

**Abstracts of  
Royan International Twin Congress**

**16<sup>th</sup> Congress on Reproductive Biomedicine  
2-4 September 2015**

**10<sup>th</sup> Seminar on Nursing and Midwifery  
2-4 September 2015**



**Royan Institute**

**Reproductive Biomedicine Research Center**

**Tehran, Islamic Republic of Iran**

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

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## Congress Chairman



**Farid Dadkhah**

**O**n behalf of organizing committee, it's my great pleasure to welcome you to 16<sup>th</sup> Reproductive Biomedicine and 11<sup>th</sup> Congress on Stem Cell Biology and Technology in Tehran (September 2-4, 2015). Royan scientific congress has long been an outstanding annual event encompassing "Reproductive Biomedicine" and "Stem Cell Biology and Technology" as its two main congresses. Since 2005, the congress has welcomed the most distinguished scientists and researchers to share their latest research breakthroughs, exchange experiences and promote knowledge. The congress consists of diverse and simultaneous scientific events as plenary sessions, symposia, poster sessions, and workshops concentrating on and addressing the most recent researches on reproductive biomedicine. It is believed that translating research findings into practical and clinical experiments is the vital opportunity to achieve desirable scientific and research-based outcomes. Congress achievements are essentially taken-for-granted for the future treatment of infertility and also in hard-to-cure diseases.

Royan Congress scientific program relies on the scientific contributions and novel investigations by the most accredited scientists.

Looking forward to meeting you in Tehran, Iran.

**Farid Dadkhah, M.D.  
Congress Chairman of  
16<sup>th</sup> Congress on Reproductive Biomedicine**

**Abstracts of  
16<sup>th</sup> Congress on Reproductive Biomedicine  
2-4 September 2015**



**Royan Institute**

**Tehran, Islamic Republic of Iran**



## Invited Speakers

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### Andrology

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#### I-1: Male Infertility; Past, Present and Future

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1<sup>st</sup> century A.D.: Celsius reported that swollen veins over the testis associated are with small testis.

Mid-19<sup>th</sup> century: Inflammation of male and female reproductive organs was treated with ointments, douches and massages.

1881: Dr. Levy a German gynecologist performed a post coital test for an infertile couple and determined that the husband was sterile (no sperm on microscopic exam).

Late 19<sup>th</sup> century: Repair of varicocele for infertility described.

1929: First description of modern sperm count; Macomber and Sanders the sperm count NEJM 200 981, 1929.

1931: Sperm mature in the epididymis; W C Young J Exp Biol 8 151 1931.

1939: MacLeod J; The metabolism of human sperm; Soc Exper Biol and Med 42 153.

1943: MacLeod states reactive oxygen species may affect fertility; Am. J Physi 138 512, 1943.

1943: Klinefelter et al. described Klinefelter's syndrome; J Clin Endo Meta 2 615 1942.

1952: One of the first clinical books; Etiology and diagnosis in the treatment of Infertility in the men; Robert Hotchkiss.

1954: Thaddeus Mann; The biochemistry of semen and the male reproductive tract.

1976: Beer Et Al described immunologic infertility.

1978: B. P. Setchell published a definitive book on testis physiology; The mammalian testis.

1985: IVF was described; Cohen J, Edwards R, Fertility and Sterility 43:422-432, 1985.

1990-91: ICSI described

1998: Micro dissection for sperm retrieval described. Human Reproduction Update Schlegel PN1998, Vol. 4, No. 4 p. 439.

2001: The relevance of environmental factors in male infertility described.

2002: The value of Aromatase inhibitors for male infertility describe by JD Raman, PN Schlegel.

2004-2009: Gene micro deletion in AZFa region of the Y chromosome described.

#### I-2: Current Evaluation and Treatment of The Infertile Men

Howards SS

University of Virginia Charlottesville VA, Wake Forest Medical Center Winston Salem NC, USA  
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Fifty percent of infertility involves a male factor. The evaluation begins with a history, physical exam and semen analysis x

2. The history should include questions about exposure to gonad toxins. The differential diagnosis of no ejaculate includes; retrograde ejaculation, failure of ejaculation (rare), failure of emission and psychogenic. Evaluation of absence of the vas deferens which occurs in 2% of infertile men includes; genetic testing for CFTR mutations of the female partner. If the female is negative testing of the male is optional. Male fertility slowly declines with age and the incidence of miscarriage increases.

The physical exam should include; check for gynecomastia, visual fields, varicocele, and evaluation of the genitalia and virtualization. Normal testis size is  $\geq$  than 4 Cm x 2 Cm or 20 ml. Endocrine evaluation should be done if there is evidence in the history or physical exam of endocrine disease, decreased libido or a sperm count of  $<5-10$  million. It should not be done routinely. If AM total testosterone (T) level is low check free or bioavailable testosterone. Prolactin should be checked only if T is low and if it is low consider pituitary MRI. Treat hypogonadotropic hypogonadism with GnRH or HCG and FSH. Clinical infections should be treated. A positive semen culture is very difficult to interpret because there are bacteria in the normal distal urethra. Men with clumping of sperm or unexplained poor motility should have an immunologic evaluation. This is a very rare cause of male infertility. It is best treated with ICSI. Testis Biopsy is usually only indicated in men with azoospermia, normal size testis without evidence of obstruction and several oligospermia in men with one small and one normal sized testis. Azoospermia should be diagnosed only after there are no sperm on 2 centrifuged samples. Semen analysis does not determine fertility unless there are no sperm. However it does provide the ability to estimate the likelihood of spontaneous pregnancy. WHO standards and interpretation will be discussed. Reactive oxygen testing is of little clinical value. DNA fragmentation testing should not be done routinely since it does not change clinical care except in candidates for ICSI. Strict criteria morphology is difficult to interpret since it does not predict pregnancy after sexual intercourse. It does correlate with IVF fertilization. Additional tests of sperm function are of little clinical value except for a post coital test for men who for religious reasons cannot provide a semen specimen. Retrograde ejaculation should be treated with alkalization and retrieval of a post void ejaculate. There are many options to retrieve sperm from men with obstructive azoospermia. We use testis biopsy either open or percutaneous. Retrieval for non-obstructive azoospermia should be done with micro dissection after checking for chromosomal abnormalities and micro gene deletions on the Y chromosome. Ejaculatory duct obstruction will be discussed. Varicocele should be repaired (we do a microscopic repair) if the semen quality is subpar and the female partner has been evaluated. Sub clinical varicocele should not be fixed. Men with a low T and a normal E-2 should be treated with Clomid and if this fails HCG. If the E2 is high or the T/E-2 ratio is less than 10 they need an aromatase inhibitor. Failure of ejaculation usually can be treated with two headed vibratory stimulus. If this fails try electro-ejaculation. Microscopic vasovasostomy and vaso-epididymostomy have a good success rate in experienced hands for the treatment of obstructive azoospermia. Surgical techniques will be discussed  
Future:

Preliminary data is available for; molecular agents to treat low T production by stimulating the Leydig cells, technology to allow less expensive IVF, improved education for patients and clinicians to increase the percent of patients who would benefit from sperm banking achieve this goal, retrieval of spermatogenic stem cells from pre pubertal patients who face future infertility because of radiation, chemotherapy, Klinefelter's syndrome etc., better diagnosis and treatment because of increased knowledge of the genetic origins of male infertility, better methods to select sperm for IVF and ICSI that do not have DNA fragmentation; improve the predict of which adolescent with varicocele will benefit from surgical repair. Speculative future developments; more insight in to unexplained infertility, better antioxidant Rx, better understanding of the male contribution to recurrent pregnancy loss, ICSI with male DNA obtained from circulating leukocytes, *in vitro* generation of sperm cells.

### **I-3: Tale of The Tail: Candidate Genes Involved in Sperm Flagella Formation**

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ISTS defect in which sperm tail is short and fibrous sheath and axoneme are disorganized, is one of the syndromes that cause male infertility. Although a few studies have been done in this regard, its exact etiology in human is unclear yet. Four candidate genes causing ISTS are SPEF2, RABL2B, and A-kinases anchoring proteins genes (AKAP3 and AKAP4). Proteins which coded by SPEF2 and RABL2B are essential for correct sperm tail assembly and development, besides, AKAP3 and AKAP4 are most abundant structural proteins of the fibrous sheath. In the present study, the variations of candidate genes were investigated in 35 men with ISTS and 40 fertile men. To study the genetic variations, DNA was extracted from peripheral blood of patient (with more than 80% short tail sperms in at least two spermograms) and control groups, initially primers were designed for each target segment of candidate genes, and then PCR sequencing was done. Sequence analysis of SPEF2 did not identify any mutation in exon 3 and 28. However, one polymorphism (363A>C) was identified in exon 3 in four of patients and three persons of controls (P>0.05). Analysis of genetic data revealed that no mutations or single-nucleotide polymorphisms in exon 4 of RABL2B was identified, but an intronic variant [(C) nucleotide deletion (rs: 144944885)] was found in heterozygote form in 5 patients (P<0.05). No alteration was identified in controls. Moreover, a polymorphism 1499T>C in AKAP3 was seen in 5 patients whereas none of the individuals in the control group had this alternation (P<0.05). No genetic alteration but one was found in AKAP4 gene which was a >1350 bp deletion in exon 5. Our results revealed that some specific gene alterations in AKAP3, AKAP4 and RABL2B can take part an important role in sperm tail malformation and can be assumed as the etiology of ISTS although genetic alterations in SPEF2

gene are not involved in this defect.

### **I-4: Male Fertility Preservation Options, Current State-of-The ART and Future Implications**

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Male infertility management has made significant progress during the past three decades, especially after the introduction of intracytoplasmic sperm injection (ICSI) in 1992. However, many boys and men still suffer from primary testicular failure due to acquired or genetic causes. New and novel treatments are needed to address these issues. Spermatogenesis originates from spermatogonial stem cells (SSCs) that reside in the testis. Many of these men lack SSCs or have lost SSCs over time as a result of specific medical conditions or toxic exposures. Loss of SSCs is critical in prepubertal boys who suffer from cancer and are going through gonadotoxic cancer treatments, as there is no option of sperm cryopreservation due to sexual immaturity. The development of SSC transplantation in a mouse model to repopulate spermatozoa in depleted testes has opened new avenues of research in other animal models, including non-human primates. Recent advances in cryopreservation and *in vitro* propagation of human SSCs offer promise for human SSC autotransplantation in the near future. Ongoing research is focusing on safety and technical issues of human SSC autotransplantation. This is the time to counsel parents and boys at risk of infertility on the possibility of cryopreserving and banking a small amount of testis tissue for potential future use in SSC transplantation.

### **I-5: Multicellular Human Testicular Organoid: A Novel 3D In Vitro Germ Cell and Testicular Toxicity Model**

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**Background:** Mammalian spermatogenesis is regulated through paracrine and endocrine activity, specific cell signaling, and local control mechanisms. These highly specific signaling interactions are effectively absent upon placing testicular cells into two-dimensional primary culture. The specific changes that occur between key cell types and involved spermatogenesis signaling pathways during primary culture

remain to be elucidated. However, current protocols to produce mature germ cells *in vitro* are inefficient and are limited in supporting post-meiotic cells. In order to address these limitations we have developed a 3-dimensional testis organoid *in vitro* by combining stem cell and novel tissue engineering approaches. This model can be utilized as a means to evaluate potential gonadotoxic agents, and act as a means to address critical deficiencies in our understanding of basic human spermatogenesis. The overall goal of this study is to establish, characterize, and culture a multicellular, 3D, human testis organoid and to assess its functionality and spermatogenic capacity over time.

