonymous SNP,rs3775290 (C/T) in fragment1, while no SNP was observed in fragment2 in 100 cases. The present study showed that the SNPs frequencies between patients and controls, were not significantly different.

Conclusion: Endometriosis is a common type of chronic inflammatory disease and both of these SNPs are very common in inflammatory disorders, but these were not significantly different between patient and control group in our studied population.

Keywords: TLR3, Endometriosis, Polymorphism, Gene Variation

Genetics

P-226: Association of β -Defensin 126 Gene Alteration with Intra Cytoplasmic Sperm Injection and *In Vitro* Fertilization Outcome in Unexplained Infertile Men

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Background: Infertility is a common problem. Despite improved methods for evaluation of sperm quality, It remains unexplained in about 20% of affected couples. During sperm maturation, a Cysteine-rich secretory glycoprotein β -defensin126 secreted by the epididymal epithelium absorbed to the entire sperm surface. It remains on the sperm until sperm become capacitated in the female reproductive tract. Its removal from over the head of sperm is required for sperm zona recognition. It is located in the subtelomeric region of chromosome 20(20p13). A cytosine dinucleotide deletion in the open reading frame of second exon of DEFB126 gene generates an abnormal mRNA. Men homozygous for this mutation have reduced chances of successful fertilization.

Materials and Methods: We examined the relationship between the ICSI and IVF outcome with the genotype of unexplained infertile men. Genomic DNA from the peripheral blood of 80 male partners of unexplained couples who underwent ICSI (first group) and IVF (second group), were extracted. PCR was performed and molecular genotyping for the DEFB126 variant was done by SSCP (single strand conformational polymorphism), tetra PCR and DNA sequencing. ELISA and Immunocytochemistry by indirect immunofluorescence antibody performed for the assessment of this protein expression on sperm cells.

Results: According to obtained data and statistical analysis, no significant differences were found between homozygote mutation and wild type carriers in fertilization rates, implantation rates and clinical pregnancy of IVF and ICSI. Our results by ELISA and Immunocytochemistry showed that the protein expression was less in men with del/del genotype in comparison to other genotypes ($p < 0.005$).

Conclusion: Although previous studies were found DEFB126, variation would affect sperm function and male fertility rate. In the present study, no significant differences were found between homozygote mutation and IVF and ICSI outcome. Further confirmation in a larger scale study is needed.

Keywords: Unexplained Male Infertility, Deletion, DEFB126, IVF, ICSI

P-227: Functional Analysis of The I.a,I.b, I.c and I.d (PII) Promoters of CYP19 (aromatase) Gene in Granulosa Cells of Polycystic Ovaries Patients and The Role of Letrozole and Antisensearom on CYP19 Gene Expression Inhibition

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Background: The key enzyme of estrogen biosynthesis, aromatase cytochrome P450, is encoded by the CYP19 gene. CYP19 plays an important role in the development, function, and regulation of the female reproduction cycle. Thus, it is the potential candidate gene affecting fertility performance in human. CYP19 transcripts are expressed mainly in the ovary, testes, breast, adipose tissue and brain. Tissue expression of the CYP19 gene is determined by the use of tissue-specific promoters. The object of the present study was to determine first, the distribution of various exon-specific CYP19 transcripts and second, the inhibitory effect of letrozole and antisense aromatase on CYP19 gene expression level in granulosa cells of polycystic ovaries (PCO) patients.

Materials and Methods: CYP19 transcript levels on four promoters were measured from granulosa cells of PCO patients by competitive RT-PCR and the effect of letrozole and antisensearom on potent promoters were evaluated by real-time PCR.

Results: Antisensearom was potent inhibitor of CYP19 transcription, while letrozole inhibited moderately. Promoter 1.d (PII) proved the most potent in driving gene expression. Promoter 1.c displayed moderate activity, while promoters 1.a and 1.b were weak.

Conclusion: Two regulator regions of promoters 1.d (PII) and 1.c

relevant to CYP19 expression in human granulosa cells in ovary. Letrozole and antisensearom modulate transcription through these regions.

Keywords: CYP19, Antisensearom, Letrozole, PCO

P-228: Altered Expression of Follicle-stimulating Hormone Receptor and Luteinizing Hormone Receptor in Granulosa Cells from Women with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common complex genetic endocrinopathy, affecting 5 - 10% of women at reproductive age. PCO granulosa cells seem to have abnormal responses to follicle-stimulating hormone (FSH). FSH is considered to be a pituitary glycoprotein that plays an important role during folliculogenesis as it promotes the proliferation and differentiation of granulosa cells and the maturation and development of follicles. The effect of FSH is believed to be mediated by the binding of the hormone with a specific receptor, FSHR, which is situated on the granulosa cells. Through the cascade effect, FSHR transfers the biological signals of FSH to the downstream network. Accordingly, activation of the LH receptor (LHR) in ovarian granulosa and theca cells initiates signaling cascades regulating transcription of genes which is necessary for ovulation and luteinization. LHR expression requires appropriate hormonal stimulation by FSH and estradiol. Objective: The aim of this prospective study was to analyze the expression of these genes at the human granulosa cells of women with PCOS.

Materials and Methods: After the approval of Iran University of medical sciences ethics committee and written informed consent of the patient, follicular fluid was collected from patients undergoing oocyte retrieval. 80 patients were divided into two groups, PCOS and control according to Rotterdam criteria. A series of isolation and purification methods was performed, including density gradient centrifugation, MACS (use of antibody bead complexes) and RNA extraction. Reverse transcriptase PCR (RT-PCR) was performed and quantification of gene expression levels was achieved by real-time quantitative PCR.

Results: The expression of FSH receptor was significantly different between case and control groups, with the lower expression in the PCOS group ($p < 0.001$). Considering all the women with and without PCOS undergoing controlled ovarian hyper stimulation, significant relationship was observed between the body mass index and FSHR level ($p < 0.05$). There was a significant difference in LHR expression levels in PCOS women compared to control women ($p < 0.02$). In both PCOS patients and controls, a strong positive linear correlation was observed between the FSH and LH receptor mRNA levels ($r = 0.92$, $p < 0.05$).

Conclusion: Findings from the previous studies show that the elevated expression of FSHR mRNA can be regarded as one of the earliest markers for differentiating granulosa cells. Also, the expression of LHR mRNA has been proposed to be associated with follicle selection. Since the selection of follicle and its further maturation to a dominant follicle does not occur in PCOS patients, it seems that PCO granulosa cells may have abnormal responses to FSH and LH. The results of our study are consistent with these findings.

Keywords: FSH Receptor, LH Receptor, Granulosa Cell, PCOS

P-229: Chromosomal Analysis of Parthenogenetic Mouse Embryos Generated from In Vitro Activated Oocytes by Hydrostatic Pressure and Ethanol and Cytochalasin B

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Background: Studies of preimplantation stage embryos by classic cytogenetic techniques have limitations, starting with the need for good metaphase stage when only one third of all analyzed embryos may show good quality metaphases. The incidence of chromosome anomalies in embryos produced by *in vitro* procedures is generally higher than that of embryos formed *in vivo*. Pressure specifically affects centriole microtubules and that proximal microtubule triplets are more resistant than distal microtubule doublets. The present study has been carried out to investigate the effects of hydrostatic pressure in the absence or presence of ethanol on the improvement of chromosomal complements in 2-cell parthenogenetic embryos.

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 were collected from oviduct 14 hr after HCG injection. Oocytes transferred to T6 medium and randomly assigned to following groups: non-treatment (control), HP exposure (treatment I), ethanol exposure (treatment II) and ethanol 7% with HP exposure (treatment III). Groups of activated oocytes were further treated with 5 mg/ml cytochalasin B for 4 hours. About 24-hours post oocytes activation, Slides were prepared according to an 'air drying' technique and the chromosomal complement of 2-cell embryos was studied by Giemsa-staining.

Results: Results indicated that in embryos treated by ethanol and HP observed the most incidence of normal chromosome (diploidy and tetraploidy) (64%) and the fewest incidence of anormal chromosome (haploidy, triploidy and mixoploidy) (26%) compared with control group and other treatments ($p < 0/05$).

Conclusion: Induction of Ca fluxes promotes the resumption of meiosis and extrusion of the second polar body, but not pronuclear formation. Treatment with a diploidization agent is required to either suppress second polar body extrusion (CB). Hydrostatic pressure probably by affecting on centriole microtubules could improve chromosomal complements.

Keywords: Chromosomal Analysis, Parthenogenetic Activation, Hydrostatic Pressure, Ethanol, Mouse

P-230: Analysis of TEX15 Expression in Testis Tissues of Severe Oligozoospermic and Non-Obstructive Azoospermic Men Referred to Royan Institute

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Background: TEX15 is a novel protein that is required for chromosomal synapsis and meiotic recombination. Human TEX15 is located on chromosome 8(8p12 region) and expressed in testis and ovary, as is its mouse ortholog. Loss of TEX15 function in mice causes early meiotic arrest in males but not in females. Specifically, TEX15 deficient spermatocytes exhibit a failure in chromosomal synapsis. In Tex15 -/- mutant spermatocytes, DNA double-strand breaks (DSBs) are formed, but localization of the recombination proteins to meiotic chromosomes is severely impaired. These results indicate that TEX15 regulates the loading of DNA repair proteins onto sites of DSBs and thus its absence causes a failure in meiotic recombination.

Materials and Methods: The samples retrieved from patients who underwent diagnostic testicular biopsy in Royan institute. 10 patients with severe oligozoospermia and 10 patients with non-obstructive azoospermia (with SCOS syndrome) were recruited. Total RNA was extracted with Trizol reagent and cDNA was synthesized. Quantitative real-time RT-PCR for TEX15 was performed using Power SYBR Green kit.

Results: Normalizing the relative amount of TEX15 transcript by the amount of GAPDH transcript in the same sample, indicated that expression of TEX15 in the testis samples of patients with non-obstructive azoospermia(SCOS patients) is significantly reduced as compared with oligozoospermic patients (p value=0.0009).

Conclusion: The same expression pattern and sequences homology of TEX15 among mouse and human indicated that TEX15 is a highly conserved gene that might have an important role in mammalian testis functions like spermatogenesis. Although its function in human is unknown, its role in mouse is spermatogenesis. It can be concluded that TEX15 can also have the same role in human. According to the results, we can conclude that TEX15 expression levels are essential for normal spermatogenesis and deficiency in this gene can cause spermatogenic failure and infertility in men.

Keywords: Male Infertility, Non-Obstructive Azoospermia, Meiotic Arrest, TEX15

P-231: Androgen Receptor Gene Expression in Azoospermia Men

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Background: Androgens are critical steroid hormones in progression of spermatogenesis process and determine the male phenotype that their actions are mediated by the androgen receptor (AR), a member of the nuclear receptor superfamily. In the Androgen

receptor, transactivation domain encoded by exon 1, DNA binding domain encoded by exons 2 and 3, hinge region encoded by part of exon 4, and C-terminal ligand-binding domain encoded by exons 4 to 8. AR is a ligand-dependent transcription factor and regulates expression of androgen-responsive genes. The binding of androgens to AR result in receptor dimerization and its entering to the cell nuclear. Then AR dimer binds to its response elements and regulate its expression by transcribe of target genes. AR expression has been observed in Leydig, Sertoli, Pritubular Myoid cells but there was no expression in Germ cells. In this study we evaluated the expression of AR in 30 azoospermic infertile men.

Materials and Methods: Samples were categorized in three groups: 10 obstructive and 10 non-obstructive Sertoli cell only syndrome and 10 Maturation arrest gene expression of AR determined by Real-time PCR technique.

Results: This study demonstrates that expression of AR mRNA in SCO patients increased due to the lack of Germ cells.

Conclusion: To this end, AR gene expression can be used as a biomarker gene for diagnosis azoospermia SCO in testis tissue of sub fertile men and azoospermia men. However, it seems that in the absence of sperm, AR gene expression increases, but this hypothesis should be confirmed by future studies.

Keywords: Androgen Receptor, Maturation Arrest, Sertoli Cell Only, Transcription

P-232: Gene Expression Analysis of the Histon Variant H2BFWT in Testis Tissues of Non-Obstructive Azoospermic Patients Referred to Royan Institute

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Background: During the later stages of spermatogenesis, spermatid nuclear remodeling and condensation are associated with histone modifications and the sequential displacement of histones by transition proteins and then by protamines. In humans, approximately 15% of the sperm DNA remains packaged by histones in sequence-specific areas. The histone variant H2B, member W, testis-specific (H2BFWT) gene encodes a testis-specific histone that becomes incorporated into sperm chromatin. The aim of this study was to investigate the expression levels of H2BFWT mRNA in testis tissues of infertile men with non-obstructive azoospermia and their relations with spermatogenesis disturbances.

Materials and Methods: Samples were collected from infertile men who referred to Royan Institute. They underwent testicular sperm extraction (TESE). Using qRT-PCR method, mRNA expression levels of H2BFWT gene in patients with obstructive azoospermia (as control group, n=8) were compared with sertoli cell only syndrome (SCOS, n=8) and complete maturation arrest (CMA, n=8) patients.

Results: Our data revealed lower expression levels of H2BFWT

in the two SCOS and CMA patient groups rather than control group.

Conclusion: In this study, it can be concluded that the lower levels of H2BFWT gene expression may be associated with spermatogenic failure in non-obstructive azoospermia patients, as a susceptibility factor for male infertility.

Keywords: H2BFWT, Male Infertility, Spermatogenesis Impairment

P-233: Study of Chromosomal Alterations and Polymorphisms of MTHFR, Factor V and Prothrombin Genes in Patients with Recurrent Miscarriage Referred to Royan Institute

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Background: Recurrent miscarriage (RM) is defined as two or more consecutive pregnancy losses before 20 weeks of gestation as an important clinical problem, with an incidence of 1-3% among couples wishing to have children. There are several factors in the etiology of recurrent miscarriage. One of the main genetic causes which involve in the pathogenesis of RM is balanced chromosomal rearrangements in one or both of partners. In 4-8% of couples with RM, at least one of the partners has chromosomal abnormality. Next to chromosomal abnormalities, thrombophilia was identified as a major cause of RM, with a rate of up to 40%, especially in the first half of pregnancy.