**Materials and Methods:** Development of our model system consisted of (1) identification and analysis of specific cellular components necessary for use of our 3-dimensional culture method, (2) establishment of basic design parameters, culture conditions, and (3) characterization of human testicular organoids using live cell imaging, immunofluorescence, immunohistochemistry, cell type and stage-specific gene expression, and viability assays.

**Results:** Human spermatogonial stem cells (SSCs), Sertoli, and Leydig cells were isolated, characterized, and expanded from tissue obtained through the National Disease Research Interchange (Philadelphia, PA, USA). These cell types were integrated successfully into 3-dimensional organoids and maintained viability as determined by ATP and Live/Dead assays for over 4 weeks in culture. During extended culture, qPCR analysis revealed a significant upregulation of spermatogenic markers including DAZL, ACR, and PRM1, as well as an upregulation of the Leydig cell functional marker HSD3B1 and Sertoli cell functional marker FSHr.

In addition, these organoids secrete androgens and are responsive to hCG stimulation *in vitro*. In order to establish enhanced feasibility of this system for high-throughput *in vitro* drug screening applications, we further demonstrated that these organoids can be successfully cryopreserved for future use without sacrificing proliferative capacity or functionality.

**Conclusion:** Testicular *in vitro* organoids were successfully generated by using isolated human SSC, Sertoli, and Leydig cells and maintained long term. Future directions will include optimizing the spermatogenic capacity of the organoids and evaluating their use as a novel testicular toxicity model.

## I-6: Azoospermia Factor in Male Infertility

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The human Y chromosome is essential for human sex determination and male germ cell development and maintenance. In 1996, Vogt et al. identified three recurrently deleted regions in Yq11 termed the azoospermia factor (AZF). The AZF region is subdivided into three non-overlapping sub-regions called AZFa, AZFb and AZFc and microdeletion in these regions is the most

important etiology of male infertility. AZF deletions are known to be associated with a various spermatogenic alteration. Microdeletions in AZFa lead mostly to Sertoli cell-only syndrome; mutations in AZFb provoke an interruption in meiosis I, and mutations in AZFc result in hypospermatogenesis, progressing to oligospermia or severe azoospermia. The development of ICSI has allowed patients with severe oligozoospermia to have children. However, the inheritance of Y chromosome microdeletions from father to son through ICSI is a risk of concern.

Our study aimed at determining the consequence of AZFc microdeletion in infertile men. We followed the patients for the result of ART. Retrospective data were gathered from patient's records. From 404 men who were consulted for infertility from 2009 to 2014 at Royan institute, 226 men (56%) had AZFc microdeletion and enrolled in this study. Semen analysis was performed according to the WHO manual for all patients. Conventional chromosomal karyotype analysis was conducted to analyze chromosome abnormalities via peripheral blood.

Karyotype analysis was available for 162 of 226 (72%) patients with AZFc deletion. Abnormal karyotypes were found in 11 cases (6.8%). Ten patients with abnormal karyotype were azoospermic and one of them had severe oligozoospermia. From 151 cases with normal karyotype, 86 patients were azoospermic, 44 patients oligozoospermic, 20 patients showed rarely sperm in their spermogram and 1 patient had normal sperm concentration.

AZF deletion provides essential information for genetic counseling and also in prediction of surgical sperm retrieval.

## Embryology

### I-7: Fatty Acids and Male Reproductive Function

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**Background:** The fatty acid composition of the sperm membrane changes drastically during spermatogenesis and may be key to its function. Previous data has shown that intake of long chain poly-unsaturated fatty acids can change the fatty acid composition of tissues, including testes and sperm. However, whether these changes in composition translate into changes in semen quality or male reproductive potential is not clear.

**Materials and Methods:** We evaluated the association between intake of fatty acids and semen quality parameters in a cohorts of young, healthy men (the Murcia Young Men Study –MYMS) and a cohort of men in couples presenting for infertility treatment (the EARTH Study).

**Results:** Subfertile men with the highest intake of saturated fat had 38% lower sperm concentration than men in the lowest intake of saturated fat while intake of omega 3 fatty acids was associated with a higher sperm morphology (Attaman et al. Hum Reprod 2012) and intake of fish, the main dietary source of these fats, was positively related to sperm count (Afeiche et al. J Nutr 2014). In addition, intake of trans fatty acids was inversely related to total sperm count among young healthy men (Chavarro et al. Hum Reprod 2014). Furthermore, sperm

membrane fatty acid levels of omega 3 fatty acids were positively related to sperm concentration while sperm membrane levels of trans fatty acids were inversely related to sperm concentration (Chavarro et al. Fertil Steril 2011).

**Conclusion:** Dietary fatty acids may play an important role modulating human spermatogenesis through mechanisms that may involve direct incorporation of fatty acids into the testes and sperm (omega 3 and trans fatty acids) and mechanism independent of direct local incorporation of fatty acids in the testes or sperm (saturated fat). Whether these effects on semen quality translate into effects on fertility remains to be determined.

### **I-8: Folic Acid and Female Fertility in Natural and Assisted Reproduction**

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**Background:** Pre-conceptional supplementation with folic acid is recognized as essential for the prevention of neural tube defects. However, the reproductive effects of folic acid may extend well beyond this well-recognized effect and may have an important role in improving fertility and survival of the conceptus and fetus.

**Materials and Methods:** We evaluated the association between pre-conceptional intake of folic acid from foods and supplements in relation to fertility-related outcomes in two prospective cohort studies: the Nurses' Health Study II and the EARTH Study. In the Nurses' Health Study II we evaluated the association between pre-conceptional folic acid intake and risks of ovulatory infertility and pregnancy loss. In the EARTH Study, a prospective cohort of couples undergoing infertility treatment, we evaluated the association between pre-treatment folic acid intake and treatment outcomes focusing on live birth rates.

**Results:** Folic acid intake was associated with lower risk of ovulatory disorder infertility (Chavarro et al. Fertil Steril 2008) and clinical pregnancy loss (Gaskins et al. Obstet Gynecol 2014a) and was positively related to live birth rates among women undergoing assisted reproduction (Gaskins et al. Obstet Gynecol 2014b). Specifically, compared to women in the lowest quartile of folic acid intake, women in the highest quartile of intake had a 39% lower risk of ovulatory disorder infertility, 20% lower risk of pregnancy loss (spontaneous abortion and stillbirth) and 20% higher live birth rate among women undergoing assisted reproduction. The benefit of folic acid was apparent at intakes substantially higher than those currently recommended for the prevention of neural tube defects.

**Conclusion:** Supplemental folic acid at doses substantially higher than those currently used for the prevention of neural tube defects is associated to better reproductive outcomes among women trying to conceive naturally and those undergoing assisted reproduction. These results should be further evaluated in randomized controlled trials.

### **I-9: Artificial Ovary and Infertility: Appli-**

### **cation of Natural Extra Cellular Matrix in Ovarian Follicular Growth *In Vitro***

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### **I-10: Transcriptomics in Oocyte Mediated Cellular Reprogramming**

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Early embryonic development in mammals begins in transcriptional silence with an oocyte-mediated transcriptional reprogramming of parental gametes occurs during a so called across-the-board process of "erase-and-rebuild". In this process, the parental transcription programs are erased long before (maternal) or soon thereafter (paternal) fertilization to generate a relatively naïve zygotic chromatin upon which the transcription program of new life cycle is rebuilt *de novo* after activation of zygotic genome. Any perturbation in either process will result in ill/fatal transcriptional phenotypes of the resultant embryos, and correspondingly, the very few viable clones obtained at the end of a typical cloning experiment underscores substantial differences exist between transcriptional reprogramming of nuclei somatic cells and gametes. Even though, a single defect in transcription reprogramming may cause a "ripple" effect on aberrant expression of entire networks of downstream target genes, and therefore, analysis of a small number of transcripts is of limited value for systematic study of genetic interactions in a complex trait and needs post genomic area approaches including genome wide analyses and network investigation. Here, we review the current status of transcriptomics in understanding oocyte mediated cellular reprogramming to propose that only a limited number of genes deregulated during oocyte mediated reprogramming. Accordingly, we believe that cloning's serendipity lies in great capability of ooplasm to reprogramming almost all genes of somatic cell and cloning unpredictability lie in few certain unstable genes that resist reprogramming.

**Keywords:** Oocyte Mediated Reprogramming, Somatic Cell, Transcriptomics

### **I-11: Optimal Strategy Toward Fertility Preservation**

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There are several indications of human female gamete cryo-

storage including sub-fertile and fertile patients. But our focus will be in women at risk of losing their reproductive function caused by oncological treatments or premature ovarian failure that could benefit greatly from this practice. Fertile women may take advantage of this technology to electively delay childbearing.

The overall outcome of the oocyte using vitrified oocytes in fertility preservation is satisfactory although clinical experience is still limited. Survival rate after warming is over 90%. The potential of vitrified oocytes is consistently similar than the one attained for fresh ones. All the evidences described in this lecture indicate that nowadays oocyte and embryo cryopreservation by means of vitrification is providing a highly effective tool within ART, attaining similar outcomes than those obtained with fresh oocytes-embryos, thus allowing its application into clinical practice.

Special attention will be done also in sperm freezing-thawing process on Vitrification. This method for cryopreservation, without the use of conventional cryoprotectants, by plunging the sperm suspension directly into liquid nitrogen is an optimal protocol for sperm cryopreservation devoted to male fertility preservation.

In this lecture we will discuss some basic and practical aspects of vitrification and show evidences on outcomes of sperm, oocytes and embryo vitrification and its contribution to the improvement of fertility preservation.

### **I-12: Objective Embryo Assessment Utility of Time-Lapse**

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Traditionally, embryo incubation and assessment daily has been under a light microscope, these observations are inevitably restricted to specific times and considering that the development of the embryo is a dynamic process, several critical stages in between observations may go unnoticed. For this reason, the new technologies, time lapse monitoring, have focused on the research for additional markers of viability to supplement current criteria for embryo selection and thus, achieve a reduction in the number of embryos transferred and so multiple pregnancies, making the selection procedure even easier for the embryologist. However, for wide adoption of time-lapse technology in IVF practice, there are two key considerations. First, validation of the time-lapse technology is needed to demonstrate its safety and effectiveness by prospectively designed clinical trials in a multiple-clinic setting. Second, time-lapse technology must be compatible with the busy workflow of any standard IVF laboratory and practical-to-use for any embryologist.

### **I-13: The Domestic Hen as Model to Studies Ovarian Cancer**

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Few preclinical animal models exist that simulate the development of ovarian tumors in humans. At present, the egg-laying hen appears to be the most promising model to study the spontaneous occurrence of ovarian tumors in the clinical setting. A flock of domestic laying hens begins to exhibit the ovarian cancer phenotype after approximately two years of age, and at four years of age cumulative reproductive malignancies may constitute up to 45% of a flock. However, age, genetic strain, reproductive history and diet have a significant impact of overall reproductive tumor incidence rates. Ovarian cancer in the domestic hen is similar to ovarian cancer in women since there is similar histopathology, and common molecular markers, such as CA125 and p53 mutations. The hen is the ideal model system for studying ovarian cancer in women since hens of a homogenous genetic background are readily available, and the onset of ovarian cancer in a flock of known genetic background is readily predictable. Furthermore, the domestic hen is an excellent model system to test chemo preventive agents that specifically target p53 or other molecular targets that predispose women to the cancer phenotype because management systems for domestic laying hens are readily adaptable to affordable large scale animal trials to test the efficacy of chemo preventive agents.

### **I-14: Novel Concepts in Molecular Pathology May Open A New Era in Treatment of Clinical Varicocele**

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Despite the long history associated with varicocele, it remains one of the most controversial issues in the field of Andrology. The main base of this is our current understanding of the pathophysiology of this disease. This has hampered treatment and management of varicocele, especially regarding why, when and to whom varicocelectomy should be applied. The main molecular pathology of varicocele is related to increased reactive oxygen species (ROS). But recent evidence suggests that other molecular mechanisms, like epigenetic modification, heat shock proteins, apoptosis and ubiquitination-proteasome are also affected in individuals with varicocele. Could understanding of these mechanisms help us with treatment of varicocele? These issues will be discussed during this talk.

### **I-15: Assessment of Transcript and Protein Profiles of Infertile Individual May Help to Select Individuals with Low Fertilization Potential Candidate of Artificial Oocyte Activation**

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Following sperm penetration, oocyte is activated by sperm oocyte activating factors (SOAFs) released by sperm. Sperm-specific phospholipase C isoform  $\zeta$  (PLC $\zeta$ ) and post acrosomal WW binding protein (PAWP) are two nominees for the SOAF. PLC $\zeta$  is located back-to-back with another testis-specific gene called CAPZA3. These two genes share a common bidirectional promoter. In this study we assessed the relative expression of these three genes in fertile individuals, in couples with low and high fertilization rate and also globozoospermic individuals. A significant correlation was observed between the expression of these genes and fertilization rate. We further showed that the expression profiles of these genes are good candidates for selection of individuals with low ability to induce oocyte activation. The selection of these individuals makes these a suitable candidate for artificial oocyte activation. We further showed that through expression analysis of these genes, it is possible to select individuals with genetic mutation in the bidirectional promoter region of PLC $\zeta$  and CAPZA3.

### **I-16: Tubulin Reversible Acetylation – Driving The Moves and The Moves Behind The Drive**

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**Background:** Asthenozoospermia accounts for almost 50% of the cases of male infertility. Our study investigating phosphoproteins differentially expressed in asthenozoosperm has identified the phosphoproteins relevant to sperm motility and the signature molecules likely to be altered in asthenozoospermia. The 66 phosphoproteins differentially expressed included four alpha tubulin isoforms which were differentially expressed in individuals with poor sperm motility; an increase in TUBA3E and TUBA4A and decrease in TUBA3C and TUBA8. Isoforms TUBA3C, TUBA4A and TUBA8 are reported to be abundantly present in the testis. Of these, TUBA3C and TUBA4A are known to be acetylatable isoforms. Their differential expression in asthenozoosperm prompted us to investigate the role of reversible acetylation of alpha tubulin in sperm motility.

**Materials and Methods:** Alpha tubulin, acetylated  $\alpha$  tubulin, and isoforms TUBA3C, TUBA4A, and TUBA8 were investigated in Percoll separated human sperm and HDAC6 in rat sperm by Western blot analyses, Flow cytometry, Real-time RT-PCR and IIF localization and data statistically analyzed. The observations were analyzed in silico for obtaining further insights. Interaction between HDAC6 and  $\alpha$  Tubulin was elucidated by IIF co-localization and Co-immunoprecipitation studies. Sperm HDAC6 activity, motility and status

of Ac  $\alpha$ -tubulin was investigated in the presence of HDAC inhibitors Trichostatin A, Tubastatin A and Sodium Butyrate.

**Results:** The differential expression of these isoforms was validated in the normal- and asthenozoosperm at protein and transcript level. Investigation of acetyl  $\alpha$  tubulin expression revealed a reduction in tubulin acetylation in asthenozoosperm. The decrease in TUBA3C and increase in TUBA4A transcripts, both being acetylatable isoforms of alpha tubulin, could be elucidated on the basis of transcription factors binding to the promoters of the respective isoforms while the reduction in acetyl alpha tubulin in asthenozoosperm could be explained on the basis of decreased TUBA3C and the association of HDAC6 with TUBA4A. However the presence of HDAC6 on sperm had not been hitherto reported. In this study we have demonstrated for the first time the presence of HDAC6 transcript and protein in testicular- and caudal-sperm of rat and further by co-localization and coimmunoprecipitation studies we showed that HDAC6 interacts with alpha-tubulin and they colocalize in the mid piece and principal piece of sperm flagella. Using HDAC inhibitors we further demonstrated that HDAC6 in sperm is catalytically active and inhibitors of HDAC6 increase acetylation and restrict sperm motility.

**Conclusion:** Our data suggests an association between reversible  $\alpha$  tubulin acetylation and sperm motility. We show that alpha tubulin acetylation is reduced in sperm of asthenozoospermic individuals. Paradoxically, our experiments in the rat sperm show that inhibition of HDAC6 increase alpha tubulin acetylation but restrict sperm motility. The persistent expression of HDAC6 on the sperm flagella in the presence of HDAC6 inhibitor hints at a possible role for HDAC6 possibly as a microtubule associated protein(MAP). On the basis of our observations with the human and rat sperm and the available literature on microtubule stability in atal1 KO mice, we conclude that HDAC6 may act as a MAP and maintain dynamic instability in sperm flagella, and that dynamic instability may be a pre-requisite for normal sperm motility.

**Keywords:** Acetylated Alpha-Tubulin, Deacetylase Activity, HDAC6, HDAC Inhibitor, Sperm Motility

### **I-17: The Mechanism of Gonadal Sex Determination**

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**Background:** In mammals, a single exon gene *SRY* on the Y-chromosome is activated in the XY gonadal primordium and initiates a cascade of molecular and morphological events leading to testicular differentiation. *SRY*-encoded protein (*SRY*) is a transcription factor harboring a HMG-box DNA-binding motif that upregulates *SOX9*, which encodes another transcription factor sharing the DNA binding motif with *SRY*. *SOX9* upregulates other genes, such as *FGF9* and *PGD2*, which in turn stabilize *SOX9* expression and contribute to testis cord organization. By contrast, in the absence of *SRY* or *SOX9*, ovarian differentiation is initiated in the XX gonad by

the activation of genes, such as *WNT4* and *FOXL2*, whose protein products antagonize testicular differentiation in gonadal somatic cells. These molecular mechanisms of sex determination have been well established by examining the mice with a deletion or mutation of individual genes involved. However, naturally occurring sex reversal often involves asymmetric gonadal sex, e.g., development of a testis and an ovary within an individual, the mechanism of which remains largely unexplained. The goal of our study is to understand the mechanism of sex determination in the B6.Y<sup>TIR</sup> mouse, which develops various combinations of gonadal structures.

**Materials and Methods:** The B6.Y<sup>TIR</sup> mouse was established by repeating backcrosses to place the Y chromosome of a local *Mus. musculus* domesticus variant caught in Tirano, Italy (TIR) onto the *C57BL/6J* (B6) genetic background, and propagated in our mouse colony (currently at the *N60* backcross generation). B6.Y<sup>TIR</sup> males were crossed with B6 females, and bilateral urogenital complexes were isolated from each fetus at 11.5 – 14.5 days postcoitum (dpc) while cranial biopsy was taken for genotyping by PCR amplification of the *Zfy* sequence. The accurate developmental stage at 11.5 – 12.5 dpc was determined by the number of tail somites (ts) from the base of the genital tubercle. The urogenital complex was fixed and processed for immunofluorescence localization of SRY, SOX9, and MVH (DDX4) in histological sections whereas the gonad separated from the mesonephros was subjected to RT-PCR of *Sry*, *Sox9*, and *Wnt4*. Gonadal structures beyond 14.5 dpc were evaluated morphologically.

**Results:** In the B6.Y<sup>TIR</sup> gonad, *Sry* was expressed in a comparable manner to the normal B6.XY gonad, but consequent *Sox9* expression was delayed and decreased after the peak at 20 ts. Although SOX9-positive cells were seen in the entire gonad at 19-20 ts, they disappeared except for the central region, in which testis cords were formed (ovotestes) by 14.5 dpc, or disappeared from the entire gonad, in which ovarian structures developed (ovaries). *Wnt4* expression pattern was similar to that in the normal B6.XX gonad. A fetal ovotestis usually developed into a small testis by regression of ovarian components by birth while a fetal ovary continued to develop into a mature ovary full of follicles. The frequency of ovotestis development was more frequent on the left side and influenced by the maternal age.

**Conclusion:** Inefficient upregulation of *Sox9* allows for partial or complete sex reversal despite normal expression of *Sry/SRY*. However, the ultimate gonadal sex is influenced by non-genetic factor(s).

**Keywords:** mouse, XY Sex Reversal, Testis, Ovary, SRY, SOX9

### I-18: The Role of Sex Chromosomes in Female Germ Cell Differentiation

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**Background:** When gonadal sex reversal occurs in mammalian species, the resultant XX males and XY females become infertile or subfertile, suggesting critical roles of sex chromosomes in germ cell differentiation. The objective of our study is to clarify the mechanism of infertility in the B6.Y<sup>TIR</sup> (XY) sex-reversed female mouse, which can be attributed to a failure in the second meiotic division in its mature oocytes. In the present study, we examined the orientation of MII-spindles and the time course of cell cycle progression after oocyte activation.

**Materials and Methods:** XX and XY females at 29-31 dpp were injected with 5IU eCG and sacrificed 45-47 hours later. GV-stage oocytes surrounded by cumulus cells (CCs) were collected from large follicles and cultured for 19 hours. At the end of culture, the oocytes were denuded of CCs and the MII-oocytes that had extruded the first polar body were subjected to following experiments: 1. After oocytes were fixed and stained with anti- $\alpha$ -tubulin-FITC and DAPI, the orientation of spindles was analyzed by confocal microscopy; 2. After oocytes were activated with 10 mM SrCl<sub>2</sub>, their cell cycle progression and polar body extrusion was monitored by live-image video for 5 hours, followed by Hoechst staining for visualizing pronuclei.

**Results:** No difference was found in the orientation of MII-spindles between the oocytes from XY females and those from XX females. Only 37.4% (n=99) of MII-oocytes from XY females extruded the second polar body within 4 hours post activation while 69.7% (n=89) of oocytes from XX females did so. The time required for the degradation of the first-polar-body was significantly longer while those for both the extrusion of the second polar body and formation of pronuclei were shorter in the oocytes from XY females, compared to those from XX females.

**Conclusion:** A lack of coordination between sister chromatid segregation and the second polar body extrusion in the MII oocytes after activation is the major defect responsible for infertility of the B6.Y<sup>TIR</sup> female mouse.

**Keywords:** Mouse, Oocyte, Meiosis, Spindle, Fertility

### I-19: Identifying and Overcoming an Epigenetic Barrier for SCNT Reprogramming

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**Background:** Despite successful cloning of many mammalian species, the cloning efficiency is extremely low compared to that of IVF raising the possibility of the existence of epigenetic barrier preventing successful cloning.

**Materials and Methods:** Using comparative transcriptome analysis comparing transcriptomes of IVF and SCNT embryos and that of donor cells, we identified epigenetic barrier and find a simple way to overcome the barrier.

**Results:** We identified that the Suv39h deposited H3K9me3

in somatic cells is an epigenetic barrier preventing zygotic genome activation and thus preventing successful development of the SCNT embryos. We find that by injecting mRNA encoding a H3K9me3 demethylase can overcome this epigenetic barrier to achieve high efficient cloning.

**Conclusion:** Suv39h deposited H3K9me3 in somatic cell is an epigenetic barrier for SCNT, which can be overcome by injecting mRNA of Kdm4d into the one-cell embryo to achieve high efficient cloning.