Materials and Methods: The patient groups included 1100 Iranian couples (2200 individuals) referred to Royan Institute between 2004 and 2013. Karyotyping was performed using standard cytogenetic techniques. Besides, thrombotic gene polymorphisms were studied in 128 women who had a normal karyotype. The results were compared with 70 healthy women as control group.

Results: Abnormal karyotypes were found in 124 people, 83 women (3.77%) and 41 men (1.86%). The frequency of FV Leiden, ProthrombinG20210A, MTHFR C677T and MTHFR A1298C mutations in patients were 10.93, 4.68, 43.75 and 60.15%, respectively. These frequencies in control group were 2.85, 2.85, 34.28 and 5.71% respectively.

Conclusion: Sex chromosome mosaicism is the most commonly detected chromosomal abnormality in couples with RM who are candidates for offering PGD. Patients with combined thrombotic mutations are at higher risk of RM than women without these mutations. The most important issue with hereditary thrombophilia is the prevention of maternal thrombosis.

Keywords: Recurrent Miscarriage, Thrombophilia, Karyotype

P-234: Altered Epigenetic Marks on Chromatin Condensing Genes of Sperm in TESE Negative Men Referred to Royan Institute

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Background: Development of assisted reproductive technology (ART) opened new hopes for couples with male factor infertility. By these methods of ART, fertilization can be achieved with sperm recovered from the seminiferous tubules. Testicular sperm extraction (TESE) is an open testicular biopsy and one of the common methods for retrieving testicular sperm. As critical role of successful spermatogenesis in fertilization, evaluation of these testicular biopsies is very valuable to understand the mechanisms of spermatogenesis and (in) fertility. One of the most important mechanisms of spermatogenesis is compaction of chromatin in sperm. Of responsible genes of this event are transition proteins (TNPs) and protamines (PRMs). High expression of these genes through last stages of spermatogenesis is regulated by epigenetic factors such as histone acetyl transferases and demethylases. So, evaluation of epigenetic activating/repressive modifications of acetylated/di-methylated lysine 9 of histone H3 (H3K9ac/me2) on regulatory regions of TNPs and PRMs may represent better insight into mechanisms of (in) fertility.

Materials and Methods: For this respect, based on spermogram and presence/absence of sperm in TESE process, testis tissue samples of infertile men referred to Royan institute were collected as two groups of TESE negative and TESE positive. Expression of TNPs and PRMs were evaluated by qRT-PCR quantitatively. Also, chromatin immunoprecipitation (ChIP) coupled with real time-PCR was performed to evaluate the incorporation of H3K9ac/me2 into regulatory regions of aforementioned candidate genes. Consent was obtained from patients according to local ethical approval.

Results: Results showed significant decrease in expression of TNPs and PRMs in TESE negative in comparison to TESE positive group. These results were confirmed by ChIP data which revealed low levels of acetylation versus methylation of H3K9, on regulatory regions of studied genes in TESE negative versus TESE positive group.

Conclusion: This study showed that altered epigenetic marks of genes which are involved in chromatin condensing of sperm is associated with male infertility.

Keywords: Spermatogenesis, Male Infertility, TESE, Epigenetic

P-235: No Association of Endothelial Nitric Oxide Synthase (eNOS) -786T/C Polymorphism with Unexplained Recurrent Abortion in Iranian Women

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Background: This is a case-control study to determine the relationship between endothelial nitric oxide synthase (eNOS) gene -786T/C polymorphism in women with unexplained recurrent abortion in comparison with healthy women.

Materials and Methods: 95 women with history of at least 2 unexplained recurrent abortion in the reproductive age range 20-35 years as patients group and 95 healthy women (age-matched) who had at least one live birth and no history of abortion were chosen. Genotyping of eNOS -786T/C polymorphism was analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: Genotype frequencies of eNOS -786T/C polymorphism for the patient group were as follows TT(27.3%),TC (65.2%) and CC (7%) while in control group genotype were 37.8, 50.5 and 11.5%, respectively. Allele frequencies of the eNOS -786T/C polymorphism among patient and control groups were 0.60 and 0.63, respectively for the T allele and 0.40 and 0.36 respectively, for the C allele.

Conclusion: Our result demonstrated no association between unexplained recurrent abortion and -786T/C polymorphism of eNOS gene in Iranian women.

Keywords: Endothelial Nitric Oxide Synthase, Polymorphism, Unexplained Recurrent Abortion

P-236: Haplotype Analysis of The H2B.W Gene in Severe Oligospermic and Azospermic Infertile Men Referred to Royan Institute

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Background: Recent studies demonstrated the multifactorial and chronic nature of male infertility, including mutations of some known spermatogenesis-related genes. The H2B family, member W (H2B.W) gene is one of the testis specific histone variant genes that encodes a sperm telomere-binding protein, required for reorganization and integration of sperm chromosomes. The objective of the present study was to find the association of -9C>T (rs7885967) and 368A>G (rs553509) (CA, TA, CG, TG) haplotypes in H2B.W gene with Iranian male infertility.

Materials and Methods: Haplotype frequencies of these single nucleotide polymorphisms in the peripheral blood samples of 92 infertile men suffering from severe oligospermia (46) and azoospermia (46) (including patient with Sertoli cell only syndrome (SCOS) (21), complete maturation arrest (CMA) (17) and hypo spermatogenesis (8) according to testicular biopsy) and 60 fertile men with normal semen parameters referred to Royan Institute were analyzed by Polymerase Chain Reaction and restriction fragment length polymorphism technique (PCR-RFLP). The frequencies of these haplotypes were assessed using logistic regression analysis.

Results: The haplotype TA compared with haplotype CA in patients suffering from CMA significantly increased, compared with patients who had SCOS (p<0.05). However, our data showed that in general, the distribution frequencies of these haplotype had no significant difference between the infertile groups and control.

Conclusion: As this gene was expressed in the late stages of spermatogenesis, it can be concluded that haplotype TA might arrest maturation process of spermatids during spermatogenesis.

Keywords: H2B.W, Male Infertility, Haplotype, PCR-RFLP

P-237: Elucidation The Role of Chromosomal Aberrations in Ovarian Reserve: A Retrospective Clinical Report

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Background: Constitutional chromosome abnormalities are among the major contributors to the genetic causes of reproductive disorders. Despite all of worldwide efforts have been made so far, the prognosis for mosaic X chromosome aberration below 30% of unemployed has yet to be established. The purpose of this study was to assess the quantity and quality of chromosomal aberrations that may negatively affect ovarian health causing premature ovarian failure (POF) and diminished ovarian reserve (DOR).

Materials and Methods: In this purpose, retrospective observational study of clinical features and biological parameters was performed. A total of 531 individuals who referred to our ward from 2007 to 2014 because of amenorrhea and poor responders to gonadotropins were selected. High-resolution chromosome analysis by GTG banding was carried out on peripheral blood lymphocyte cultures. Supplementary tests also were performed when required.

Results: Of the 531 cases who were assessed for chromosomal defects, 52 showed abnormal karyotype. 22 cases were found to have cell lines with different level of X chromosome variation. Seven cases were sex reverse sex determining region Y (SRY) negative, five cases with abnormal X chromosomes, three cases with structurally abnormal autosomes and four individuals carrying X-autosome translocation were diagnosed. The overall prevalence of chromosomal abnormalities was 9.8% which 2.1% of it belongs to normal variable chromosome features. The frequencies of chromosomal alterations were 5% and 1.7% in POF and DOR females, respectively.

Conclusion: The results confirm previous observations and emphasis on the critical role of chromosome abnormalities as one of the possible etiologies for ovarian follicular attrition.

Keywords: Diminished Ovarian Reserve (DOR), Premature Ovarian Failure (POF), Cytogenetic, Chromosome Abnormalities

P-238: Lack of Association of Estrogen Receptor Polymorphisms with Male Infertility

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Background: Estrogen is recognized as one of the significant

regulator of spermatogenesis. Estrogens are synthesized in the male reproductive system (sertoli and leydig cells). Estrogen function is mediated by estrogen receptors (ER- α or ER- β). Some studies have suggested an association between single nucleotide polymorphisms (SNPs) rs2234693 (ESR1 pvull C>T), rs1801132 (ESR1 325 C->G) of ER α gene are associated with human spermatogenic defect.

Materials and Methods: In a case-control study, including 264 infertile (149 Nonobstructive Azoospermia and 115 oligo-asteno-teratozoospermia) and 90 fertile men, we investigated pvull C>T and 325 C->G variants. Semen analysis was performed according to WHO. All patients underwent a comprehensive examination; karyotyping and molecular test for Y chromosome microdeletion. All enrolled subjects had a normal karyotype and intact Y chromosome. Polymerase chain reaction was performed on DNA isolated from peripheral blood samples. The rs1801132 (C325G), was detected by sequencing while RFLP was applied to determine rs2234693 (ESR1 pvull C>T). Statistical analysis was performed by using the Chi-square test.

Results: Our results showed that allele frequencies of C and G of rs2234693 were approximately 0.42 and 0.58 in infertile men, respectively, which were similar to fertile group. Allele frequency distribution of rs1801132 (G=0.35 and C=0.65) in our studied population was similar to those observed in the Asian populations (G= 0.50) whereas varied from those in European and American Caucasians (G= 0.05-0.20). Therefore, two studied of SNPs of ESR1 were not significantly associated with semen parameter (sperm concentration, motility and morphology), either by allelic or genotypic frequencies in our population.

Conclusion: We found no evidence for association of both Pvu II T/C and C325G polymorphisms with infertility susceptibility. Therefore, the hypothesis that studied estrogen receptor alpha gene variants could influence on semen quality which is not supported by our data.

Keywords: Male Infertility, Estrogen Receptor Alpha, Single Nucleotide Polymorphism

P-239: NGS Mapping Breakpoints of a Familial Chromosome Insertion (18:7) (q22.1; q36.2 q21.11) to DPP6 and CACNA2D1 Genes in An Azoospermic Man

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Background: Aberrant chromosomes can cause azoospermia but little is known about its molecular mechanism. Our aim is to explore any possible genetic defective to explain a given male infertility.

Materials and Methods: An azoospermic male was identified in a 23 years old male. G-banding and FISH confirmed the karyotype as chromosome insertion (18:7) (q22.1; q36.2q21.11). NGS was performed to analyze the breakage points and around sequences.

Results: The analysis revealed that the breakage in chromosome 7 disrupts two genes, dipeptidyl aminopeptidase-like protein 6 (DPP6) and contactin-associated protein-like 2 (CACNA2D1), the former participates in regulation of voltage-gated potassium channels, and the latter is one of the components in voltage-gated calcium channels. The deletion and duplication were not identified equal or beyond 100Kb, but 4 homologous DNA elements were verified proximal to the breakpoints. One of the proband's sisters inherited the

same aberrant karyotype and experienced recurrent miscarriages and consecutive fetus death, while in contrast, another sister with a normal karyotype experienced normal labor and gave birth to healthy babies. The insertional translocation is confirmed with FISH and the Y-chromosome microdeletions were excluded by genetic testing.

Conclusion: This is the first report describing chromosome insertion inv ins (18; 7). Our NGS analysis showed that DPP6 and CACNA2D1 may attribute to azoospermia, and Alu-like sequences may mediate the chromosome insertion.

Keywords: Male Infertility, Azoospermia, Insertional Translocation, DPP6, CACNA2D1

P-240: Effect of Oxidative Stress on DNA Fragmentation Index in Sperm of Mature Mice

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Background: One of the major factors affecting on male infertility is oxidative stress (OS). Two main sources of ROS production are leukocytes and spermatozoa. The ROS such as tertiary-butyl hydroperoxide (TBHP) may damage and fragmentate to sperm DNA, which can lead to complications in the offspring. In this study, we will investigate the effect of od on DNA fragmentation index (DFI) in sperm of mature Balb/C Mice.

Materials and Methods: OS in adult mice testis was induced by injection of the 1:10 concentration of tertiary-butyl hydroperoxide. Adult male Balb/ mice were randomly selected. Case group included treated mice by TBHP for 2 weeks and control group treated only by Injection of DH2O. Induced ROS levels in testes tissue samples of all mice measured by flow-cytometry. Consequently, TUNNEL assay performed to detect sperm DNA damage using fluorescence microscopy.

Results: According to flow-cytometry results, an increase in level of oxidative stress in ROS treated mice in comparison to control group was observed. Moreover, the level of DFI measured by fluorescence microscopy increased in TBHP treated mice compared with control mice (p \leq 0.05).

Conclusion: As ROS is produced naturally in the body due to oxidative stress, it is likely that oxidative stress can cause DNA fragmentation in human-spermatozoa. Also, the balance between ROS production and antioxidant levels in the body can protect DNA against damages. This experiment shows that there is significant association between ROS and DNA fragmentation.

Keywords: Sperm, Oxidative Stress, DNA Fragmentation Index

P-241: Association of ITPA Polymorphisms rs1127354 with Infertility

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Background: Infertility is a relatively common problem that affects couples worldwide. It is estimated that approximately 1 in 6 couples will experience difficulties in reproducing, defined as a failure to conceive after two years of unprotected sexual intercourse. The molecular and genetic factors underlying the cause of infertility remain largely undiscovered. ITPA is an inosine triphosphatase (ITPase), which is reported to hydrolyze (d) ITP and XTP to the corresponding nucleoside monophosphates. It is documented that ITPA deficiency could lead to miscarriage in mice, so it seems that ITPA deficiency may be a candidate of infertility in human.

Materials and Methods: The human genomic DNA of all patients was extracted from white blood cells using salting out method. In order to determine the single nucleotide polymorphism (SNP) of ITPA (rs1127354), genotyping was performed by RFLP-PCR.