**Keywords:** SCNT, Epigenetic Barrier for Cloning, Suv39h, Kdm4d, ZGA

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## Ethics and Reproductive Healths

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### I-20: Quality of Life Measures in Reproductive Health

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Quality of life or more correctly health-related quality of life is currently receiving more attention in biomedical literature and outcome studies. However, there are several issues that we should be dealt with. First, measuring quality of life needs valid and reliable instruments. In addition, it is important to know how we conceptualize quality of life and reproductive health. Finally, a challenge remains whether we should use general measures, specific instruments, or both? This lecture will focus briefly on introducing quality of life instruments (both general and specific) that were used in reproductive health investigations. A few recommendations will be made for new investigators.

### I-21: Development and Validation of A Questionnaire Measuring Attitude toward Oocyte Donation

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### I-22: The Process for Justification of ARTs in Iran Health System: Ethical and Legal Trends

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## Femal Infertility

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### I-23: Mild Stimulation in ART

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The conventional ovarian stimulation protocol aims to provide the maximum number of oocytes for fertilization which is lead to several embryos for selecting to transfer. There are some complications in this case like; OHSS, Multiple gestation, Time consuming, Patients discomfort, High costs, Emotional distress and Reduced implantation rate due to the embryo-endometrial asynchrony.

Recently, an increased scientific interest is focused on mild approach for ovarian stimulation in clinical practice. Milder stimulation aims to develop safer and more patient-friendly protocol which is less expensive, less drug use, more physiological and the risk of treatment is highly minimized.

On the base of recent systematic reviews and meta-analysis, mild stimulation could be applied for the poor, high and normoresponders which are going to be discussed in detail at Royan Award 2015.

### I-24: New Molecular Aspects in Recurrent Pregnancy Loss

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Recurrent pregnancy loss (RPL) is usually defined as three or more consecutive pregnancy losses before 20<sup>th</sup> gestational week. Many etiological factors have been considered as cause of RPL including genetic factors, uterine anatomical defects, endocrine abnormalities, thrombotic and immunologic factors. Nevertheless, the cause of RPL remains unknown in around half of the patients despite extensive workup, and thus termed idiopathic RPL (IRPL). As endometrium is considered as a fertility determining factor, different molecules and cytokines are suggested to be involved in pathogenesis of RPL. In this presentation, some of important molecular aspects of RPL will be discussed.

**Keywords:** Recurrent Pregnancy Loss, Immunologic Factors, Cytokines

### I-25: Recurrent Pregnancy Loss; Updates in Etiologies, Diagnosis and Management

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Recurrent pregnancy loss defined as two or more miscarriage before 20 weeks of pregnancy affecting 1-5 % of women in reproductive age. There are many etiologies have been suggested, like Genetic, Immunologic, Thrombophilia, Endocrine and Anatomic; but in 50% of cases, the exact etiology remains uncertain. Endometrium acts as biosensor of embryo quality and endometrium itself contribute in embryo implantation and growth. Recently It has been shown that embryo selectivity impaired in the endometrium of patients with recurrent abortion, so poor embryo allowed to be implanted. There are many conflicting results provided from prevailing studies about the effectiveness of current treatment modalities. There are many suggested diagnostic and treatment options selected from recently published papers which will be presented in the lecture.  
**Keywords:** Pregnancy Loss, Embryo, Endometrium

### **I-26: Surgical Techniques in Tissue Transplantation for Fertility Preservation**

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Ovarian tissue cryopreservation is an option for patients who require immediate gonadotrophic treatment of cancers. when there is insufficient time to conduct ovulation induction This procedure can be used in patients with genetic mutations with a high risk for premature ovarian failure. This strategy is the only option for fertility preservation in prepubertal girls or in women who have hormone-sensitive malignancies. prophylactically cryopreserve ovarian tissue can be used for hematopoietic stem cell transplantation for the treatment of benign hematologic diseases or autoimmune diseases that haven't respond to immunosuppressive treatment. Ovarian tissue should be obtained before a woman initiates treatment. Obtaining a small volume of cortical tissue by a laparoscopic or minilaparotomy is the most common way to get ovarian tissue, then the tissue is transferred to the laboratory on ice and cut then cryopreserved. In cases in whom complete ovarian failure after treatment is anticipated, whole-ovary cryopreservation may be considered in these patients the whole ovary is removed with a large part of the vascular pedicle left attached. Both slow-freezing and vitrification can be used to cryopreserve in this situation. Autologous ovarian cortical tissue transplantation can be performed into orthotopic or heterotopic site. Orthotopic transplantation of ovarian tissue involves transplantation of strips of thawed ovarian tissue into either the medullary portion of the remaining ovary or the peritoneum of the ovarian fossa. Heterotopic transplantation of ovarian tissue has been reported in the forearm, abdominal wall, and chest wall. There is no report of a successful transplantation of a previously cryopreserved whole ovary in

humans. blood-borne cancers have the highest of risk for malignancy following transplantation of ovarian tissue. whereas metastatic disease was less common in most other cancers. ovarian tissue transplantation is not recommended for patients with blood-borne malignancies or malignancies that metastasize to the ovary.

### **I-27: GnRH Agonist Triggering and Luteal Phase Support**

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GnRH agonist triggering is one of the strategies for ovulation triggering and final maturation of oocytes. So, should be notice for luteal phase support in these cycles.

At the first it was began for prevention of severe OHSS but it was associated with luteal phase problem and lower pregnancy rate due to luteolysis effects of GnRH agonists. So, two other alternative strategies have been suggested, the first was dual triggering with GnRH-a and low dose hCG and the second was 1500 units of hCG on the day of oocyte pick up to replace the actions of early luteal LH to sustain implantation and endogenous luteal ovarian steroidogenesis. Sometimes the physician avoids hCG injection and instead focuses on correcting the abnormal luteal steroid profile by providing intensive luteal-phase support with oestradiol and progesterone. Although the pregnancy rate is lower in the fresh embryo transfer but in donor recipient and frozen embryo cycles there was no significant differences between GnRH-a triggering and hCG. It shows that the oocyte quality is not disturbed by GnRH agonist triggering. It may be associated to endometrial gene expression. The endometrial gene expression after the GnRH agonist trigger and a modified luteal phase support (with 1500 IU of hCG on the day of OPU) was similar to the pattern seen after the hCG trigger and standard luteal phase support but it does not eliminate the risk of OHSS.

However, Regarding the optimal results of segmental IVF, it is recommended to use from GnRH agonist for ovulation triggering and postpone the embryo transfer to the another cycle.

**Keywords:** GnRH Agonist, Luteal Phase, Ovulation Triggering

### **I-28: Recent Strategies in COS in Endometriosis**

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Endometriosis is a prevalent, chronic, and debilitating condition in women of reproductive age. Endometriosis is commonly associated with infertility. Approximately 25-40% of

infertile women have endometriosis.

Indications for assisted reproductive technology (ART) in endometriosis are tubal factor, male factor, failure of other treatments, and finally low ovarian reserve.

The effect of endometriosis on ART outcome is controversial. Most studies agree on longer duration of stimulation, and higher gonadotrophin requirements, and lower number of oocytes, but the same live birth rate compared to tubal factor infertility.

There is no ideal protocol for COS in endometriosis. There are several studies trying to improve the pregnancy outcome in ART cycles. Most of them suggest prolong (3-6 month) GnRH agonist therapy before ART cycle. Although the real benefit and the exact mechanism are not known.

A recent manufactured progestin; Dienogest at the dosage of 2 mg/day taken orally has been shown to alleviate the endometriosis symptoms equivalent to GnRH agonist. It has strong progestational activity but no androgenic activity and mild hypo estrogenic effects. This product in addition to aromatase inhibitors might be effective newer drugs for medical treatment of endometriosis and also can have similar benefit and less side effects and cost compared to GnRH agonist in prologue endometrial suppression before ART cycle.

### **I-29: Fertility Preservation in Early, Low Grade Endometrial Cancer**

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Endometrial cancer is the most common gynecologic malignancy in the United States, with over 40,000 cases diagnosed each year, typically in the postmenopausal women. 25% of cases affect premenopausal women. 14% of endometrial cancers are diagnosed in women younger than 45 years old. 5% of these tumors are diagnosed in women younger than 40 years old. In this talk the learning objectives are ; 1. To review the basic about the endometrial cancer and its epidemiology with focus on the young age patients, 2. To discuss the feasibility and efficacy of conservative therapy options of endometrial cancer in women who desire fertility preservation. 3. To analyze the different ART options in Endometrium cancer patients after conservative therapy.

### **I-30: Late Onset Severe Hemoperitoneum after Transvaginal Oocyte Retrieval: Incidence, Course, and Management**

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Severe ovarian bleeding after transvaginal oocyte retrieval is

a rare but potentially life-threatening complication. A late-onset is common and may be associated with an increased risk for ovariectomy. In the present study we analyzed our cases of ovarian bleeding (OB) after oocyte retrieval and compared it with the published cases and studies, including 31 patients. Main outcome measures were how often it happens, risk factors, course, and findings during surgery. We could find that this complication occurs in 0.08% of the cases. The first sign of OB was evident in 33.3% within the first hours after retrieval, and in 93.3% within 24 hours. During surgery, a diffuse OB was found in 13.3%. In four patients, the ovary could not be preserved which was associated with longer time intervals between retrieval and the onset of symptoms as well as between retrieval and surgical intervention. In conclusion, severe OB occurs in 0.08% after oocyte retrieval. Late-onset bleedings are common. A longer time interval between retrieval and surgical intervention might put a patient at risk for removing the ovary.

### **I-31: New Approaches for Luteal Phase Support in ART Cycles**

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During a normal menstrual cycle, progesterone prepares the endometrium for pregnancy by stimulating proliferation in response to human chorionic Gnadotropin produced by the corpus luteum. Many questions were raised about the role of follicular fluid aspiration on the granuloma cells at the time of oocyte retrieval during the ART cycles. Authors believes that oocyte retrieval might disrupt and/or diminish the number of granuloma cells undergoing luteinization, that results in a defective corpus luteum and abnormal progesterone production. As a result luteal phase would be defective in ART cycles.

Many evidences support the need for providing luteal phase support in ART cycles. Various types of regimens were tried for the luteal phase support. Luteal support is initiated on the day of oocyte retrieval or on the morning after.

Injectable form of progesterone or vaginal preparations was found as effective as repeated hCG injections. To avoid the risk of OHSS, the HCG is best avoided.

Concerning the timing for discontinuing progesterone administration, needs for E2 support, and other products such as uterorelaxing factors remain debated.

The best regimen for luteal support following triggering of ovulation by GnRH-a in GnRH antagonist cycles, remain a to be proved. This leads authors to recommend cryo-preservation when ovulation is triggered by an agonist.

It is concluded that luteal phase support is mandatory in ART for optimizing outcome. Using progesterone preparation, should be offered to all ART patients, starting on the day of oocyte retrieval until anywhere from two to eight weeks after embryo transfer.

### **I-32: Predictive Factors of Recurrent Pregnancy Loss**

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Accurate prevalence figures are not available, but it has been estimated that 1-2% of women have recurrent pregnancy loss (RPL), defined as three or more consecutive losses of intra-uterine pregnancy in the first trimester. Half of whom have no identifiable cause. Recent studies of early pregnancy loss, preeclampsia, pregnancy complications such as hydatidiform mole suggests that these are the results of a common pathophysiology. Abnormal placentation in the first trimesters leads to oxidative stress and resultant endothelial dysfunction plays a key role in the emergence of complications of pregnancy such as abortion. There are some markers for prediction of pregnancy outcome in cases of recurrent pregnancy loss, such as: serum  $\beta$ HCG, progesterone, CA125 Levels, soluble fms-like tyrosine kinase 1 (sFlt-1), PIGF (placental growth factor), pregnancy-associated plasma protein A (PAPP-A), Inhibin A, fetal fibronectin, platelet distribution width, platelet count, plateletcrit (pct), white blood cell (WBC) count, red cell distribution width (RDW), lymphocyte count, neutrophil count, neutrophils to lymphocyte (N/L) ratio and presence of the protein fractions of low-or mid-weight. Further larger studies are required to investigate the predictive efficacy of these markers.

**I-33: Current Methods for Fertility Preservation****Rashidi B**

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Increasing survival rates in patients who suffer from oncological disease and improvement in reproductive medicine techniques has led to increasing use of different fertility preservation methods.

The evidence supporting current and forthcoming options for fertility preservation consisted of two main parts as for female and male. Fertility preservation options for female and male depend on the type of treatment. For instance if the treatment includes cancer surgery, fertility-sparing surgery preserving gonads, preservation of the uterus in females, and use of cryopreservation may also be considered prior to surgery, if the risk of gonadal damage is high.

On the other hand, if cytotoxic treatment with high risk of gonadal damage is performed for the patients, then use of cryopreservation methods such as sperm banking for males, freezing of embryos and oocytes for females and gonadal tissue freezing should be considered.

Gonadal tissue cryopreservation in some conditions could be utilized in male and women specially in prepubertal and adult patients.

It should be emphasized that fertility preservation is not limited to cancer patients. There are some non oncological systemic diseases which are treated with chemotherapy or radiotherapy, such as autoimmune and hematological conditions. In addition, there are other interventions that may impair fertility, such as recurrent ovarian surgery for benign disease, like endometriosis or prophylactic oophorectomy in women with *BRCA* mutations. Therefore, today fertility preservation is also commonly utilized in non-cancer conditions, increasing the number of females who may benefit from current available and established techniques.

In this presentation we will review the current state, approach, and indications of different established as well as experimental methods for fertility preservation.

**I-34: Endometrial Secretome and Its Role in Uterine Functions**

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**Background:** Earlier it was believed that uterine fluid (UF) or secretions are not relevant after the embryo implantation in humans. However, recent reports suggest that uterine secretions continue to play important role till the first trimester of human pregnancy. Evidences also suggest that UF mirrors endometrial functions or dysfunctions. Considering the relevance of UF in endometrial functions, studies were undertaken 1. To develop human UF proteomes; 2. To identify UF proteins that display differential abundance during the receptive phase and also; 3. To assess the functional relevance of differentially abundant proteins using an animal model.

**Materials and Methods:** 2D-PAGE and gel-free Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) were employed. Samples were collected in the pre-receptive (i.e. day two post-ovulation, n=7) or receptive phase (i.e. day six post-ovulation, n=7) of the menstrual cycles, from regularly cycling healthy fertile women. Receptive phase samples were also collected from women with unexplained infertility. Further, to test the functional significance, rats (*Rattus norvegicus*) were used as an experimental model. Samples were collected in the proestrous (n=8) and metestrous (n=8) phases of estrous cycles and also from pregnant rats (n=18) during day 3-5 post-coitum (p.c.).

**Results:** Our studies demonstrated higher abundance of UF alpha-1 antitrypsin precursor and apolipoprotein A-1 in the re-

ceptive phase, than in the nonreceptive phase, in regularly cycling women (Parmar et al, 2008). iTRAQ revealed identities of 127 proteins in the human uf. Of these, 27 proteins displayed differential abundance in the receptive (R) phase, compared to the pre-receptive (PR) phase. High mobility group Binding Protein 1 (HMGB1), one of the differentially abundant proteins displayed less abundance in the R phase than in the PR phase; in secretions as well as in endometrial tissues. Interestingly rats also revealed a lesser abundance of HMGB1 in the receptive phase, compared to that in the nonreceptive phase uf (Bhutada et al, 2013). Thus, human and rat data indicated an association of endometrial receptivity with a decline in the levels of uf HMGB1. A significant decline was also observed in the expression of endometrial HMGB1 on the day of implantation in pregnant rats. Further, recombinant HMGB1 (0.25-1.5ug/horn) was administered on day three p.c. in mated rats. The horns administered with HMGB1 showed pregnancy failure, whereas those with saline remained unaffected. This indicated the detrimental effect of an excess of extracellular HMGB1 on pregnancy in rats. Also, morphological changes in the endometrium, an increase in the expression of luminal epithelial NF $\kappa$ B; and also various inflammatory molecules such as Receptor for Advanced Glycation End Products, Tumor necrosis factor-alpha and interleukin-6; were observed in HMGB1 treated rats, when compared with untreated rats (Bhutada et al, 2014). Significantly higher expression of endometrial HMGB1 was also observed during the receptive phase in the women with unexplained infertility, compared with healthy proven fertile women.

**Conclusion:** Our study, for the first time, employed iTRAQ, a gel-free approach to characterize human uf proteome. The study also demonstrated that an excess of extracellular HMGB1 in the receptive phase induces inflammatory changes in the endometrium which interfere with pregnancy.

**Keywords:** Uterine Fluid, Proteomics, HMGB1, Inflammation, Pregnancy

### **I-35: Emergency Stimulation for Onco-Fertility Patients**

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Fertility preservation for female cancer patients prior to cancer therapy has emerged as an essential component of comprehensive patient care. In the US, approximately 6% of women diagnosed with invasive cancer between 2007 and 2011 that were under 45 years old. The incidence of cancers in reproductive age women signifies a need for fertility preservation options and this need is increasing with the current trend of delayed childbearing and increasing cancer survival.

Cancer treatment is cytotoxic and may result in complete or partial ovarian failure with subsequent subfertility and premature menopause.

Studies indicate that alkylating agents are particularly gonadotoxic with significantly diminished ovaries response.

Pelvic radiation therapy is also highly gonadotoxic to oocyte. Multiple strategies are available to preserve fertility in these patients including embryo and oocyte cryopreservation, cortical and whole ovary cryopreservation, ovarian transplantation, ovarian transposition, IVM and ovarian suppression with GnRH-a controlled ovarian stimulation (COS) for oocyte/embryo cryopreservation is still the preferred method for fertility preservation due to higher success compared to other technologies.

Preserving a woman's fertility requires time for ovarian stimulation and oocyte retrieval which would delay life saving cancer therapy.

Ovarian hyper stimulation syndrome an iatrogenic sequelae of COS and in cancer patients if OHSS develops it can cause the delay in cancer treatment. Thromboembolic events are one of the most concerning events as patients with aneoplasm inherently have a hypercoagulable state that poses and increased risk of morbidity and mortality. Elevated serum estradiol levels as a result of COS with gonadotropins may promote growth of tumors in estrogen sensitive cancers.

For all the above problems we discuss about random start protocols and for the patient with estrogen sensitive cancer, we use natural cycle-aromatase inhibitors-tamoxifen and combined tamoxifen and gonadotropins.

Recent data suggest that GnRH-a used to stimulate endogenous LH surge for oocyte maturation results in reduced risk of OHSS. For prevention of embolism we can use anticoagulant such as low molecular weight heparin.

### **I-36: Updates on Matching IVF Protocols with Patient Segments**

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### **I-37: Triggering Final Oocyte Maturation and Luteal Phase Support-Innovative Approaches**

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## **Genetics**

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### **I-38: New and Old Technologies in the Modern IVF Clinic**

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PGD has been around in various forms for over a quarter of

a century now. During this time it has evolved from simple FISH and direct PCR mutation analysis to whole chromosome profiling using microarrays or next generation sequencing and improved monogene testing using STR linkage or now, recombination mapping. Modern PGD can now deliver highly accurate and reproducible results capable of identifying the best embryo to transfer with pregnancy rates unimaginable only a relatively short time ago. So, how has this all come about? And is it all necessary for the best patient outcome?

Technology has been a big driver in delivering a more complete and comprehensive suite- but it all comes at a price. Is it all essential? Is there even more that will still be needed? Is there still a place for the older technologies? Are there other considerations that can deliver the same opportunities? What is needed to be able to effectively implement a successful PGD program in a clinic?

I will attempt to answer these questions and provide context for how the most appropriate approaches to PGD can be selected and what will be required of the clinic and the laboratory for maximum gains. Different circumstances will require different solutions and this needs to be understood by all involved to ensure the most beneficial outcomes.

### **I-39: Exome Sequencing Reveals New Genes Involved in Human Infertility**

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### **I-40: The Genetic Complexities of Human Sex-Determination**

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### **I-41: Lipid Modification of Hedgehog Prote8n in Testicular Organogenesis**

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### **I-42: Origins and Differentiation of Somatic Progenitors of The Mammalian Gonad Revealed by Single Cell RNA-Seq**

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### **I-43: Expression Profile of Macrophage Migration Inhibitory Factor (MIF) Signaling Pathway as A Potential Biomarker in Pathophysiology of Endometriosis**

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**Background:** MIF via its receptor, CD74, initiates a signaling cascade that leads to proliferation and survival of cells. Also, MIF binding to CD74 activates p38 signaling pathways that lead to positive effect on the expression of COX-2. The aim of this study was to evaluate the gene expression profile of MIF, CD74 and COX-2 in normal, ectopic and eutopic endometrium during menstrual cycle. The expression level of MIF protein in peripheral blood samples of patients was another variable factor checked in this study.

**Materials and Methods:** Quantitative real-time polymerase chain reaction (Q-PCR) was performed using cDNA and primers for MIF, CD74 and COX-2. Also, protein level of MIF in blood serum was measured by ELISA assay.

**Results:** The mean relative expression of MIF, CD74 and COX-2 genes were significantly higher in ectopic endometrium in compare to eutopic and control endometrium. However, there were significantly variations in mRNA expression of these genes in normal, ectopic and eutopic endometrium during menstrual cycle. Also women with endometriosis had significantly higher circulating levels of MIF protein as compared to normal controls.

**Conclusion:** Higher expression of MIF, CD74 and COX-2 genes in ectopic endometrium can be considered as a molecular bi'omarker for endometriosis development and pathophysiology. Variation in the expression of these genes in normal, ectopic and eutopic endometrium during menstrual cycle could play an essential role in reproduction, inflammation and endometrium reconstruction.

**Keywords:** MIF, CD74, COX-2, Endometriosis

### **I-44: Concurrent Whole-Genome Haplotyping and Copy-Number Profiling of Single Cells**

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**Background:** Methods for haplotyping and DNA copy-number typing of single cells are paramount for studying genomic heterogeneity and enabling genetic diagnosis. Before analyzing the DNA of a single cell by microarray or next-generation sequencing, a whole-genome amplification (WGA) process is required, but it substantially distorts the frequency and composition of the cell's alleles. As a consequence, haplotyping methods suffer from error-prone discrete SNP genotypes (AA, AB, BB) and DNA copy-number profiling remains difficult because true DNA copy-number aberrations have to be discriminated from WGA artifacts.

**Materials and Methods:** Here, we developed a single-cell genome analysis method that reconstructs genome-wide haplotype architectures as well as the copy-number and segregational origin of those haplotypes by employing phased parental genotypes and deciphering WGA-distorted SNP B-allele fractions via a process we coin haplarithmisis.

**Results:** Our approach proved accurate on 55 embryos from 12 couples carrying either autosomal dominant, recessive or X-linked Mendelian disorders, or simple or complex translocations. The method allowed diagnosing an embryo for multiple monogenic disorders at once, and, in contrast to current PGD for translocation cases, it enabled distinguishing embryos that inherited normal chromosomes from embryos that inherited a balanced configuration of the rearranged derivative chromosomes.