Results: This study showed that the two SNPs of rs1127354 are in Hardy-Weinberg equilibrium. This study explored the association between inosine triphosphatase (ITPA) functional variants; SNPs rs1127354 (missense variant in exon 2) and the development of infertility, and explored the relationship between ITPA variants and therapeutic response.

Conclusion: It seems that rs1127354/rs7270101 variants could be a genetic determinant for decreased fertility.

Keywords: ITPA, SNP, Infertility

P-242: Sex Determination in ICSI Oocytes with Two Polar Bodies Using PGD and FISH, A Case Report

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Background: Morphology and presence of Polar body in the oocyte are denoting oocyte quality and is used as a criterion for prognosis of embryo quality. However, little is known about ICSI result of oocytes with two polar bodies. The present study reports the result of ICSI in a case having several oocytes with two polar bodies.

Materials and Methods: A 34 years old patient (K.H.) with history of familial marriage admitted to Mother's Clinic in Tabriz-Iran for preimplantation sex determination. A total of 12 oocytes was collected after superovulation protocol. From the collected oocytes 7 were at MII stage and 5 had 2 polar bodies. All oocytes including those with 2 polar bodies were underwent ICSI procedure, but were kept separate from MII oocytes. Biopsy procedure was performed on day 3 and FISH method, based on single cell model, was used for sex determination.

Results: The results revealed that all oocytes, in both groups, were fertilized and developed to embryos. The embryos had almost similar morphology in both groups and appeared normal. However, the result of FISH signals for biopsied blastomere showed that in MII group, all embryos were XX/XY but one was XO. However, in 2 polar body group one embryo was XO and the other four were NS.

Conclusion: It is concluded that the embryos obtained from oocytes with two polar bodies should not be transferred.

Keywords: Sex Determination, ICSI, FISH, PGD, Polar Body

P-243: Prenatal Diagnosis Using Array CGH: Case Presentation

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Background: Karyotype analysis has been the standard and reliable procedure for prenatal cytogenetic diagnosis since the 1970s. However, the major limitation remains requirement for cell culture, resulting in a delay of as much as 14 days to get the test results. CGH array technology has proven to be useful in detecting causative genomic imbalances or genetic mutations in as many as 15% of children with a normal conventional karyotype. We describe the use of array comparative genomic hybridization (aCGH) for prenatal diagnosis and the ability to detect abnormalities not detected by karyotype.

Materials and Methods: A 26-year-old pregnant woman referred for prenatal diagnosis and genetic counseling at 27 weeks gestation because of an ultrasound detected fetal oligohydramnios and normal karyotype. She was offered array CGH analysis using a cytochip ISCA 4x44K oligonucleotide array. Parental samples were obtained concurrently to exclude maternal cell contamination and determine if copy number variations (CNVs) were de novo or inherited.

Results: Whole-genome CGH array analysis on the DNA extracted from amniocytes detected a 17Mb deletion at 1q22-q25.1 or arr 1q22q25.1(154559773-171639287)x1. This deleted region encompassing at least 138 OMIM genes. After genetic counseling, the mother decided to continue the pregnancy. A female fetus was delivered at 37 weeks gestation by cesarean. She was diagnosed with bilateral cleft lip and palate, a transverse single palmar crease in right hand, a birth weight of 1800g, small hands and feet, hypotonia, short neck, normal head circumference, and a hemangioma on the back of the head.

Conclusion: aCGH analysis of a fetus with normal karyotype and abnormal ultrasound finding is recommended.

Keywords: Array CGH, 1q22-q25.1, Cleft Lip/palat

P-244: Analysis of Genomic and Cell Free DNA of A let-7 microRNA Binding Site of KRAS Gene Polymorphisms in Endometriosis

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Background: Endometriosis is one of the most common benign gynecological diseases which is characterized by endometrial-like tissue growing outside the uterine cavity. Although the pathology of endometriosis remains unknown, the genetic predisposition plays an apparent role. Several genes have been contributed to endometriosis, but it seems KRAS has a crucial role, because its activation

results in de novo endometriosis in mice. We screened women with endometriosis for a polymorphism in a let-7 microRNA binding site of KRAS, in genomic DNA and cell free DNA, which are associated with the risk of endometriosis in Iranian people

Materials and Methods: We investigated 58 patients with histopathologically or laparotomy confirmed endometriosis and 60 control group women who were surgically proven to have no endometriosis in Firoozgar hospital. Participants were interviewed in a structured questionnaire that included questions about demographics, weight and height, menstrual history, pregnancy history, contraceptive methods and family history of endometriosis. Genomic DNA and Cell free DNA (CFD) of cell blood and plasma of each patient were extracted separately. Identification of let-7 microRNA family complementary site 6 (LCS6) in 3'UTR of KRAS polymorphic variant (rs61764370) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The 699 bp PCR product was digested with the endonuclease Pfl (Tfil). Results were confirmed by sequencing analysis.

Results: 1 (1.72%) patient with endometriosis and 4 (6.66%) subjects without endometriosis carried minor allele at KRAS LCS6 SNP. The allele frequency of this SNP in CFD was 0 and 4.76% in case and control group respectively.

Conclusion: Although researchers in Yale university (2012) reported high frequency (31%) of KRAS LCS6 SNP as a marker for endometriosis risk, our results suggest that this SNP, in genomic DNA and CFD, have no statistically significant association with endometriosis in Iranian population and this is not a useful marker of endometriosis risk in our population.

Keywords: Endometriosis, KRAS, MiRNA Binding Site SNP, Cell Free DNA, Iranian Women

P-245: Vitamin D Receptor FOKI and BSMI Polymorphisms in Women with Recurrent Abortions in Iranian Population

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Background: Recurrent spontaneous abortion (RSA) is a frequent reproductive problem, with three or more abortions affecting 1 to 2%, two or more abortions affecting up to 5% of women of reproductive age. Some polymorphisms are supposed to be etiology in this group. Vitamin D receptor (VDR) gene polymorphism seems to be a good ones to be searched due to their function on placenta.

Materials and Methods: 150 women with idiopathic RSA who had history of two or more successive spontaneous abortions were suffering from recurrent abortion who referred to Avicenna Infertility clinic and 100 healthy controls with at least two live children. Blood samples were used to extract DNA, by using the salting out method from peripheral blood samples and then PCR-RFLP designed for BSM1 and FOKI VDR gene. The results were analyzed using Chi Square Test while a p<0.05 was considered to be significant.

Results: The frequencies of (VDR) BSMI BB, Bb and bb genotypes were 18.6% (47), 60.9% (154), and 20.6% (52), respectively, in the sample. The frequencies of BB, Bb, and bb genotypes were 13.0% (13), 64.0% (64), and 23.2% (23), respectively, in control group and 22.2% (34), 58.8% (90), and 19% (29), respectively, in RSA patients. The proportion of BSMI demonstrated no significant

differences between RSA patients and healthy subjects (p=0.171). In contrast, while FF homozygote was the largest group in FOKI genotype, FF normal, Ff heterozygote and ff homozygote are (50) 60.2% and (23) 60.3%, (7) 8.4% respectively in control group and (84) 54%, (50) 32.5%, (20) 13% respectively in RSA patients. The proportion of FOKI demonstrated no significant difference between RSA patients and healthy subjects (p=0.278).

Conclusion: In the present study, there was no significant difference between case and control group in an Iranian population of reproductive age women.

Keywords: RSA, Polymorphism, VDR

P-246: Gene Variation of TLR4 in Patients with Endometriosis

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Background: Endometriosis, defined as the presence of endometrial tissue outside of the uterus, is an estrogen-dependent chronic inflammatory condition associated with degrees of pelvic pain and infertility. Toll-like receptors play a key role in immune response, by regulating inflammatory reactions and activating adaptive immune response to eliminate infectious pathogens and cancer debris. Polymorphisms in TLR4 have been shown to be associated with increased susceptibility to diseases such as inflammation and cancer. Ectodomain of TLR4 protein consists of 21 leucine-rich repeats (LRRs) that are crucial for its dimerization and signaling. The aim of this study was to determine gene variations of TLR4 in patients with endometriosis.

Materials and Methods: In this case-control study 65 blood samples were recruited from endometriosis patients referring to Royan Institute during 2012-13, who have been confirmed by laparoscopic surgery and 45 fertile women as control group that had no history of inflammatory disorders or using any related drugs. Ethical approval forms were obtained prior to the samples collection. DNA was extracted by kit, special primers were designed, the LRR coding region was amplified by PCR and products were analyzed by sequencing.

Results: One out of 65 patients had a heterozygote single nucleotide polymorphism (SNP) in (rs4986790) Asp299Gly.

Conclusion: According to our results we suggest that AS-P299GLY polymorphism be involved in pathogenesis of endometriosis although more studies are required in this regard.

Keywords: Endometriosis, Gene Variation, Polymorphism, TLR4

Reproductive Imaging

P-247: To Evaluate The Oxytocin Effect on Maternal-Fetal Circulation during Termination of Pregnancy

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Background: To evaluate the effect of oxytocin on blood circulation of fetus and uterus by Doppler ultrasound.

Materials and Methods: Forty six pregnant women were assigned to receive for indication of delivery intravenous oxytocin. Doppler velocitometry before and after administration of oxytocin for umbilical, middle cerebral artery and uterine artery were performed, and Pulsatility index (PI), resistance index (RI) and systolic /diastolic ratio (S/D) were measured for these arteries.

Results: There was no difference between PI, RI, and S/D of umbilical, uterine and middle cerebral arteries before and after oxytocin therapy $p > 0.05$.

Conclusion: During induction of labor infusion of Oxytocin do not have any effect on uterine -fetus main arteries circulation

Keywords: Oxytocin, Doppler, Middle Cerebral Artery, Uterine Artery, Umbilical Artery

P-248: Uterine Synechiae on Hysterosalpingography (HSG)

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Background: This study showed the patterns and grading of Asherman's syndrome on hysterosalpingography (HSG). Asherman's syndrome (AS) which is termed as uterine synechiae, and intrauterine adhesions (IUA), result in trauma to the basal layer of the endometrium with subsequent scarring. This may be from previous pregnancy, dilation and curettage, surgery, or infection such as genital tuberculosis. The radiographic appearance of intrauterine adhesions varies with the sites involved and the severity of scars. Intra-uterine adhesions are typically seen on hysterosalpingogram as multiple irregular linear filling defect (that may give an irregular, multiple angulated lacunar pattern) synechiae.

Materials and Methods: The infertile women who visited Royan Institute and referred to HSG consecutively, prior to hysteroscopy, from 2011 to 2014 included in this cross-sectional study. Classification systems for Asherman's syndrome (AS) were done according to the American Society of Reproductive Medicine (ASRM). The mild synechiae was suspected when up to of endometrial cavity involved and moderate (involvement of 1/2), and severe (involvement of 3/4 or more).

Results: Uterine synechiae was found in 35 out of 3200 patients, 0.01 % of women undergoing hysterosalpingography HSG during a 3-year period. All cases of synechiae are confirmed by hysteroscopy. 16 out of 35 patients (45%) had diagnosed with mild synechia. 8/35 (22%) and 11/35 (31%) had diagnosed with moderate and severe synechiae, respectively.

Conclusion: HSG is commonly used as a first-line tool in the diagnosis of intrauterine adhesions because it is simple, safe, cost effective, sensitive, and minimally invasive procedure, allowing the visualization of the uterine cavity, revealing the extent of the scar formation, while suggesting the presence of intrauterine adhesions.

Keywords: Hysterosalpingography, Uterine Synechiae

P-249: Concordance Rate of Hystrosalpingography and Laparoscopy in Diagnosis of Tubo-Pritoneal Pathology in Infertile Women

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Background: Infertility is defined as 1 year of unprotected intercourse without pregnancy. The main causes of infertility are as follows: male factor, Both male and female, as well as unexplained etiologies. Tubal and peritoneal factors account for 30%-40% of causes of female infertility. Tubal factors include damage and obstruction of the fallopian tube, usually associated with previous pelvic inflammatory disease (PID) or previous pelvic or tubal surgery. Peritoneal factors include peritoneal adhesions which generally results from PID or surgery and endometriosis. Hystrosalpingography (HSG) is the initial test of tubal patency and the gold standard for diagnosis of tubal and peritoneal disease is laparoscopy. Concordance of H.S.G with laparoscopy is 2/3 in a lot of references and concordance of two procedures was reported poor in this center in 1998. To find the better results, this study is conducted on more cases.

Materials and Methods: In this study, the records of H.S.G and laparoscopy of 200 infertile patients was assessed from 2011 to 2012, then sensitivity, specificity, positive and negative predictive value and concordance of two procedures were calculated.

Results: In this study, sensitivity and specificity of HSG in tubal obstruction are 60, 81% respectively, and positive - and negative predictive value are 43, 89% respectively and concordance with laparoscopy is 36%. Sensitivity and specificity of HSG in hydrosalpinx are 23, 95% respectively, and positive and negative predictive values are 61, 80% respectively. Concordance with laparoscopy is 23%. Sensitivity and specificity of HSG in peritoneal adhesions, endometriosis and delayed spillage are 37, 79% respectively. Positive and negative predictive value are 51, 67% respectively. Concordance with laparoscopy is 17%.

Conclusion: With evaluation of the results, it seems that H.S.G is not the accurate procedure for diagnosis of tubo-peritoneal pathology, but because the laparoscopy is an invasive procedure, a more accurate study is recommended for better results.

Keywords: Hystrosalpingography, Laparoscopy, Peritoneal, Pathology, Infertility

P-250: A Case Report of Postabortal Hematometra

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Objective: Hematometra is a rare and delayed complication of medical termination of pregnancy. Acquired acute hematometra also termed the postabortal syndrome or the redo syndrome is a rare complication of suction evacuation with incidence ranging from 0.1 to 1 per 100 suction curettage abortions. The treatment consists of prompt evacuation of both liquid and clotted blood leading to rapid resolution. An oxytocic is administered after the repeat evacuation.