**Conclusion:** We demonstrate that the method can be applied as a generic method for preimplantation genetic diagnosis (PGD) on single cells biopsied from human embryos, enabling diagnosis of disease alleles genome wide as well as numerical and structural chromosomal anomalies. Moreover, meiotic segregation errors can be distinguished from mitotic ones. The method, therefore, facilitates genetic selection of embryos, and broadens the range of classic PGD.

**Keywords:** Single-Cell Genomics, Haplotyping, Copy Number Typing, Reproductive Genomics, Embryo Genomics

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## Reproductive Imaging

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### I-45: Color Doppler Sonography of Endometrium in Infertility Patients

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Endometrial receptivity is one of parameters which determine the reproductive outcome in *in vitro* fertilization programs. A good blood supply towards the endometrium is usually considered to be an essential requirement for implantation.

Blood vessels of the endometrium can be detected by transvaginal color and pulse Doppler sonography. Color Doppler assessment permits better visualization of endometrial vessels in infertility treatment.

Absence of subendometrial blood flow seems to be more significant negative predictor than morphological assessment of endometrium. Color mapping of endometrial vascularity were classified in various types according to the degree of penetration into the endometrial thickness. In this way we can differentiate four Zones: Zone 0 (absent), Zone1 (sub-endometrial), Zone2 (outer hyperechogenic Zone), Zone 3(inner hypoechogenic Zone).

We aimed to evaluate the role of endometrial and subendometrial blood flows; detected by Color Dopplers Sonography in the prediction of pregnancy rate during IVF treatment.

### I-46: Color Doppler Assessment in IUGR

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Fetal growth restriction is the second leading cause of perinatal morbidity and mortality.

The incidence of intrauterine growth restriction (IUGR) is estimated to be approximately 5 percent in the general obstetric population.

Abnormal uterine artery suggest a maternal cause for the growth restriction where as normal uterine artery Doppler studies suggest that a fetal cause.

Use of umbilical artery Doppler to monitor high-risk fetuses reduces perinatal morbidity and mortality.

A variety of indices of umbilical arterial Doppler waveform, such as resistance index, systolic/diastolic ratio, pulsatility index and diastolic average ratio, is used for predicting perinatal outcome.

Resistance index had the best ability to predict abnormal outcomes.

When an anomaly scan and umbilical artery Doppler are normal, the small fetus is likely to be a 'normal small fetus.

In Doppler study of pregnancy we look for fetal hypoxia, acidosis, asphyxia and anemia as well.

### I-47: Doppler Ultrasound Diagnosis of Intrauterine Lesions

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In patients with abnormal uterine bleeding, transvaginal ultrasound (TVS) is the first-line diagnostic approach to exclude endometrial pathology. If a thickened endometrium suggests endometrial pathology, sonohysterography, office hysteroscopy or dilatation and curettage are the second-line tests preferred, depending on availability and on the suspected pathology, to exclude malignancy and to aid in the decision on management of any pathology found.

However, these invasive tests cause patient discomfort and increase the cost of the examination in comparison with TVS. Discriminating between endometrial polyps and submucous myomas can cause difficulties using traditional greyscale ultrasound, even with instillation of saline. The malignancy potential of endometrial polyps is still dubious.

Power Doppler is a new technology with various advantages over conventional Color Doppler. It is based on the amplitude of the Doppler signal but not on the Doppler frequency shift. It is insonation angle independent, with no aliasing, and is more sensitive to low-velocity blood flow.

Power Doppler is useful to discriminate between submucous myomas and endometrial polyps. The use of power Doppler in the evaluation of intracavitary lesions may prove to play a role in avoiding unnecessary surgery.

To compare power Doppler flow mapping characteristics of endometrial polyps and submucosal fibroids and analyze whether two different power Doppler characteristics, single-vessel pattern and rim-like vessel pattern, can help to differentiate these focal endometrial lesions.

In this presentation, I will discuss about differentiation intrauterine lesions in Color Doppler.

### **I-48: Ultrasound Deformable Model for Virtual Surgery Simulation of Oocyte Retrieval in Infertility Programs**

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**Background:** The use of a medical simulator should enhance the goals of minimally invasive surgery: patient safety, cosmesis, shortening the length of hospital admissions, and reducing cost. Using an innovative approach to the handling of ultrasound images in virtual reality simulation, this article describes a process that employs a hybrid model of deformable models that can be applied in the teaching and practice of oocyte retrieval in infertility programs.

**Materials and Methods:** Oocyte aspiration is modeled using a spline mass model by which the collapse of tissue during

needle insertion can be modeled using tensor mass models. Organ incision can be modeled using a mass-tensor type model in the incision area and the remainder of the organ can be modeled employing a tensor pre-computation approach. The interface between the two can be designed to comply with the finite element method (FEM)-type method. The displacements of the common nodes shared by both models are hence imposed by the mass-tensor model, which, in turn, receives the resulting forces as its input.

**Results:** Analogous to the relationship between robotics and simulation, the present innovation of simulation can be applied to the simulation of ultrasound guided surgeries.

**Conclusion:** The goals of laparoscopic surgery will only be achieved if gynecologic surgeons choosing this less invasive approach have the requisite expertise. We believe that the use of this ultrasound deformable model for virtual surgery, will contribute to the attainment of these skills and improved clinical outcomes.

**Keywords:** Simulation, Oocyte Retrieval, Ultrasound Guided Surgeries, Deformable Display Models, Infertility

### **I-49: Doppler Study in Female Pelvic Tumoral Mass**

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Ultrasound is the first line modality in evaluation of pelvic masses in female patients. It is helpful in detection of masses and also in determining origin of them. Tissue characterization is possible with gray scale but Doppler study is quite necessary and considers as complimentary method.

Most of the time, it should be performed in vaginal US exam. Existence of vascularity and also spectral pattern of detected vessels may help the differential diagnosis.

We are going to review different pelvic masses and their Doppler finding.

### **I-50: Gray Scale and Doppler Features of Retained Products of Conception and Placenta Accrete**

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Postpartum bleeding is a component of the normal physiologic process that occurs after delivery or miscarriage. Normally, bleeding decreases over the first postpartum week. When bleeding is excessive and uterine atony has been excluded as a cause, the next most common cause of postpartum bleeding is retained products of conception (RPOC). The placenta usually constitutes the majority of RPOC, and, because of its highly vascular connection to the uterus, it provides a con-

duit for continued bleeding. Although accurate diagnosis of RPOC is a challenge both clinically and radiologically, it is important for guiding proper management. In this article, we discuss the placental development; the clinical, pathologic, gray scale, and Doppler diagnosis of RPOC; and some potential pitfalls that may mimic RPOC. In this article diagnosis of placenta creta will be discussed.



## Oral Presentations

### Andrology

#### **O-1: Therapeutic Effect of Silymarin, Celecoxib and Exogenous Testosterone on Varicocele-Induced Disorders; Possible Mechanisms**

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**Background:** Varicocele (VCL) is characterized by a progressive disorder which is defined by tortuosity of the pampiniform plexus veins that exerts bilateral impacts. 50-60 percent of males with infertility problems are suffering from VCL. Considering VCL-induced massive problems in human fertilizing potential, investigating different aspects of VCL-induced derangements is gaining considerable attentions. Although, elevated oxidative stress, germinal cells apoptosis and nitrosative stress are reported as some of reasons for infertility in VCL patients, the exact mechanism(s) by which VCL reduces the fertilizing potential is unknown. Roles of cytokines in testicular endocrine status (steroidogenesis) and overexpression of potent pro-inflammatory activators are illustrated in patients with varicocele. Thus, taking together the inflammation, reduced endocrine status associated with various biochemical stresses are generally known for varicocele-induced apoptosis. However, the roles of proto-oncogenes, tumor suppressors, pro-apoptotic genes, testicular steroidogenesis, physiologic interactions of estrogen receptors and genes involved in aromatization are remained unknown. Due to conflicting outcomes of varicocelectomy, the medical/drug intervention as an important alternative gained increasing interest.

**Materials and Methods:** Our previous data for this purpose showed that VCL by up-regulating inflammation ratio enhances oxidative stress and therefore it results in a significant reduction in testicular endocrine status. Moreover, the gene expression of E2F1 (gene involved in DNA fragmentation), protein biosynthesis of HSP70-2 (protein involved in protein, DNA and RNA assembling and reassembling) as well as ubiquitin (protein participating in post transcriptional regulation) were estimated in VCL alone-induced and silymarin co-treated animals. Our findings showed that silymarin (as an antioxidant and antiinflammatory agent) ameliorated the inflammation, oxidative stress, E2F1 gene expression and DNA damage, while it did not considerably affect the endocrine status.

**Results:** Thus, therapeutic properties of dexamethasone+ vitamin E in one study and testosterone+vitamin E in other research were assessed for estimating the possible major role of inflammation, oxidative stress and endocrine potential. Our lastly published data showed that simultaneous administration of testosterone with a potent antioxidant vitamin E

more significantly up-regulated HSp70-2 expression, reduced mRNA damage and enhanced testosterone biosynthesis versus dexamethasone+vitamin E-received ones.

**Conclusion:** Considering dexamethasone-induced side effects, in our last running project we were tried to analyze the ameliorative/therapeutic effects of silymarin (100 mg/kg, potential antioxidant), celecoxib (10 mg/kg, anti-inflammatory drug) and exogenous testosterone (400 µg/kg, endocrine promoter) in experimentally-varicoceled rat models. For this purpose more especial and related parameters were analyzed. The mRNA levels of Bcl-2, p53, caspase III, caspase VIII, ERα, ERβ, cytochrome p450 (CYP19), iNOS and COXII were investigated by RT-PCR. The spermatogenesis and spermiogenesis cells series presenting Bcl-2, p53, ERα, ERβ, caspase III and caspase VIII proteins were stained immunohistochemically. Testicular total antioxidant capacity, malondialdehyde content and carbonyl groups were estimated biochemically. In conclusion, reduced endocrine status, enhanced inflammatory agents and elevated oxidative stress in VCLs, promote the apoptotic pathway at germinal cell level via affecting proto-oncogenes. However, co-administrating silymarin, celecoxib and testosterone ameliorated the VCL-induced derangements

**Keywords:** Varicocele, Apoptosis, Hsp70-2, E2F1, Ubiquitin

#### **O-2: Co-Administration of Korean Red Ginseng and Ciprofloxacin May Improve The Sperm Quality and Apoptosis of Testes in Epididymo-Orchitis Rat Model Induced by Uropathogenic Escherichia Coli**

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**Background:** Adverse effects of genitourinary tracts infections on the reproductive system of male are known. Some of these effects are caused by oxidative events. Ciprofloxacin is a choice antibacterial agent in the treatment of these infections. It has been shown that this drug can also induce apoptosis through the activation of the enzyme caspase-3 and oxidative damages in target tissues. With this background, the evaluation of the effectiveness of antioxidants on the adverse effects of antibiotics, such as ciprofloxacin is expanding. It is also shown that vegetative antioxidants such as Ginseng may be useful in the treatment of male infertility and causes increase of the sperm production rate and testosterone level. Therefore, in this study, with the aid of UPEC infection model, we tried to prove the possible protective role of Ginseng on apoptosis induced by

infection and treatment with ciprofloxacin in Spermatogenesis cell type in rates testicular system.