Materials and Methods: A 31 year old woman, G2L1A1 was admitted on 19th April, 2014 in emergency with the complaint of acute pain in abdomen associated with amenorrhoea of 22 weeks, nausea, infrequent spotting, and frequent urge for defecation and micturation. She gave history of colicky pain in the lower abdomen since her induced abortion on 23rd January, 2014. Her symptoms aggravated since the last 2 days. Her BHCG test was negative on admission. On examination she was hemodynamically stable but was pale. Her pulse was 100/minute and regular, blood pressure 100/70 mm Hg, and temperature 37.40 c. Respiratory and cardiovascular systems were normal. Abdominal examination revealed tenderness in the lower abdomen without any palpable lump. Vaginal and speculum examinations revealed healthy vulva, vagina, and cervix with very scanty dark blood stained discharge. The uterus was tender, retroverted, 7 to 8 weeks size with closed cervical os, and with tender right adnex. On interrogation, she gave history of suction curettage at 9 weeks gestation for IUFD on 23rd January, 2014 after which she had no menstruation. Her previous menstrual cycles were of 30 days with 4 days bleeding. She had a full term vaginal delivery 6 years ago. Investigations showed hemoglobin 10.5g/dL, WBC count 8900/mm³, differential count – P72 L28, blood group B+, nonreactive HIV and HbsAg, BHCG negative.

Results: Conservative treatment was started with intravenous fluids and antibiotics. Her vital signs remained stable but colicky pain continued which needed injectable analgesic at frequent intervals. Vaginal sonography showed thin endometrium and intra cavity fluid 15 mm with right adnexal cystic mass 2.5 cm and free fluid 7-8 mm. On 20th April, 2014 hematometra was drained by intra cervical canal catheter then she received misoprostol 200 µgq4h for 24 hrs. She was discharged on 5th April, 2014 with metronidazole and doxycycline. Until follow up pain has not any pain or symptom.

Conclusion: This case showed that postabortal hematometra can be treated by intra-cervical catheter drainage.

Keywords: Postabortal Hematometra, Suction Curettage, Intracervical Catheter

P-251: Effects of Indomethacin on Post-natal Development of Mouse Testes

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Objective: Indomethacin is a non-selective cyclooxygenase (COX) inhibitor that is commonly administered to extremely low gestational age neonates for symptomatic patent ductus arteriosus. It suppresses prostaglandins which modulate growth and secretion of various hormones. We examined the hypothesis that early postnatal administration of Indomethacin may influence growth and development of testes in neonatal mice.

Materials and Methods: The mice were acclimatized for seven days prior to coupling and were housed in an air conditioned animal house at 22 ± 2°C with exposure to 10–12 hours of daylight. Day zero of pregnancy was determined by vaginal plug test. In this study, mice pups received intraperitoneally injection of 25, 50 and 100 mg/kg Indomethacin which was dissolved in 0.09% NaCl on the day of birth. After pup's delivery, male pups were sacrificed on day 35 and testes were removed, fixed in 10% neutral buffered formalin and embedded in paraffin wax. Serial sections (5µm) of the medullary area were obtained in each group and stained with hematoxylin-eosin. The sections were observed under light microscopy. For all experiments, at least 6 to 8 replicates were performed. The data

were analyzed statistically using analysis of variance (ANOVA). A level of (p<0.05) was accepted as significant.

Results: Our results indicated that intraperitoneally injection of 25, 50 and 100mg/kg Indomethacin decreased the number of spermatogonia, spermatocyte and sperm in seminiferous tubule. The number of seminiferous tubules decreased in comparison to control (p<0.05).

Conclusion: These results provide evidence for the involvement of prostaglandins in the regulation of development and suppressing the growth of testis in the sucking mice. Therefore, early postnatal exposure to Indomethacin as a prostaglandins inhibitor may further exacerbate postnatal growth restriction of testis and ability to cope with stress.

Keywords: Indomethacin, Neonate, Testes, Mouse

P-252: Protective Effects of Imedeen on Oxidative Stress Induction Spermatogenic Disorders and Fertility Potential of Cyclophosphamide-Treated Male NMRI Mice.

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Background: cyclophosphamide is anticancer agent that has been toxic effects on male reproductive system. The antioxidant agents in imedeen such as lycophence GS and Biomarine complex play important role in preventing the direct and indirect effects of free radicals caused by CP; therefore, the purpose of this study was to evaluate the effects of Imedeen on sperm characteristics of mature male mice that treated by CP.

Materials and Methods: In this experimental study, 60 mature male mice were assigned to six groups, 10 mice in each. The animals allocated to control group, CP treated in 12mg/kg/day, Imedeen treated in a first dosage 111 µg/kg/day, Imedeen treated in second dosage 222 µg/kg/day, CP treated and Imedeen treated in first dosage and the last group CP treated and Imedeen treated in second dosage. Sperm analysis (motion, count, morphology and viability) were evaluated at the end of experiment after 35 days. By sperm motion was assessed by computer – Assisted sperm Analysis (CASA). The data were analyzed using GB stat software. Probability values of p<0.05 and p<0.01 were considered significant.

Results: The results obtained from the caudal epididymal sperm analysis revealed that treated with CP caused significant decrease in sperm count, 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 caudal epididymal spermatozoa as evidenced by Acridine Orange (AO) and Aniline Blue staining respectively. Notable administration Imedeen caused a considerable recovery in above-mentioned parameters (p<0.05).

The results suggest that Imedeen as an antioxidant could diminish the adverse effects of cyclophosphamide in the reproductive system of male mice during cyclophosphamide administration.

Keywords: Cyclophosphamide, Caudal Epididymal Spermatozoa, Imedeen, DNA Damage, Mouse

P-253: Assessment of Some Minerals In Seminal Fluid of Asthenospermic Patients

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Background: The aim of present study was to estimate the levels of some minerals (Zn, Se, Ca, Mg, Na and K). Also relationships between mentioned components and some sperm parameters and the infertility period were studied as well.

Materials and Methods: This study was performed on human semen specimens obtained from Asthenospermic patients (101 specimens) and Normospermic males (61 specimens) who were attending to the laboratories of Fertility center in ALSader Hospital of ALNajaf ALAshraf city, during the period extended from December 2009 to September 2010.

Results: The results revealed significant decrease ($P < 0.05$) in the concentrations Zn, Se and Ca. A significant increase ($P < 0.05$) of Na while insignificant difference of Mg and K values were found in asthenospermic specimens compared with those of Normospermic specimens. Correlation study showed negative relationship between infertility period and sperm motility percent, normal sperm morphology percent and Grad activity A, and positive relationship with Grade activity D.

Conclusion: The results showed negative relationship between the abnormal sperm morphology percent and concentration of Se, Na and Ca in seminal plasma. Sperm motility percent correlated positively with Zn and Ca concentrations. The current study concluded that the change in the concentration of some of the elements lead to change parameters and sperm quality in terms of movement.

Keywords: Asthenospermia, Normospermia, Minerals

P-254: The Effects of Zinc in Vitrification Medium on In vitro Maturation of Oocytes Derived from Vitrified-Warmed Mouse Ovary

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Background: Cryopreservation of ovarian tissue is an important option for preserving the fertility of cancer patients undergoing chemotherapy and radiotherapy. Researchers have used different cryoprotectants and various techniques to improve the cryopreservation of ovaries. However, despite significant recent progress, the efficiency of ovary cryopreservation is still low. Zinc is essential for many biological processes, including proper functioning of gametes. Researches describe major changes in the zinc physiology of the mammalian oocyte as it matures and initiates embryonic development. In this study, the effects of zinc in vitrification medium on in vitro maturation of oocytes derived from vitrified-warmed mouse ovary were examined.

Materials and Methods: In this experimental study, the ovaries of 2-4 week-old NMRI mice randomly assigned to following groups: V0 (vitrified-warmed ovaries without any zinc in vitrification solution), V1, V2, V3 (vitrified warmed ovaries with 100, 150 and 200 µg/dl zinc concentration in vitrification solution, N-v (none vitrified ovaries). Ovaries in the vitrified groups were frozen sequentially by immersion into two vitrification solutions VS1: 7.5% ethylene glycol

(EG) + 7.5% DMSO in holding medium (α -MEM + 20% FBS) for 7 minutes and VS2: 15% EG + 15% DMSO for 3 minutes in holding medium and vitrified by straw and were kept in LN2 tank for a week. After one week, the ovaries were thawed at temperature room in 1.0, 0.5 and 0.25 M sucrose, vitrified ovaries as well as non-vitrified ovaries were serially sectioned and examined histologically. Immature oocytes were isolated mechanically from ovaries, they were put in maturation medium and evaluated for in vitro maturation (IVM).

Results: The results show that the presence of zinc in vitrification solution is effective and can reduce the traumatic effects of vitrification on ovarian tissues. In addition viability of oocyte and oocyte maturation rate was higher in the presence of 200 µM zinc concentration in comparison to other vitrified-warmed groups, nevertheless it was lower than non vitrified group (ANOVA, p -value < 0.05).

Conclusion: Zinc supplementation of vitrification medium has a positive effect on the in vitro maturation of oocyte.

Keywords: Vitrification, Zinc, Oocyte Maturation, Ovary, Mouse

P-255: The Effects of Vanadium in Vitrification Medium on in vitro Maturation of Oocytes Derived from Vitrified-Warmed Mouse Ovary

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Background: Since the freezing method was offered, much effort has been done to improve the freezing conditions in different studies and the impact of different freezing methods and mediums on oocyte, embryo and ovarian tissues has been examined. Based on studies, minerals play an important role in oocyte maturation and subsequent embryo development. Vanadium is one of the minerals, which is very important as a trace element for normal cell function as well as development. It has been proven that vanadium can act as an insulin mimicking factor. In this study, the effects of vanadium in vitrification medium on in vitro maturation of oocytes derived from vitrified-warmed mouse ovary were examined.

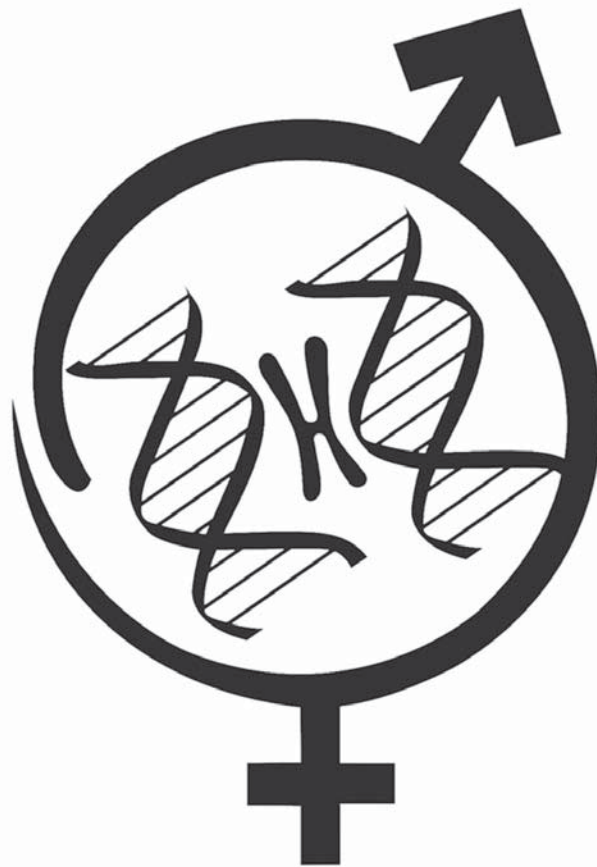
Materials and Methods: In this study, the ovaries of 2-4 week-old NMRI mice randomly assigned to non vitrified and vitrified-warmed with different concentrations (0, 10, 100 and 250 µM) of ammonium metavanadate groups. Ovaries in the vitrified groups were frozen sequentially by immersion into two vitrification solution, equilibration solution: 7.5% EG, 7.5% DMSO in holding medium (α -MEM+10%FBS) for 7 minutes and vitrification solution: 15% EG, 15% DMSO, 0.5 M sucrose in holding medium (α -MEM+10%FBS) with different concentration of ammonium metavanadate for 3 minutes in room temperature. The ovaries transported to the straw and were kept in a nitrogen tank for a week. After this period, ovaries were transferred to thawing medium with falling concentration of sucrose (1, 0.5, 0.25) at 37°C. Oocytes derived from ovaries were put in maturation medium for 24h.

Results: The results show that the presence of ammonium metavanadate in vitrification solution is effective and can reduce the traumatic effects of vitrification on oocyte in vitro maturation. In addition, viability of oocyte and oocyte maturation was higher in the presence of 100 µM ammonium metavanadate compared to the other vitrified-warmed groups, nevertheless it was lower than non- vitrified group (ANOVA, p -value < 0.05).

Conclusion: Vanadium supplementation of vitrification medium has a positive effect on the in vitro maturation of oocyte.

Keywords: Vitrification, Ammonium Metavanadate, Oocyte Maturation, Mouse

Abstracts of
9th Royan Nursing and Midwifery Seminar
3-5 September 2014



Royan Institute

Tehran, Islamic Republic of Iran

Invited Speakers

I_{nm}-1: Blastocyst Culture: Advantages and Disadvantages

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Both cleavage-stage and blastocyst-stage embryo transfer policies have advantages and drawbacks. Potential advantages of blastocyst culture and transfer in human IVF include: i. synchronization of the embryo with the female tract leading to increased implantation rates; thereby, reducing the need for multiple embryo transfers, ii. assessment of viability of an embryo before transfer. This can be achieved by both the identification of those embryos with little developmental potential, as manifest by slow development or degeneration in culture, and by the introduction of non-invasive tests of developmental potential to select the most viable embryos from within a cohort for transfer. Furthermore, culture of the human embryo beyond the 4-8-cell stage, the time at which the genome is activated, will facilitate the quantification of true embryonic markers as opposed to those inherited from the oocyte, i.e. after the 8-cell stage one is assessing embryo physiology, while prior to this the physiology of the cleavage stage embryo reflects that of the oocyte, iii. an increase in the time available between cleavage stage embryo biopsy and embryo transfer. This is of particular importance where the biopsied material has to be sent to a separate locale for analysis, and iv. facilitation of the introduction of trophectoderm biopsy for the screening of genetic diseases. Trophectoderm biopsy represents the earliest form of genetic diagnosis of non-embryonic material.

I_{nm}-2: Evaluation of Informing Patients of The Royan Institute

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Background: To ensure appropriate acknowledgment consistent with patient's diagnosis and treatment is essential for safety and quality of service. Although it is an overwhelming process but it obviously has an obligation to promote due to the valuable outcomes to empower patients to make decisions after obtaining the necessary information.

Materials and Methods: This study was a descriptive study based on information which was collected with questionnaires. Study population was infertile patients who were referred for treatment for the first time. Questionnaire designed by faculty members of Obstetrics and epidemiological groups and its validity and reliability have been tested. Data were analyzed using SPSS software with the use of appropriate statistical indicators.

Results: In terms of cause of infertility, 67 (33.5%) studied patients were male factor, 31 (15.5%) female factor, 54 (27%) male and female factor and 36 patients (18%) unexplained. Average Patients comments in terms of satisfaction on the quality and quantity of informing, in wom-

en and men were respectively as follows: about cause of infertility in 3.5 and 4.2, about the proposed treatment 3.5 and 4.2 and about Diagnostic methods 3.5 and 1.8; if the lowest satisfaction Scoring 1 and the most satisfaction scoring 5. Meanwhile, the greatest effect was from physician and the least effect was from reception.

Conclusion: Clinical knowledge is the basis of patient's interaction with treatment and all patients have the right to receive proper training about maintaining and improving health and prevent disease.

Keywords: Informing, Infertility, Patient

I_{nm}-3: Infertile Couples Attitude

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I_{nm}-4: The Effect of Midwives Training in Stress Reduction of Infertile Couples

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Background: A study to assess the effectiveness of patient education program focused on improving knowledge in reducing stress among primary infertile couples undergoing ART in Royan Institute.

Materials and Methods: In total, 50 primary infertile Couples fulfilling the inclusion criteria undergoing ART, 25 each in experimental and control groups completed the intervention. A well educated and experienced Midwife conducted educational courses offered. The effectiveness regarding infertility-related stress was assessed by questionnaire immediately before and after the intervention in experimental group; whereas control group received no training.

Results: It seems that there are no differences in infertility-related stress at base line between the two groups studied. Women and men changed occurrence, frequency and content of communication with clinical team. The intervention resulted in important perceived improvement in the participants' competence to actively manage changes in their process.

Conclusion: The performance of new reproductive technologies will make education and training necessary before treatment initiation.

Keywords: Assisted Reproduction, Infertility, Patient Education, Stress

I_{nm}-5: Comparison in Prenatal Care for Infertile Patients and Normal Pregnancy

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Antenatal care seems to be a significant contributor to low birth weight (LBW) babies, preterm birth, obstetric complications, and neonatal mortality. Widespread apply of prenatal care is often re-

lated to high-risk pregnancies; though the effects of prenatal care on unpleasant pregnancy outcomes in such pregnancies have not been widely discovered.

Suitable prenatal screening and counseling are of vital significance for pregnant women with a history of infertility; these women are mostly older than average pregnant women, have a greater number of chronic situations, and are at risk for spontaneous abortion and chromosomal abnormalities. Studies indicated that even after adjusting for age and parity, sub fertile women are at higher risk of preeclampsia, placenta previa, and placental abruption, and are more probable to go through induction of labor, have a cesarean section (CS), and deliver LBW and preterm infants. Researches demonstrated that women with singleton births who used various treatment modalities for infertility, including assisted reproductive technologies (ARTs), have worse pregnancy outcomes despite the sufficient prenatal care, compared to the general population. However, impacts of prenatal care on adverse birth and obstetric outcomes in infertile women have seldom been investigated.

I_{nm}-6: Molecular Genetic Basis of Infertility

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Background: Sexual reproduction affords the stands for conserving genetic characteristics and sequentially, genetic inconsistency may influence the capability to imitate.

Materials and Methods: Research was conducted by subject in PubMed and other databases.

Results: A significant number of genotypes have been related with infertility phenotypes and evaluation of precise genes in humans and model systems illuminate the essence of the polygenic and multi-factorial basis of infertility. Single-gene defects are almost certainly to be found among hypogonadotropic hypogonadism patients caused by defects in the KAL genes or the gonadotrophin-releasing hormone receptor genes. An increased risk of having a premutation of the Fragile X syndrome gene is observed in premature ovarian failure. Complex genetic inheritance may elucidate the variable familial associations 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. Genetic factors implicated in male infertility evident as chromosomal disorders, mitochondrial DNA (mtDNA) mutations, monogenic disorders, multifactorial disorders and endocrine disorders of genetic origin. Genetic disorders like Klinefelter syndrome and Y chromosome microdeletions are known genetic causes of male infertility

Conclusion: Accepting the methods used for genetic diagnosis is becoming a standard obligation for the clinical follow of reproductive medicine.

I_{nm}-7: Hypothyroidism and Pregnancy

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A normal pregnancy results in a number of important physiological and hormonal changes that alter thyroid function. These changes mean that laboratory tests of thyroid function must be interpreted with caution during pregnancy. Levels of thyroxine binding globulin (TBG) and production of T3 and T4 hormones and the daily requirement of iodine in pregnancy are increased to 50%, TSH receptor stimulated by HCG and TSH levels reduced. The thyroid gland can increase in size during pregnancy especially in iodine-deficient areas. This is usually only a 10-15% increase in size and is not typically apparent on physical examination by the physician. In fact, pregnancy is a stressful situation for the thyroid gland that is the cause of hypothyroidism in women with limited reserve of thyroid hormone (due to autoimmune destruction or iodine deficiency).

Hypothyroidism is a common disorder of the thyroid in pregnancy. The most common cause is chronic autoimmune thyroiditis (Hashimoto's thyroiditis), but in iodine deficient areas, deficiency of this element can be associated with goiter or hypothyroidism.

For the first 10-12 weeks of pregnancy, the fetus is completely dependent on the mother for the production of thyroid hormone. By the end of the first trimester, the fetus thyroid begins to produce thyroid hormone on its own. However, remains dependent on the mother for ingestion of adequate amounts of iodine, which is essential to make the thyroid hormones. A small amount of thyroid hormones reaches from the mother to the fetus is essential for brain development. Untreated maternal hypothyroidism can lead to reduction in IQ and mental retardation in the fetus. In addition to abortion, preterm labor, preeclampsia, pregnancy-induced hypertension are complications of hypothyroidism in pregnancy. These complications can be prevented by timely and appropriate treatment of hypothyroidism during pregnancy.

It is important to note that levothyroxine (LT4) requirements frequently increase during pregnancy, often times by 25 to 30 percent. Occasionally, the LT4 dose may double. Ideally, hypothyroid women should have their LT4 dose optimized prior to becoming pregnant. Women with known hypothyroidism should have their thyroid function tested as soon as pregnancy is detected and their dose adjusted by their physician as needed to maintain a TSH in the normal range. Thyroid function tests should be checked approximately every 6-8 weeks during pregnancy to ensure that the woman has normal thyroid function throughout pregnancy. It is also important to recognize that prenatal vitamins contain iron and calcium that can impair the absorption of thyroid hormone from the gastrointestinal tract. Consequently, LT4 and prenatal vitamins should not be taken at the same time and should be separated by at least 3-4 hours. As soon as delivery of the child occurs, the woman may go back to her usual pre-pregnancy dose of LT4 and 6-8 weeks postpartum, thyroid function tests should be rechecked.

I_{nm}-8: The Effectiveness of OHSS Patient's Management in Reducing Complications and Symptoms

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Background: The present research investigated the clinical

efficacy of active outpatient management on procedural outcomes and time to resolution of ovarian hyperstimulation syndrome (OHSS) symptoms in women at increased risk, following ART programs.

Materials and Methods: Retrospective analysis of the 50 patients underwent ART and developed OHSS requiring management. Patients were managed on outpatient basis by frequent office visits, daily phone calls, and received IV normal saline aimed at achieving fluid balance, restoring plasma volume and improving renal function when required as well as an early resort to ascitic fluid aspiration, which will improve the feeling of wellbeing.

Results: It seems that Active outpatient intervention in the early stages of OHSS, including modifying the dose of gonadotrophins, coasting, use of an agonist trigger, cycle cancellation, cryopreservation of all embryos and albumin administration, can avoid hospitalization while minimizing the progression and complications of OHSS.

Conclusion: Patients should be counseled that the condition is self-limiting. An effective strategy is required to prevent the episode of OHSS without necessitating the cancellation of the cycle or cooperate success rates along with preventing the need for inpatient hospitalization.

Keywords: Complication, OHSS, ART, Patient Management

I_{am}-9: Global Approach toward Banking Cord Blood Stem Cells

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Umbilical cord blood is the baby's blood left over in the umbilical cord and placenta after a baby is delivered and the cord is cut. Cord blood has all of the features of regular blood, but it is also a rich source of hematopoietic stem cells. Hematopoietic stem cells are unspecialized cells that produce all blood cells. These include:

- Platelets, which are needed for blood clotting
- Red blood cells, which transport oxygen to the cells
- White blood cells, which help fight diseases

These stem cells are similar to those found in bone marrow. In addition, cord blood is immunologically tolerant and can be transplanted without full HLA matching, increasing access to transplantation for patients lacking matched adult donors.

The full potential of cord blood is currently unknown. Cord blood contains rare stem and progenitor cells for tissues that are different from the blood. Researchers are investigating the possibility that cord blood cells could be used to repair damaged tissues including those in the heart, brain and pancreas.

In recent years, umbilical cord blood, which contains a rich source of hematopoietic stem and progenitor cells, has been used successfully as an alternative allogeneic donor source to treat a variety of pediatric, genetic, hematologic, immunologic, and oncologic disorders. Because there is diminished risk of graft-versus-host disease after transplantation of cord stem cells using matched related donors, the use of less-than-completely matched HLA cord blood stem cells may incur less risk of graft-versus-host disease than mismatched cells from either a related or unrelated "walking" donor, although this remains to be proven. Gene-therapy research involving modification of autologous cord blood stem cells for the treatment of childhood genetic disorders, although experimental at the present time, may prove to be of value. These scientific advances have resulted in the establishment of not-for-profit and for-profit cord blood-banking programs for allogeneic and autologous cord blood transplantation.

Many issues confront institutions that wish to establish or participate in such programs. Parents often seek information from their physicians about this new biotechnology option. This document is intended to provide information to guide physicians in responding to parents' questions about cord blood donation, banking, the types as well as quality of cord blood banks. Provided also are recommendations about appropriate ethical and operational standards, including informed consent policies, financial disclosures, and conflict-of-interest policies for physicians, institutions, and organizations that operate or have a relationship with cord blood-banking programs

Today, expectant parents may choose to have the blood remaining in their baby's umbilical cord and placenta (cord blood) collected and stored with either a Public Cord Blood Bank or a Private Cord Blood Bank, to donate it for research, or simply have it discarded as medical waste.

there are hundreds of cord blood banks, serving both the public and family clients, around the world. Since the first cord blood transplant 25 years ago, more than 30,000 have been performed worldwide. The biggest cord blood banks have hundreds of thousands of units in inventory, and many banks rely on automated equipment to facilitate stem cell separation and storage.

The FACT-NetCord accreditation is end-to-end, covering every aspect of cord blood handling from the moment of collection at birth, through the laboratory where the cord blood is processed to store stem cells, and to end with patient care at hospitals where the cord blood is used for therapy.

FACT is the Foundation for the Accreditation of Cellular Therapy, formed by medical doctors who perform stem cell transplants. Since 2004 they have partnered with the International NetCord Foundation, a consortium of the world's leading public cord blood banks.

Oral Presentations

O_{nm}-1: Self-Confidence in Women with and without Polycystic Ovary Syndrome

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Background: Polycystic Ovary Syndrome (PCOS) as other chronic diseases causes dependence and decreasing body control. It also causes unworthy unsafely, negative suggestions and decreasing of Self-confidence. The aim of this comparative study was comparing Self-confidence in woman with and without PCOS according to their ages.

Materials and Methods: This comparative study conducted between 100 women with and 300 women without PCOS in Tehran, 2013. For analyzing the data SPSS (version16) software was used.

Results: This study showed the most women in two groups (98 percent in PCOS and 93 percent in non-PCOS) had average self-confidence with score 15-25. None of women in PCOS group and 6.7 percent of non-PCOS women had high self-confidence (score>25). There was a significant difference between two groups in term of self-confidence level ($p<0.001$).

Conclusion: Impaired self-confidence among PCOS in comparison with non-PCOS women shows need to an urgent attention to psychological issues more than ever.

Keywords: Polycystic Ovary Syndrome, Self-Confidence, Self-Reliance

O_{nm}-2: Effect of Antioxidant Supplementation Like Ascorbic Acid, Vitamin E and β -Carotene Supplementation on Semen Quality and Biochemical Parameters

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Background: Semen quality may be impaired by environmental exposures, lifestyle or dietary factors. The aim of the present study was to determine the effects of dietary supplements intake of antioxidants like ascorbic acid (vitamin C), vitamin E and β -carotene, supplementation is associated with semen quality and sperm characteristics.

Materials and Methods: This paper is a case-control study. 20 men with poor semen quality (case subjects) and 20 normospermic control subjects of couples attending our fertility clinics. Dietary habits and nutrient consumption with using a food frequency questionnaire adapted recorded. The associations between intake of antioxidants in quartiles and the semen volume, sperm concentration, sperm motility, sperm morphology, total sperm count and total

motile sperm count were assessed using multivariate linear regression. Hormones levels were also analyzed in case and control subjects.