**Materials and Methods:** In this study, 72 male Wistar rats were selected and randomly divided into 9 groups (8 rats each) such as; control (Con), vehicle (V), infection (M39), ciprofloxacin (C), ginseng (G), ciprofloxacin-ginseng (CG), infection-ciprofloxacin (CM), infection-ginseng (GM), infection-ciprofloxacin-ginseng (CGM). First by injection of 50µl (OD=0.06) *E. coli* suspension (M39) in the beginning of Vazodeferrence tubes, bilaterally, we created UPEC infection models. 48 hours after infection was made, ciprofloxacin applied orally 150 mg/kg daily for 10 days via gastric duct. Then Ginseng 15 mg/kg daily through intraperitoneal (ip) injection applied for 10 days. 14 days later, all animals were anesthetized and the epididymis tissues were immediately removed. Epididymis tail was used for sperm analysis. Testes excised and after weight measurements, the samples immersed in formaldehyde solution. After tissue processing, for tissue structure evaluation, the Miller criteria (to assess layers of Seminiferous tubes of epithelial cells) and Johnson (for the classification of spermatogenesis) and for testicular apoptosis evaluation TUNEL kit were used.

**Results:** M39 infection caused weight gain and testes and accessory sex organs (epididymis, seminal vesicles and prostate gland) weight lost and also reduces the sperm indices such as sperm count, motility and percentage of normal cells and increased apoptosis indices and sperm abnormality index ( $P<0.05$ ). Despite the negative effects of ciprofloxacin on spermatogenesis and weight indices in group C than in the control group, a significant improvement in the CM group than in the M group were seen in most indices ( $P<0.05$ ). Ginseng had a positive effect on all spermatocytics indices and also had additive effect with ciprofloxacin on sperm related indices ( $P<0.05$ ). Despite a reduction in weight indices in Ginseng group compared to control group, Ginseng improved the weight indices in CGM, GM and CG groups compared to M and CM groups. M39 also reduced the miller and increase Johnson criteria, and These effects improved by the aid of ciprofloxacin and Ginseng.

**Conclusion:** By co-administration of Ginseng and ciprofloxacin, the adverse effects of infection caused by UPEC reduced significantly. In future researches we recommend to use different dosage of antibiotic in a much larger group to reach more precise results. In subsequent studies suggested tubules diameter of Seminiferous also be considered. We also suggest evaluating Ginseng administered orally.

**Keywords:** Ginseng, Ciprofloxacin, Spermatogenesis, Apoptosis, Infection

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## Animal Biotechnology

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### O-3: Effect of Melatonin Treatment on Developmental Potential of Somatic Cell Nuclear- Transferred Mouse Oocytes *In Vitro*

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**Background:** Melatonin (N-acetyl-5- methoxytryptamine) is mainly synthesized and secreted in the pineal gland, ovary, testes, bone marrow, retina and lens in mammalian species. It is involved in the detoxification of ROS and protects embryos from oxidative damage. Melatonin acts as a potential free radical scavenger, including peroxy radical and hydroxyl radical. In addition, it can stimulate the activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. Though the beneficial effect of supplementing culture medium with melatonin has been reported previously, to our knowledge, there are no reports on the effect of melatonin on mouse SCNT during *in vitro* embryonic development under culture medium. Therefore, in the present study melatonin was added to the culture medium and its effect on mouse SCNT embryos was investigated.

**Materials and Methods:** In this study, we assessed the effects of various concentrations of melatonin (10<sup>-6</sup> to 10<sup>-12</sup> M) on the *in vitro* development of mouse somatic cell nuclear transfer embryos for 96 h. Embryos cultured without melatonin were used as control.

**Results:** There was no significant difference in cleavage rates between the groups supplemented with melatonin, dimethyl sulphoxide (DMSO) and the control. The rate of development to blastocyst stage was significantly higher in the group supplemented with 10<sup>-12</sup> M melatonin compared with the control group ( $P<0.05$ ).

**Conclusion:** Thus, our data demonstrated that adding melatonin to pre-implantation mouse nuclear-transferred embryos can accelerate blastocyst formation.

**Keywords:** SCNT, Melatonin, Embryo, *In Vitro* Culture

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## Embryology

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### O-4: Morphological Analyses and Apoptosis Genes Expression Evaluation in Vitrified Human Ovarian Tissue after Warming, Long Term Culturing and Xenotransplantation

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**Background:** *In vitro* culturing and retransplantation of vitrified-warmed ovarian tissue are two ways to restore fertility after radiation or chemotherapy. This study aimed to evaluate

the incidence of apoptosis in vitrified human ovarian tissue after warming, long term culturing and xenotransplantation by morphological analyses and apoptosis genes expression evaluation.

**Materials and Methods:** We obtained human ovarian tissue biopsies from 30 women who underwent elective caesarean sections. Tissues were transported to the laboratory, then were cut into small pieces and were divided into two groups, vitrified and non-vitrified. Apoptosis incidence was assessed by light microscope and apoptosis related gene expression in both groups after warming, after 14 days in vitro culture and 30 days after xenotransplantation to  $\gamma$ -irradiated mice.

**Results:** We observed no morphological differences between nonvitrified and vitrified samples before and after culturing. But vitrified grafted tissues showed significantly less normal follicles than grafted-non-vitrified group ( $P < 0.05$ ). The expression of some pro and anti-apoptotic genes in vitrified-warmed tissues were not changed compared to non-vitrified ones but the expression of Fas and caspase8 was increased and the expression of BRIC5 was decreased in this group ( $P < 0.05$ ). In transplanted vitrified group the Bcl2, FasL and BRIC5 gene expression was high and caspase8 was low ( $P < 0.05$ ). The expression of all genes in both grafted groups was more than non-grafted tissues and lower than cultured groups except for caspase8 and BIRC5 ( $P < 0.05$ ).

**Conclusion:** This study provides the first evidence for the effects of vitrification on apoptosis in non-cultured, cultured and grafted human ovarian tissue at mRNA level. We concluded that vitrification could induce apoptosis incidence during long term cultivation and we should improve vitrification protocols and culture conditions to achieve favorite result.

**Keywords:** Vitrification, Apoptosis Gene Expression, Transplantation, Long Term Culture, Human Ovarian Tissue

### O-5: Reprogramming of Paternal DNA Methylome during Spermiogenesis

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**Background:** Chromatin of male and female gametes undergoes a number of reprogramming events during the transition from germ cell to embryonic developmental programs in the zygote. This process involves reorganisation of the patterns of 5-methylcytosine (5mC), a DNA modification associated with regulation of gene activity. Notably, both maternal and paternal genomes undergo Tet3-dependent oxidation of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) in one-cell embryos. Although the precise biological functions of these oxidised forms of 5mC remain elusive, they may play specific roles in active demethylation and transcriptional regulation.

**Materials and Methods:** Here we present the results of genome-scale analysis of 5mC/5hmC/5caC distributions in round spermatids and spermatozoa and demonstrate that reprogramming of the paternal methylome begins during spermatid maturation.

**Results:** We show that patterns of 5caC genomic distribution are highly dynamic during spermiogenesis. Whereas 5caC is eliminated from LINE1 retroposons and transcriptionally active spermiogenesis-specific genes during spermatid maturation, it is simultaneously accumulated at promoter regions and introns of the genes involved in embryo development. Importantly, a large fraction of 5caC-enriched genes also retain nucleosomes marked with bivalent histone modifications in spermatozoa chromatin.

**Conclusion:** Our results suggest that embryonic patterns of DNA modifications are prearranged during spermatid maturation and imply a role for 5caC in poising the activity of developmental genes in mammalian embryogenesis.

**Keywords:** DNA Methylation, Epigenetics, Spermiogenesis, Reprogramming, 5-Carboxylcytosine

### O-6: Maturation of Spermatogenic Cells in Artificial Seminiferous Tubules

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**Background:** This study aimed to investigate whether Artificial Seminiferous Tubules (AST) coated with testicular extra cellular matrix (ECM) support maturation of spermatogenic cells from immature testes.

**Materials and Methods:** Medical grade tubes coated with testicular ECM were used. Testes from immature mice were loaded in the concentration of 10000- 20000/ml. Tubes were then immersed in germ cell culture medium supplemented with a mixture of growth factors and cultured at 32oC for 30 days. After 7 days in culture maturation inducing factors including FSH, SCF and Retinoic Acid were added. After 14 days in culture, the contents of some tubes were collected and samples were taken for histology, DNA content and gene expression analyses. Also some cells were co-cultured with the Sertoli cell monolayer. After 3-4 weeks, cells with the morphology of spermatids and sperm were collected and their ability to fertilize eggs was determined by ICSI and IVF. Furthermore, the ability of the fertilized eggs to develop to embryos was investigated.

**Results:** After 7 and 14 days, small cells similar to the morphology of round and elongated spermatids were present. Localization of the Peanut Agglutinin (PNA) as well as PAS staining revealed the presence of acrosomal structure in these cells. Cells matured in AST expressed meiotic and post meiotic markers and on average 75% of the cells reached to haploid stage after two weeks of maturation. After 18 days in culture more elongated spermatids and cells with the appearance of mature sperm containing head, acrosome, mid piece and tail

were formed. Injection of round spermatids developed in AST into MII eggs resulted in GFP embryos.

**Conclusion:** AST coated with testes ECM created a micro milieu similar to testicular seminiferous tubules and supported maturation of spermatogenic cells.

**Keywords:** Germ Cell, Maturation, Spermatogenesis, *In Vitro*

### **O-7: Detrimental Effects of Dietary Fish Oil without Vitamin E Supplementation on Cryopreserved Sperm of Iranian Mehraban Rams**

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**Background:** Although several studies confirmed positive effects of fish oil on semen quality, the antioxidant status in omega-3 supplemented diets of several studies is a fuzzy point. The aim of this study was to investigate the effect of dietary fish oil and (or) vitamin E supplementation on cryopreserved sperm in Mehraban rams.

**Materials and Methods:** Sixteen fertile rams were randomly allotted to four groups and fed either of four diets during 13 weeks: I. control diet without fish oil and vitamin E supplementation (CON), II. diet containing 200 IU/day/ram vitamin E (VE), III. diet containing 2.5% (dry matter basis) fish oil (FO), and IV. diet containing 2.5% fish oil and 200 IU/day/ram vitamin E (OVE). Semen was collected at 14-d intervals during the breeding season. Semen samples were frozen in a Tris-based diluent, cooled to 4°C, frozen in liquid nitrogen vapor, and stored in liquid nitrogen. Finally, straws were thawed in a water bath (37°C) for 30 sec and evaluated by the CASA system. Data were analyzed using the MIXED procedure of SAS Institute (2003) with a repeated measurement analysis.

**Results:** Sperm motility, viability, progressive motility, type A (rapid progressive) as well as type B (medium progressive) sperm were higher in frozen-thawed semen of rams fed fish oil and vitamin E ( $P < 0.05$ ). Hyperactive cells percent were lower in the FO group. Progressive motility was decreased by feeding dietary fish oil alone, but feeding of fish oil and vitamin E supplementation obliterated the detrimental effect of fish oil on progressive motility. The CASA parameters, (VCL, VSL, VAP, ALH, LIN, STR, WOB, and BCF) were not affected by the dietary treatment.

**Conclusion:** This study elucidates the importance of vitamin E supplementation once fish oil is to be consumed, especially for frozen-thawed semen.

**Keywords:** Antioxidant, CASA, Frozen-Thawed Semen, Progressive Motility

### **O-8: Study of Peroxynitrite Levels, Argin-**

### **ase Activity and NO Synthase Activity in Seminal Plasma of Iraqi Leukocytospermic Patients**

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**Background:** Leukocytes linked directly and indirectly to reactive oxygen species (ROS) formation. Although leukocytospermia is defined as the presence of  $\geq 1 \times 10^6$  white blood cells/mL (WBC/mL) in a semen sample, the presence of less than  $1 \times 10^6$  WBC/mL (low-level leukocytospermia) still can produce a detectable amount of ROS, impairing sperm function and lowering the chances of pregnancy. Low concentrations of nitric oxide (NO) are necessary for the biology and physiology of spermatozoa, but high levels of NO are toxic and have negative effects on sperm functions. Our objective was to assess the effect of leukocytospermia on semen quality, NO levels, NOS activity, and Arginase activity in infertile men.