Results: In the multivariate adjusted linear regression models, there was a positive association between dietary intakes of vitamin E (Ptrend 0.05), vitamin C (Ptrend 0.04), and β -carotene (Ptrend 0.06) and total motile sperm count. Treatment with Vit C, Vit E, β -carotene and their combination significantly ($p<0.05$) increased libido (reaction time), ejaculate volume, sperm concentration, total sperm output, sperm motility index, total motile sperm, packed sperm volume, initial hydrogen ion concentration (pH), and semen initial fructose concentration. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were significantly ($p<0.05$) decreased, whereas glutathione S-transferase (GST) showed a significant increase in seminal plasma of treated men compared with the controls.

Conclusion: A high intake of antioxidants was associated with better semen quality but, there was no clear dose relationship in that moderate intake groups had the poorest semen quality. This study indicated that supplementation Vit C, Vit E, β -carotene and their combination reduced the production of free radicals and can improve semen quality, but the greater improvement seemed to be from Vit E.

Keywords: Male Infertility, Semen Quality, Antioxidants, Vitamin E, Ascorbic Acid

O_{nm}-3: The Impact of Sex Educational Program on Marital Satisfaction in Women with Sexual Dysfunction

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Background: Sexual function and relationship is the factors that interfere with marital satisfaction. This study is about the effect of sex education on marital satisfaction in women with sexual dysfunction who were referred to health centers in Qazvin city.

Materials and Methods: This is a randomized control trial study with an intervention group and a control group with 90 participant. The demographic questionnaire - sexual function index (FSFI), the Beck Depression and marital satisfaction Inventory completed through structured interviews, and after that the women who had sexual dysfunction and did not have moderate to severe depression were chosen and randomly assigned into two group. Sex education programs in this study were done in four sessions of 90 minutes once a week. The impact of training sessions were evaluated two months after the completion of marital satisfaction questionnaire and compared the results of the completed questionnaires before and after the intervention.

Results: Before the intervention, Two groups in terms of demographic characteristics and dimensions of sexual function and marital satisfaction were similar. Two months after intervention, significant differences were seen between two groups in terms of marital satisfaction.

Conclusion: These findings suggest that sex education affect the quality of sexual relationship and increase marital satisfaction.

Keywords: Sex Education, Marital Satisfaction, Female Sexual

Dysfunction

O_{nm}-4: A Simple Screening Approach for Assessing Prevalence and Clinical Characteristics of Polycystic Ovary Syndrome among Students of Babol Universities in Iran

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Background: Studies have revealed that polycystic ovary syndrome (PCOS) usually begins in adolescence, and it might manifest differently in different populations. In Iran, prevalence and phenotypes of PCOS among women in the community are unknown. The aim of the study was to estimate prevalence and clinical characteristics of Polycystic Ovary Syndrome in a community setting in Iran in 2013-2014.

Materials and Methods: Present work was a community-based cross-sectional study. A random sample of all students aged 18-33 years was selected by cluster sampling proportionate to population size. Detail menstrual history, marital status, and clinical manifestations of hyperandrogenism were recorded. PCOS be defined presence of least two criteria were considered diagnostic of PCOS, after exclusion of other etiologies.

Results: A total of 410 students, 33 (8.2%) previously diagnosed cases already present and 43 confirmed newly diagnosed cases of PCOS based on the 2003 Rotterdam diagnostic criteria, with total prevalence were 76 (19.0%). The most common clinical characteristics of PCOS were hirsutism 43.9, alopecia 40.8%, acne 39.5%, and oligo/amenorrhea 23.7%. Of 150 students with regular cycles in the absence of clinical hyperandrogenism 91.3 % were confirmed as normal. While 60.0 % women with irregular cycle and clinical hyperandrogenism were confirmed to have PCOS.

Conclusion: Hirsutism is a predisposing factor for PCOS. Over 40 % of women affected with PCOS have hirsutism.

Keywords: PCOS, Hirsutism, Screening, Alopecia

O_{nm}-5: Sonographic Assessment of Female Infertility: Instruction for Midwives and Nurses

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Background: The aim of this article was to describe an instruction for sonographic assessment of female infertility for midwives and nurses.

Materials and Methods: A narrative review was performed within articles published at "PubMed", "Elsevier", "SID" and original text books to reach the aim.

Results: Sonography is an accurate, non-invasive, and cost-effective

tool that provides information for detecting and characterizing possible factors of female infertility such as: endometrial polyp, polycystic ovary syndrome (PCOS), congenital uterine malformations, uterine fibroma, endometriosis, hydrosalpinx, etc. Furthermore, in case of assisted reproduction treatment cycles, ovarian and endometrial responses to hormonal therapy can be assessed by means of sonography. Therefore, every midwife and nurse working at infertility centers needs to learn about application of which. A key point is that pelvic organs are influenced by cyclic changes during menstrual cycle and hormone therapy. Thus, pathologic conditions of pelvis need to be well assessed depending on the day of the cycle. In this article, we introduced a proper guideline for planning the best time of sonographic evaluation and managing the patients based on sonography findings.

Conclusion: Sonography is known as the first imaging modality in the investigation of the female pelvis which helps midwives and obstetricians to evaluate infertile women and make better treatment choices. Therefore, every midwife needs to learn about the application of which and how to manage patients based on sonography reports.

Keywords: Female Infertility, Sonography, Menstrual Cycle, Assisted Reproduction Techniques

O_{nm}-6: Evaluate The Effectiveness of Training on Anxiety in Infertile Women

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Background: The problem of infertility as a public health problem of global dimensions of physical, mental and social. Since the causes of anxiety and depression in infertile women lack adequate knowledge about the causes of infertility and its treatment, so we decided to investigate the effect of training on reducing anxiety in infertile women.

Materials and Methods: This study was conducted as a quasi-experimental study and 74 infertile women were available for sampling. The samples were randomly divided into 2 groups: 37 patients were divided questionnaire. Anxiety was assessed using self-administered Spielberger's state-trait anxiety questionnaire. Using a paired t test, independent of the effect of face to face training on anxiety in infertile women were studied.

Results: The mean scores of anxiety in the experimental group before and after training was reduced, which was statistically significant (p value=000/0). Means of general anxiety in the group before training, 106.08 and two weeks after completion of the training program 87/81 was significantly decreased (p value=000/0) of the control group, the mean anxiety scores of the control group did not change before and after training.

Conclusion: Lack of awareness of the causes and treatment of infertility, infertility is involved in the development of depression and anxiety in individuals. Education infertile patients with emphasis on the cognitive, affective and behavioral attitudes towards infertility is suggested.

Keywords: Infertility, Anxiety, Training

O_{nm}-7: Association between Infertile Women's Adjustment and Coping Strategies with Assisted Reproductive Techniques Success Rates

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Background: Half of the infertile couples that use assisted reproductive techniques will not succeed. Psychological Adjustment and Coping Strategies are the most important factors influencing the stress of infertility treatment. This study aimed to determine the relationship between infertile women Adjustment and Coping Strategies with the success rate of ART.

Materials and Methods: This is a cohort study of 204 infertile women undergoing ART procedures. Before starting treatment were asked to fill Fertility Adjustment Scale and Infertility Coping Strategies. The medical conditions of the patients were followed until pregnancy outcome. Two positive pregnancy test results within 48 hours were considered as the success of ART. Statistical analysis was performed using SPSS v.18.0 and descriptive statistical tests, spearman correlation and logistic regression model are used.

Results: The mean Fertility Adjustment Scale (FAS) is 34.1 ± 8.3 with range of 55-15. Most coping strategies that participants can use to match their infertility problems are Belief in miracles (76%) Belief in God (72.5%), Hope (71.1%) and efforts to obtain medical advice (71.1%) and the lowest coping strategies that more than 50% of our subjects never have selected are receiving information from the Internet (52%) and others advises (50.5%). Spearman correlation test results demonstrated that However, the average adjustment score in the pregnant group (36.8 ± 4.3) was higher than the non-pregnant group (33.5 ± 2.8), but this difference is not statistically significant ($p=0.612$).

Conclusion: Although infertile women are faced with many psychological problems, they have little adjustment with them. The most coping strategies are used to overcome their fertility problems is in spiritual believes dimension. Therefore, we can recommend strengthen this aspect during treatment period.

Keywords: Infertility, Psychological Adjustment, Coping Strategies, Assisted Reproductive Technologies

O_{nm}-8: Association between Reproductive and Medical and Human Papilloma Virus Infection in Iranian Women

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Background: Human Papilloma viruses (HPV) is the most common sexually transmitted infection (STI) and the most important pathogenic factor for cervical intraepithelial lesions and carcinoma therefore a significant source of morbidity and mortality worldwide. This study was designed to investigate reproductive and medical factors associated with HPV infection in women referred to Colposcopy clinic of Gynecological hospital in Tehran, Iran in 2102.

Materials and Methods: The case-control study includ-

ing 201 women with abnormal pap smear referred to clinic of Colposcopy. Case group composed of 01 women with diagnosis of Condyloma Acuminata in biopsy specimen of cervix or positive HPV Typing test for types 6, 00, 06, 01, 10, 13, 31- 61 and control group composed of 140 women without diagnosis of Condyloma Acuminata in biopsy specimen of cervix or negative HPV Typing for types 6, 00, 06, 01, 10, 13, 31- 61. Two group were matched according to Iranian-descent, being of reproductive age (03-42 years) and had LSIL/HPV result on Pap Smear Test. Their demographic characteristics, reproductive and medical history were gathered by standard questionnaire then analyze data were done by SPSS version 21, t test, Chi-square test, fisher exact and Logistics Regression.

Results: Our finding showed that demographic characteristics including reduction of age ($p=0.000$), increasing age at first marriage ($p=0.000$), Self-employed spouse ($p=0.001$), reproductive factors including reduce number of pregnancy ($p=0.000$), reduction of sexual intercourse number per week ($p=0.006$), no consumption of OCP ($p=0.001$), history of withdrawal contraception method ($p=0.002$), medical factors including no history of urinary tract infection ($p=0.002$) and non genital warts ($p=0.001$) were significant risk factors for HPV infection.

Conclusion: Some reproductive and medical factors associated with increasing incidence of HPV infection. So knowledge about these factors helps us to identify women at risk and follow them consciously.

Keywords: Sexually Transmitted Diseases (STD), Human Papilloma Virus (HPV), Cervical Cancer (CC), Genital Wart

O_{nm}-9: Sexual Experiences of Infertile Women: Qualitative Study

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Background: Evaluation and treatment of infertility is critical to can be affected all aspects of life, particularly women, the Including how their knowledge of fertility and assisted reproduction treatment can disrupt marital and sexual Infertile women. Hence this qualitative study was performed to explore experiences of infertile women about sexual life.

Materials and Methods: This is a qualitative study with a phenomenological approach. Participants were 20 infertile women, selected through purposive sampling, referring to health care centers and infertility clinics of Isfahan. Data were collected through tape recording of deep interviews and analyzed by Collaizzi method

Results: Analysis of the participants' experiences led to the main concepts: "Concerns of a planned sexual relation during treatment", Suppression of sexual desire after infertility treatment failure", Sexual behavior has changed

Conclusion: The findings showed that the evaluation and treatment of infertility, are affected infertile women's sexual lives and they "Concerns of a planned sexual relation during treatment", Suppression of sexual desire after infertility treatment failure", Sexual behavior has changed" face. Accordingly, with regard to sexual problems following treatment of assisted reproduction, seems to provide sexual counseling in department infertility and infertility specialists in this field can help.

Keywords: Experiences, Sexual Life, Treatment Infertility, Phenomenology, Qualitative Research

Poster Presentations

P_{nm}-1: The Relationship between Spiritual Health and Life Satisfaction in Infertile Women

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Background: Spiritual is the most important dimension of existence in more than a third of the people and it has been at the center of their lives as strong force and so may have an influence on a person's Life satisfaction. As regards infertile women are at risk so much stress and anxiety, the purpose of this study was to investigate the relationship between spiritual health and Life satisfaction in infertile women referred to Isfahan infertility.

Materials and Methods: In this cross-sectional study, 210 infertile women 20 to 45 years who were referred to Isfahan infertility centers were selected through convenience sampling. To assess the spiritual health and quality of life was used the spiritual well-being scale (SWBS) and (SWLS) Questionnaire.

Results: The results of this study showed that the mean total score of spiritual health was (97.7) and Life satisfaction (23.5). Between Spiritual health and Life satisfaction ($r=0.643$, $p<0.001$) there is a direct relationship.

Conclusion: As regards infertility is a multi-faceted problem and can cause multi damage to women and influence their Life satisfaction; we can improve Life satisfaction in infertile women with promotion the spiritual health.

Keywords: Life Satisfaction, Spiritual Health, Infertility

P_{nm}-2: Unprotected Sexual Relationship and Socially Damaged Women

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Background: Socially damaged women population is reportedly at higher risk for STIs than the general population. The objective of this study was to explore the cause of unprotected sexual relationship among socially damaged women.

Materials and Methods: Qualitative method was utilized to determine family's role in HIV/AIDS/STD risk behaviors. Between February to May 2013 two focus groups and eighteen semi-structured in-depth interviews were conducted with socially damaged women, 15-45 years old, at shelters and Drop-in-Centers in Tehran, Iran. Total interview was recorded and typed word to word. Data was analyzed using the content analysis approach.

Results: Women in this sample ranged in age from 17 to 43 with the majority being in their Thirties. More than half of them had primary or

middle school education. Slightly more than three quarters of them, 14 (77%), were divorced, and more than four fifth of them, 16 (88%), were drug abusers. Two of the participants were injection drug users and four of the participants were consuming alcohol. All participants were heavy smokers. Two third of the interviewees, 12 (66%), were homeless. More than half of them lived in shelters and three of the interviewees lived in parks. More than two thirds of the interviewees were sex workers, while all of participants were sexually active. Two third of the interviewees, 12 (66%) had Imprisonment record. Most of participants (88%) had an unprotected sexual relationship. The reasons of having an unprotected sexual relationship included the need to have financial and emotional support, disturbance in pleasure, and misconception about the use of condoms. Financial need was the most important reason for a majority of the participants to have sexual relationships.

Conclusion: The study results emphasizes on the need for affiliated organizations to conduct preventive and damage-reducing programs continuously and accurately, to offer effective training to exposed group, and to provide expert consultation.

Keywords: High Risk Behaviors, Women, Iran

P_{nm}-3: A Cross Sectional Study to Determine Effective Factors on Prevalence of Anemia in The First and Second Trimester of Pregnancy

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Background: Iron deficiency anemia is a major nutritional problem throughout the world. The incidence of anemia is especially high during pregnancy and lactation due to an increase in the need for iron. This study aimed to investigate the prevalence of anemia in the first and second trimester of pregnancy and determine of demographic and laboratory factors that can effect on prevalence of anemia.

Materials and Methods: This study is a cross sectional study that is done between 1390 and 1392, in pregnant women who live in southern Tehran- Rey. Maternal hemoglobin (Hb), fasting blood sugar (FBS), pre pregnancy body mass index (BMI), plasma lipid levels were measured in all women (n=800) in the first trimester (12.6 ± 4.2 weeks) and singleton pregnant women without anemia (Hb≥11g/dl) were enrolled. After 15 weeks and 6day, all pregnant women received iron supplementation with 60 mg of ferrous sulfate. Maternal Hb was checked for 495 women in the second trimester (25.94 ± 2.48 weeks). All confident intervals were calculated at the 95% level. Data was analyzed using student's t test, paired samples test and chi-square test.

Results: In the second trimester maternal Hb concentration were reduced significantly ($p<0.001$). The prevalence of anemia in the first trimester (Hb<11g/dl) and in the second trimester (Hb<10.5g/dl) was 14.05% and 14.77%, respectively. Maternal pre pregnancy BMI and FBS were significantly lower in anemic women in the first and second trimester ($p=0.001$, $p=0.02$), ($p=0.0001$, $p=0.005$), respectively. In the first trimester in anemic women, age was lower than others ($p=0.01$). There were not any relationship between gravidity, parity, the number of abortion and plasma lipids with prevalence of anemia in the first and second trimester.

Conclusion: We must provide education about proper nutrition and the proper iron supplementation to all women, especially in women with low BMI.

Keywords: Anemia, BMI, Hb, Hct, Pregnancy

P_{nm}-4: The Association of Depression and Fetal Sex in Pregnant Women with Sleep Disorder

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Background: 79% of the pregnant women suffer from sleep disorders and can affect disorders before, during and after child-birth can be involved in causing depression during pregnancy, according to studies the poor quality of sleep in the second trimester of pregnancy is directly related to depressive symptoms in late pregnancy. Also gender difference may have a role in depression. This study aimed to evaluate the association of depression and fetal sex in pregnant women with sleep disorder.

Materials and Methods: This is a cross-sectional study conducted on 972 pregnant women with sleep disorders referring to two selected health care centers in Makou affiliated to Urmia University of Medical Sciences during Jun-Oct 2012. Data collection tools included demographic characteristics questionnaire, Pittsburgh Standard Sleep Quality questionnaire, and Beck Depression standard questionnaires. Sampling was done by convenient sampling. Firstly, Pittsburgh Standard Sleep Quality Questionnaire was completed and the pregnant women with sleep disorders were selected as the subjects then demographic characteristics questionnaire and Beck Depression standard questionnaires was completed by women, and statistical methods (Frequency tables, Pearson, independent t test, ANOVA, Chi-square and Fisher) used to analysis.

Results: The results indicated that depression of pregnant women with sleep disorder reported 67.3 percent and there were significant association Between Depression and fetal sex. Pregnant women with male fetus experience depression more than women with female fetus ($R=0.067$).

Conclusion: Given that a large percentage of pregnant women suffer from sleep disorders and along with it from depression. Hope this period, in addition to usual care, special programs for research, Structured psychiatric interview, changing the social circumstances that many women face them, diagnostic and troubleshooting cause of the disturbance takes place.

Keywords: Depression, Pregnant Women, Sleep Disorder, Fetal Sex

P_{nm}-5: The Comparative Study of Yaz and Ovocept-ld on Patients with Simple Ovarian Cysts Referring to Shariati Hospital of Isfahan

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Background: Functional ovarian cysts include follicular, corpus luteum, and theca lutein cysts are the most common adenexal masses (about 50%) in women of reproductive age. Treatment with the combined monophasic oral contraceptives reduces functional

ovarian cysts. Yaz (drospirenone/ethinyl estradiol) is a low-dose combined oral contraceptive pill containing 20 µg ethinyl estradiol and 3 mg drospirenone. In addition to contraceptive effects, Yaz has anti-mineralocorticoid and anti-adrenergic effects. Ovocept- low-dose LD is also a low-dose combined oral contraceptive drug containing 30 µg ethinyl estradiol and 3 mg norgestrol. Ovocept-LD has some side-effects such as weight gain, spotting, breast tenderness, nausea, and headache.

Materials and Methods: Being a clinical study, the present research was carried out on 42 patients with the simple ovarian cysts from 2010 to 2012. 84 Patients were assigned to A and B groups. Group A received Yaz once a day for a period of 28 days and group B received Ovocept-LD once a day for a period of 21 days. After treating by Yaz and Ovocept-LD, Cysts were evaluated by ultrasound. Results were analyzed by the SPSS software. A $P < 0.05$ was considered the significance threshold.

Results: Obtained results indicated that both Yaz and Ovocept-LD had an effect on the simple ovarian cysts. Statistical tests, however, has shown that the effect of Yaz has been significantly more than that of Ovocept-LD.

Conclusion: Given the faster and better recovery effect, and the lesser side effects of Yaz as compared to Ovocept-LD, it is recommended to use Yaz for the simple ovarian cysts.

Keywords: Ovocept-LD, Simple Ovarian Cyst, Yaz

P_{nm}-6: Sexual Dysfunction in Women Seeking Fertility Treatment

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Background: To determine the impact of infertility on all aspects of female sexual function.

Materials and Methods: A literature search was conducted on PubMed. Several methods have been used for evaluation of female sexual functions one of them is the questionnaire included of the female sexual function index (FSFI). FSFI is currently the most frequently used female sexual dysfunction questionnaire, which is a short questionnaire that provides an objective assessment of the quality of sexual functioning and sexual relationships.

Results: Though, it is now understood that female sexuality is multi-factorial in nature, comprising neurologic, psychosocial, hormonal, and vascular processes. The explanation of sexual dysfunction is a recurrent or persistent sexual complaint that causes a woman personal suffering. Several studies show that women with infertility reported a lower sex-life satisfaction than controls. They also represent significantly sexual function especial to arousal and desire domain scores, indicating worse sexual functioning, compared with women without infertility. There are some reasons to support these findings. The consequences of an infertility diagnosis may include the development of personal distress, reduced self-esteem, depression and anxiety. It is not completely clear whether it is the assessment of infertility, the treatment, or the interaction of the two that has the greatest impact on female sexual function. Some studies report that women with infertility experienced a higher prevalence of negative emotions associated with previous failed conception attempts and a decrease in sexual satisfaction compared with women without infertility.

Conclusion: Women with a diagnosis of infertility were found to be at higher risk for sexual function. The interaction of sexual function and infertility is complex and deserves further study. Therefore there is a need for screening and psychosocial interventions in the setting of infertility to prevent and identify the factors that may contribute to the development of sexual dysfunction.

Keywords: Female Sexual Function, Female Infertility, Female Sexual Dysfunction

P_{nm}-7: Intimate Partner Violence and Antenatal Depression among Pregnant Women in Iran

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Background: Domestic violence against women is a significant public health issue in both developed and developing countries of the world. Intimate partner violence during pregnancy can result in adverse outcomes for both mothers and their infants. Partner violence against women is common during pregnancy and might have an adverse effect on the mental health of women during antenatal pregnancy. Objective: This study aims to determine the frequency and risk factors of domestic violence in pregnant women the association of antenatal depression with psychological, physical, and sexual violence against women by their intimate partners during pregnancy.

Materials and Methods: This cross-sectional study was done on 1097 pregnant women referring in health centers in jahrom south of Iran between April and October 2013. All women were invited by the midwife to participate in the study during their visit to the clinic for their first checkup or during a follow-up visit. This research was implemented through questionnaires including the demographic characteristic. The form of partner violence in pregnancy including emotional abuse, physical violence and sexual violence was assessed with a validated questionnaire, and the Beck depression scale was used to measure antenatal depression. Data was analyzed using Student's t test and one-way ANOVA tests.

Results: The overall prevalence of violence during pregnancy was 79.1%. For this rate, 69.7, 28.1 and 30.9% were emotional violence, sexual and physical violence, respectively. The most common form of partner violence was psychological. Domestic violence was significantly associated with, age ($p=0.005$), Length of marriage years ($p=0.004$), Husband's Educational level ($p=0.023$), pregnancy number ($p<0.001$), Previous mode of delivery ($p=0.03$), Demands of pregnancy ($p=0.001$), depression during pregnancy ($p=0.02$). Between depression and psychological violence ($p=0.016$), sexual violence ($p=0.027$) was statistically significant.

Conclusion: This study indicated that the frequency of domestic violence in pregnant women is very high; therefore it is suggested women clinic to be established to protect pregnant women suffering from domestic violence during pregnancy. Also in this study, it is clear that violence against women increases the risk of mental health problems. Routine screening for violence and depression is warranted during pregnancy to aid in the early detection and violence of depression.

Keywords: Violence, Pregnancy, Antenatal Depression, Women

P_{nm}-8: Is Parental - Fetal Attachment Behaviors Different in Pregnant Women and their Husbands who Had Infertility?

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Background: Parental - fetal attachment behaviors play important role in pregnancy outcome, maternal and paternal identity and also children development. It seems that these behaviors are different in infertile couples. Knowing the relationship between infertility and parental - fetal attachment was the aim of this study

Materials and Methods: For this cross - sectional study that was done in 12 centers of health and medical centers, affiliated to Tehran University of medical science, 400 pregnant women and their husbands were selected in 2012. Data were gathered by using 3 questionnaires; first one was demographic questionnaires and second one was MFA for mothers and PFA for fathers. Data were analyzed by using statistical tests, ANOVAs and post hoc test. The $p<0.05$ denoted statistical significance.

Results: The mean (SD) of MFA in fertile women was 84.81 (17.16) and in infertile women was 85.25 (12.33). The mean (SD) of PFA in fertile men was 76/86 (17.87) and in infertile men was 75.15 (13.55). There was no significant relationship between MFA and infertility ($p=0.36$) There was no significant relationship between PFA and infertility ($p=0.51$).

Conclusion: Pregnancy is a kind of crisis during life especially after infertility. It is important to notice the feeling of infertile couples about their unborn baby. Midwives are in a unique position to promote the prenatal care by assessing parental- fetal attachment and referring them to psychologist when it is necessary.

Keywords: Maternal - Fetal Attachment, Paternal - Fetal Attachment, Infertility

P_{nm}-9: The Effect of Maternal Age (40 and Over 40 Years) on The Birth Weight, Mode of Delivery and Newborn's Sex

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Background: Age of mother during pregnancy and delivery is important factor in maternal, child and community health. The aim of study is the assessment of effect of maternal age (40 and over 40 years) on birth weight, mode of delivery and newborn's sex.

Materials and Methods: This descriptive, analytic study was performed on 1840 primiparous term women without maternal and obstetrics complications that delivered from April 2011 to March 2014 in Rouhani Hospital of Babol. Data were collected from register files in la-

bor. Then data were analyzed by ANOVA and Chi-square tests in SPSS.

Results: 447 pregnant women were under 20 years, 1372 women between 20-40 years and 21 women 40 and over 40 years. The results showed difference between maternal age and birth weight in three groups (the mean of birth weight in under 20 years: 3197 gr, between 20-40 years: 3247 g and 40 and over 40 years: 3080 gr), but this difference was not significant statistically ($P=0.52$). Also 48.3% pregnant women under 20 years, 66.5% women between 20-40 years and 90.5% women 40 and over 40 years delivered by cesarean, these results showed significant difference between maternal age and mode of delivery ($p<0.001$). There was no significant difference between maternal age and Sex of newborns ($p=0.84$). (Sex of newborns were 51% female, 49% male in women under 20 years, 49.6% female, 50.4% male in groups between 20-40 years and 52.4% female, 49% male in women 40 and over 40 years).

Conclusion: Maternal Age of 40 and over 40 years is an important and effective factor on mode of delivery but there is not any relation between age of women pregnant and newborn's sex and birth weight.

Keywords: Newborn, Weight, Cesarean, Sex

P_{nm}-10: The Relationship of Sexual Function and Marital Adjustment with Menstrual Pattern and Factors Affecting in Infertile Women

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Background: One of the important components reproductive Health that can has some effects on physical and psychological aspects of couple's life is Infertility. Infertile women are concerned about their sexual and physical health, it is even possible, and use and evaluation of assisted reproductive techniques have negative effects on women feelings about the sexual value and marital relationships. This study therefore aimed to the relationship of sexual function and marital adjustment with infertile women menstrual pattern and factors affecting in infertile women in 2011 in Mashhad, Iran. One of the important components reproductive Health that can has some effects on physical and psychological aspects of couple's life is Infertility. Infertile women are concerned about their sexual and physical health, it is even possible, and use and evaluation of assisted reproductive techniques have negative effects on women feelings about the sexual value and marital relationships. This study therefore aimed to the relationship of sexual function and marital adjustment with infertile women menstrual pattern and factors affecting in infertile women in 2011 in Mashhad, Iran.

Materials and Methods: This correlation study was carried out on 130 infertile women referred to Monasteries' Infertility Research Center, Mashhad who selected using convenient sampling. Research tools were consisted of valid and reliable demographic questionnaires including personal and infertility-related information, menstrual pattern, ROSEN female sexual function index (FSFI) and Spanier Marital Adjustment Scale (DAS), which were completed by the subjects. Data analysis was performed by SPSS software using t test, one way ANOVA, and Spearman and Pearson correlation tests.

Results: The mean score of duration of information and treatment of infertility were 5.24 ± 4.12 and 4 ± 3.95 years respectively and causes of infertility were 46.9% female, 38.7% male and common and

unknown factors. 45.4% of the women had high sexual function and 76.9% had high marital adjustment. 71.7% and 65.4% of the women had regular menstrual and premenstrual distress respectively. 66.9% of the women had dysmenorrhea. There was not significant relationship between sexual function with menstrual cycle, but there was significant relationship between marital adjustment with regular menstrual periods in infertile women ($p=0.001$). 87.7% of the women with high marital adjustment have regular menstrual pattern.

Conclusion: The findings showed that in infertile women with improved marital adjustment will be better regular menstrual pattern, which will help to improve infertility; therefore we can use these results in implement training programs and consulting, special in infertile women who have marital disorders.

Keywords: Sexual Function, Marital Adjustment, Menstrual Pattern, Infertility

P_{nm}-11: Lifestyle Modification; Impact on Infertility Treatment of PCOS Women

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies, affecting 5-10% of women of reproductive age. The treatment of infertile women with PCOS is surrounded by many controversies. This paper reviews the lifestyle modifications in these patients prior to assisted reproductive techniques (ART).

Materials and Methods: In this review, English studies published from 2000 to 2014 in MEDLINE data base were identified and recruited. Articles were searched using the combination of the terms "lifestyle", "infertility" and "PCOS". In addition, the bibliographies of selected references were searched and utilized.

Results: Before any intervention is initiated, pre-conceptual counseling should be provided emphasizing the importance of life style, especially weight reduction and exercise in overweight women, smoking and alcohol consumption. Researches show that weight loss is associated with improved spontaneous ovulation rates in women with PCOS even after losing as little as 5% of initial body weight. It is clear that regular physical activity is an important component of weight loss program, because it is associated with better long-term weight loss maintenance. Regarding diet for PCOS women, it is generally agreed that energy restriction is required for weight loss. Increasing evidence suggest that diets with reduced glycemic load may be beneficial in alleviating hyperinsulinemia and its metabolic consequences.

Conclusion: Given that Obesity adversely affects reproduction and is associated with anovulation, pregnancy loss and late-pregnancy complications and also since treatment of adverse lifestyles, including obesity and physical inactivity, should precede ovulation induction, it is highly recommended to encourage PCOS women to lifestyle modifications. Although the best diet and exercise regimens are unknown, but caloric restriction and increased physical activity are strongly recommended.

Keywords: Lifestyle, Infertility, PCOS

P_{nm}-12: Can Stress Lead to Infertility?

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Background: The role that stress plays in infertility remains controversial and despite medical advances a large percentage of infertility remains unexplained. Clinicians and researchers consistently report that infertile women view infertility and its treatment as extremely stressful. Basic science has elucidated the linkages between the hypothalamic-pituitary axis (HPA) and hypothalamic-pituitary gonadal axis such that it is now accepted that physical stressors can perturb women's menstrual cycles. What is less clear is whether psychological stress can have the same effect. This study performed to evaluate the relationship between stress and infertility.

Materials and Methods: This study is a review article that is provided with library and internet resources.

Results: Stress arousal may compromise the feedback regulation of the hypothalamo-pituitary-adrenal axis (HPA), releasing stress-related biomarkers and thereby affecting establishment of pregnancy. For the HPA axis, blood cortisol levels increase which subsequently results in an increase in salivary cortisol. The fact that both of these biomarkers can be detected in saliva makes them ideal for use in population-based studies. Recent work suggests that psychological stressors produce a more pronounced alpha-amylase response than physical stressors and Higher levels of stress as measured by salivary alpha-amylase are associated with a longer time-to-pregnancy (TTP) and an increased risk of infertility. Another side some study established severe depressive symptoms were significantly associated with increased infertility-related distress at both the individual and partner level.

Conclusion: These results suggest that stress is a risk factor of infertility. Health professionals should explore the quality of social networks and encourage seeking positive support from family and partners.

Keywords: Infertility, Stress, Psychological Status

P_{nm}-13: Survey to The Impact of Relaxation on Anxiety and The Result of IVF in Patients with Infertility that Have Been Referred to The Infertility Center of Tehran Medical Sciences during 2012-2013

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Background: The process of infertility comes along with various social, mental, physical and financial stresses. The process of IVF treatment makes about 1.3% of women depressed having much anxiety in terms of psychiatric health. This also makes an intervention with the process of treatment in this regard. The present study is to determine the impact of relaxation on anxiety and the result of IVF in patients with infertility that have been referred to the infertility center of Tehran medical sciences during 2012-2013.

Materials and Methods: This experimental study is a clinical-based examination type that has been carried out on 100 infertile women using IVF treatment ranging from 25-35 year old into the hospitals affiliated on Tehran Medical Sciences University during 2012-2013. The sampling method is an available way and the related data was gathered by the use of Newton infertility anxiety question-

naire into two groups of case and observation. The related data was analyzed by the descriptive statistics, t test and covariance analysis in $p < 0.05$ level.

Results: The mean score of anxiety in two groups before the relaxation was 163.24 and 165.48, respectively. The related t test did not show a significant difference between two groups ($p > 0.05$). After the relaxation, the mean score of the anxiety was obtained between two groups as 148.38 and 156.18, respectively. The t test showed a significant difference between two groups after the relaxation ($p < 0.05$). Also, the covariance results showed significant statistical difference between two groups in terms of anxiety reduction. Therefore, the number of positive results was 20 cases in observation group and 19 cases in experimental group in this regard. The number of negative replies was 30 cases in observation group and 31 ones in experimental group in this study. The results of chi-square test did not show a significant difference between two groups in relation to the positive replies of pregnancy test ($p > 0.05$).

Conclusion: Due to the result of the present study, the relaxation process is an effective way on reducing the related patients' anxiety degree. This reduced a considerable degree of the case group anxiety but it does not have an essential role in its own success.

Keywords: Infertility, Anxiety, IVF, Relaxation

P_{nm}-14: Investigation and Comparison of Pop-Smears Due to Pregnancy Inhibiting Methods, in Urban and Rural Women Who Have Visited in Hygiene Centers of Yazd State

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Background: Vaginitis is the most common reason of women attendance in gynecology clinics. Many factors can lead to this type of infection, so that the treatment procedure would be different according to the producing factor. Pop-smear is a cytological test which got created by Papanicolaou for the first time in 1941. It's based on sampling from cervix and smears providing and direct observation under the microscope. Vaginal infections can be produced by many reasons which as the most important we can mention; bacterial vaginosis, candida Albicans, Gardnerella vaginalis and Trichomonas vaginalis. In this investigation, the infectious caused by definite most are called "specific infectious" and the cases caused by unknown most are called "non-specific infectious".

Materials and Methods: In this study, totally 600 files of pop-smear cases involving 300 urban pop-smear and 300 rural cases. These files got selected randomly throughout the patients who had attended to hygiene centers of Yazd during the first 3 month of 2013.

Results: After analysis of data by SPSS software declared that; 25.7% of urban infections is caused by Gardnerella V., 4% by Candida A., 3.7% by both most together that totally 32.4% got reported as specific infectious and 41.7% as non-specific. There was no significant correlation between rate of infection and pregnancy inhibiting methods, and the some between rate of infection and age groups in urban population. In rural population, 27.3% of infections are caused by Gardnerella V., 4% by Candida A., 4.3% by the both, totally 35.6%

of infections were specific and 42.3% of them were Non-specific.

Conclusion: Among this population the most infected age was from 30 to 39 and the second grade related to 20-29 age group with a tiny difference, that there was no significant relation between the rate of infection and age groups, also in this population the most infected group among the pregnancy inhibition methods users were them who used condoms. Because of the less usage of pregnancy inhibition methods in rural population than the urban, significant of relation between the rate of infection and pregnancy inhibition methods is acceptable.

Keywords: Pop-Smears, Pregnancy Inhibiting Methods, Urban, Rural

P_{nm}-15: Studying about The legal nature of Embryo donation

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Background: Embryo donation is one of the methods of infertility treatment. With this way, Infertile couples use a donated Embryo formed from an egg of another woman and the sperm of another man. The embryo is then placed into the woman's womb. In order to determine the legal nature of embryo donation, a comparison has been made between this legal institution and those mentioned in our Civil Law. The result will be compared to Article 10 of the Civil Law to begin to determine the rights and obligations of both sides.

Materials and Methods: Due to the imperfection of the Law in this regard, general rules of contracts have been used to study the subject of the essay. The method that has been used to write the essay is called descriptive-analytic.

Results: Considering the analysis and determination of the procedure of Embryo Donation, it seems that a distinction should be made between the different steps involved. For the first step, the Owners of the sperm and the egg offer their sexual cells to Infertility Centers. During the following step, the egg and the sperm are fertilized in a laboratory environment at an Infertility Center. Then the resulting embryo is transferred to the woman's womb. The first step, which includes the giving of sperm and egg to Infertility Centers, has reasonable profit and can be done in the form a contract of sale. However, it is not appropriate to call the next step a contract of sale, because it is contrary to Human Dignity. It is actually a step in the treatment process.

Conclusion: In order to systematize the legal relations of the parties, the law maker should either accept the act of transferring an embryo from the owners of sperm and egg as a form of a legal institution recognized by Civil Law or define the act by using a new contract.

Keywords: Donation, Embryo, Owners of The Egg and The Sperm

P_{nm}-16: Endometriosis: Diagnosis and Intervention (All Things that A Midwife Should Know)

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Background: To describe the evaluation and management of endometriosis for midwives.

Materials and Methods: A narrative review was performed within articles published at "PubMed", "Elsevier", "SID" and original text books to reach the purpose.

Results: Many infertile women presenting at infertility treatment centers suffer from "endometriosis". The significant correlation between endometriosis and infertility has been shown in several studies and the American Society for Reproductive Medicine suggest that 25-50% of infertile women have endometriosis and 30-50% of women with endometriosis, are infertile! Although patients may be asymptomatic in early stages, endometriosis manifests as pelvic pain, presence of an adnexal mass and infertility, but clinical presentations and treatment procedures vary depending on the stage of the disease. Therefore, correct diagnosis and grading the condition helps us for effective administration of the patients and to make better treatment choices. So, every midwife working at infertility treatment centers should be aware of diagnosis procedures and therapeutic guidelines to give better care for patients. In this article, we explained the guideline for imaging evaluation and management of endometriosis.

Conclusion: Endometriosis is a widespread phenomenon among infertile patients. Therefore, every midwife working at infertility treatment centers needs to learn about the application of imaging methods in the diagnosis of the endometriosis and how to manage patients based on reports.

Keywords: Endometriosis, Infertility, Sonography, Laparoscopy

P_{nm}-17: Impact of Aerobic Training on Work Ability of Midwives Working in Health Care Centers in The City of Mashhad in 2013

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Background: Maintaining and promoting work ability is an important social objective and are challenges facing the health care and rehabilitation system, as well as employers. Physical and mental health status impact on work ability. The aim of this study is to determine the impact of aerobic training on work ability midwives to work in health care centers in the city of Mashhad.

Materials and Methods: This randomized clinical trial, health care centers 1 and 3 selected randomized, any center divided randomized to the intervention or control groups, then 60 midwives working in health centers of Mashhad were selected through purposeful methods. The intervention group did aerobic training with intensity 30 to 60 percent of maximum oxygen consumption during 24 sessions. Subjects performed the Bruce test At the baseline and At the end of the study and completed questionnaire work ability index. The data were analyzed with SPSS 19 and tests Independent t-student, Paired sample t test, Mann-Whitney and Chi squared test. $p < 0.05$ Was considered significant.

Results: The mean score work ability in intervention group (40.5 ± 4.9) significantly more than the control group (36.4 ± 5.3) ($p = 0.004$). There are significant difference between two groups in two sub scales, Work ability compared with life time best ($p < 0.0001$) and mental resources ($p = 0.036$).

Conclusion: Aerobic training increased work ability midwives. It is proposed to managers for Promotion work ability midwives to work in health care centers.

Keywords: Aerobic Training, Work Ability, Midwives

P_{nm}-18: Personality Characteristics and Psychological Well-Being in Infertile Women

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Background: To identify the relation(s) of personality characteristics and psychological well-being in infertile women and also well-being prognosis according to the personality characteristics.

Materials and Methods: 240 women who had been diagnosed with infertility and who were undergoing fertility treatment at private infertility clinic (Navid Institute), Tehran, Iran participated in the study. The study population was selected randomly from patients referring to the institute. The age of the participants was between 25 and 40 years. The patients with a history of more than once infertility treatment were excluded. All the eligible women fulfilled a short questionnaire comprising of 5 personality factors (NEO Five-Factor Inventory: NEO-FFI) and a well-being questionnaire.

Results: Evaluation and analysis of data by multivariate regression analysis indicates that personality characteristics were significantly associated with psychological well-being. Neuroticism ($r=-0.5$) is negatively and extraversion ($r=0.4$), adjustment ($r=0.4$), conscientious ($r=0.4$), openness to experience ($r=0.1$) are positively, associated with psychological well-being ($p<0.05$).

Conclusion: Regarding prognosis of psychological well-being according to personality characteristics, analysis of results expresses that neuroticism, adjustment and conscientious can explain well-being, which neuroticism (26.2%) is more significant, and the other factors (adjustment and conscientious) have a role of (5.90%).

Keywords: Personality Characteristics, Psychological Well-Being, Infertile Women