**Materials and Methods:** Semen samples were obtained from 150 patients and divided into 3 groups: no seminal leukocytes; group 2, men with low-level leukocytospermia ( $0.1-1.0 \times 10^6$  WBC/mL); and group 3, frank leukocytospermia ( $>1.0 \times 10^6$  WBC/mL). After liquefaction of the seminal fluid at room temperature, routine semen analyses were performed. The stable metabolites of NO (nitrite) in seminal plasma were measured by nitrophenol assay. Arginase activity and NO synthase activity were measured spectrophotometrically.

**Results:** Conservative semen parameters between the 3 groups were similar. Peroxynitrite levels, arginase activity, NO synthase activity and various sperm parameters were compared among leukocytospermic patients. Peroxynitrite levels and NO synthase activity were significantly elevated with increasing leukocytospermia. Conversely, arginase activity was significantly decreased in the leukocytospermic patients.

**Conclusion:** Patients presenting with leukocytospermia have elevated levels of NOS activity and peroxy nitrate levels.

**Keywords:** Leukocytospermia, Nitric Oxide Synthase, Arginase, Peroxynitrite, Oxidative Stress

### **O-9: Generation of Haploid Spermatids with Fertilization and Development Capacity from Human Spermatogonial Stem Cells of Cryptorchid Patients**

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**Background:** Infertility affects around 15% of couples, and male factors account for 50%. Cryptorchidism is one of the most common causes for azoospermia. Generation of func-

tional spermatids from azoospermia patients is of unusual significance for treating male infertility. It has been recently reported by peers and us that human spermatogonial stem cells (SSCs) can be clearly identified and cultured for a short and long term period. Round spermatids with unknown function can be derived from spermatogonia *in vitro* in mice (Feng LX, et al., 2002, Science, 297: 392-395). Recently, haploid spermatids with function to be determined are generated from human pluripotent stem cells (Easley et al., 2012, Cell Reports, 2: 440-446). Nevertheless, the generation of functional haploid spermatids from SSCs *in vitro* has not yet been achieved in humans.

**Materials and Methods:** Immunohistochemistry revealed that human spermatogonia remained whereas meiotic germ cells were rare in cryptorchid patients. RT-PCR and meiotic spread assays showed that expression of numerous markers for meiotic and post-meiotic male germ cells was enhanced in human spermatogonial stem cells (SSCs) of cryptorchidism patients by retinoic acid (RA) and stem cell factor (SCF) treatment. Meiotic spreads and DNA content assays revealed that RA and SCF induced a remarkable increase of SCP3-, MLH1- and CREST- positive cells and haploid cells. Single-cell RNA sequencing analysis reflected distinct global gene profiles in embryos derived from round spermatids and nucleus of somatic cells.

**Results:** Spermatogonia remain whereas meiotic male germ cells are rare in cryptorchid patients. - Human SSCs of cryptorchid patients differentiate to phenotypic and haploid spermatids. Distinct gene profiles exist in embryo from haploid spermatid and somatic cell nucleus. - Haploid spermatids from human SSCs have fertilization and development capacity.

**Conclusion:** Here we report an efficient approach to obtain human functional spermatids from cryptorchid patients.

**Keywords:** Human Spermatogonial Stem Cells, Differentiation, Haploid Spermatids, Fertilization and Development Potentials, Cryptorchid Patients

### **O-10: Formation and Molecular Composition of The Sperm Head to Tail Coupling Apparatus**

Hoyer-Fender S

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**Background:** According to a worldwide survey in 2010 infertility affects 48.5 million of couples. In roughly half of the cases infertility is provoked by the male mate. Thus, a significant percentage of young men are infertile but the underlying causes are mostly unknown. Male fertility and reproduction success critically depends on proper formation of the mature sperm. Transmission of the genetic information, harboured in the sperm head, is achieved by tail propelled directed movement. Since impaired sperm motility is a frequent cause of male infertility, the sperm tail cytoskeleton has come into focus in male infertility diagnostics and as a prognostic factor in

assisted conception in human. For directed movement of the sperm and fertilization, the sperm tail has to be tightly connected to the sperm head. A sophisticated structure, known as the head-to-tail coupling apparatus, or shortened HTCA, is the morphological equivalent of head to tail anchorage. The mature HTCA is well described at its ultra-structural level but information about its development during sperm formation, the precursors from which it originated, and its molecular composition is scarce. We have previously shown that the major sperm tail protein ODF1 is essential for the tight linkage of sperm head to tail. Absence of ODF1 in mice caused sperm decapitation and male infertility whereas haplo-deficiency of ODF1 provoked weakening of head-to-tail linkage and severe subfertility in male mice. We have also generated mice deficient for an interaction partner of ODF1 that suffer from male infertility. We are currently investigating haplo-deficient and knock out mice at the molecular and cytological level. Our intention is to figure out the formation of the HTCA during spermiogenesis at its molecular and ultra-structural level taking advantage of our knock out model systems.

**Materials and Methods:** We first generated and analysed knock out mice and investigated the phenotypic effects at the molecular and cytological level.

**Results:** We have generated Odf1-deficient mice on C57BL/6J genetic background and found that homozygous males are infertile. However, spermatogenesis proceeded almost normally based on histological inspection and expression analysis of marker genes by RT-PCR. Ultra-structural inspection of spermatozoa demonstrated that loss of ODF1 caused disturbance of the mitochondrial sheath, and affected the tight association of the outer dense fibers to their corresponding microtubule doublets. The most striking phenotype, however, was complete detachment of sperm heads resulting in acephalic sperm and male infertility. In contrast, haplo-deficient spermatozoa were ultra-structurally normal. However, we found that haplo-deficiency of ODF1 provoked weakening of head-to-tail linkage and severe subfertility in incipient congenic male mice. Additionally, we identified an interaction partner of ODF1 situated in the nuclear membrane that itself caused weakening of the head-to-tail linkage in the haplo-deficient condition similar to ODF1 haplo-deficiency.

**Conclusion:** We have identified the first molecular components of the head-to-tail linkage apparatus. Characterization of the molecular components of the HTCA as well as a precise analysis of HTCA formation and its disturbances will eventually be instrumental for infertility diagnostics, assisted reproduction and development of male contraceptives.

**Keywords:** HTCA, ODF1, Sperm Tail, Outer Dense Fibers, Male Fertility

### **O-11: N-a-acetyltransferase 10 Protein Regulates DNA Methylation and Embryonic Development**

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**Background:** Genomic imprinting is a heritable and developmentally essential phenomenon by which gene expression occurs in an allele-specific manner. While the imprinted alleles are primarily silenced by DNA methylation, it remains largely unknown how methylation is targeted to imprinting control region (ICR), also called differentially methylated region (DMR), and maintained. Here we show that the N-acetyltransferase 10 protein (Naa10p), whose mutation is associated with human Ogden syndrome and severe non-syndromic developmental delay, was required for mouse early embryonic development and genomic imprinting.

**Materials and Methods:** Naa10-null mice were generated for analysis of Naa10p effect on DNA methylation and embryonic development. Cell-based genome-wide studies, molecular biology assays such as chromatin immunoprecipitation and bisulfite sequencing, and in vitro gel shift assay were applied.

**Results:** Naa10-null mice showed partial embryonic lethality, growth retardation, brain disorder and maternal-effect lethality, phenotypes commonly observed in imprinting defects. Consistently, the DNA methylation level of the paternally imprinted alleles of H19-ICR and Rasgrf1-DMR was significantly reduced in Naa10-KO embryos and ES cells. RNA-seq revealed dysregulation of multiple paternally and maternally imprinted genes that correlate with their DNA methylation alteration. Mechanistically, Naa10p facilitates DNA methyltransferase 1 (Dnmt1) binding to specific imprinted alleles by colocalizing with Dnmt1 in specific imprinting control loci. Moreover, Naa10p associates with the imprinted alleles in vivo independently of Dnmt1. Wild-type Naa10p but not the S37P mutant found in the Ogden syndrome directly bound to H19-ICR and increased DNMT1 binding in vitro.

**Conclusion:** Taken together, our data not only uncover a novel function of Naa10p in imprinting gene regulation by recruiting DNA methyltransferase 1 but also links imprinting defects to the Ogden syndrome.

## **O-12: Tubulin Reversible Acetylation – Driving The Moves and The Moves Behind The Drive**

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**Background:** Asthenozoospermia accounts for almost 50% of the cases of male infertility. Our study investigating phosphoproteins differentially expressed in asthenozoospermia has identified the phosphoproteins relevant to sperm motility and the signature molecules likely to be altered in asthenozoospermia. The 66 phosphoproteins differentially expressed included four alpha tubulin isoforms which were differentially expressed in individuals with poor sperm motility; an increase in TUBA3E and TUBA4A and decrease in TUBA3C and TUBA8. Isoforms TUBA3C, TUBA4A and TUBA8 are reported to be abundantly present in the testis. Of these, TUBA3C and TUBA4A are known to be acetylatable isoforms.

Their differential expression in asthenozoospermia prompted us to investigate the role of reversible acetylation of alpha tubulin in sperm motility.

**Materials and Methods:** Alpha tubulin, acetylated alpha tubulin, and isoforms TUBA3C, TUBA4A, and TUBA8 were investigated in Percoll separated human sperm and HDAC6 in rat sperm by Western blot analyses, Flow cytometry, Real-time RT-PCR and IIF localization and data statistically analyzed. The observations were analyzed in silico for obtaining further insights. Interaction between HDAC6 and alpha tubulin was elucidated by IIF co-localization and co-immunoprecipitation studies. Sperm HDAC6 activity, motility and status of Ac alpha-tubulin was investigated in the presence of HDAC inhibitors Trichostatin A, Tubastatin A and Sodium Butyrate.

**Results:** The differential expression of these isoforms was validated in the normal- and asthenozoospermia at protein and transcript level. Investigation of acetyl alpha tubulin expression revealed a reduction in tubulin acetylation in asthenozoospermia. The decrease in TUBA3C and increase in TUBA4A transcripts, both being acetylatable isoforms of alpha tubulin, could be elucidated on the basis of transcription factors binding to the promoters of the respective isoforms while the reduction in acetyl alpha tubulin in asthenozoospermia could be explained on the basis of decreased TUBA3C and the association of HDAC6 with TUBA4A. However the presence of HDAC6 on sperm had not been hitherto reported. In this study we have demonstrated for the first time the presence of HDAC6 transcript and protein in testicular- and caudal-sperm of rat and further by co-localization and coimmunoprecipitation studies we showed that HDAC6 interacts with alpha-tubulin and they colocalize in the mid piece and principal piece of sperm flagella. Using HDAC inhibitors we further demonstrated that HDAC6 in sperm is catalytically active and inhibitors of HDAC6 increase acetylation and restrict sperm motility.

**Conclusion:** Our data suggests an association between reversible alpha tubulin acetylation and sperm motility. We show that alpha tubulin acetylation is reduced in sperm of asthenozoospermic individuals. Paradoxically, our experiments in the rat sperm show that inhibition of HDAC6 increase alpha tubulin acetylation but restrict sperm motility. The persistent expression of HDAC6 on the sperm flagella in the presence of HDAC6 inhibitor hints at a possible role for HDAC6 possibly as a microtubule associated protein (MAP). On the basis of our observations with the human and rat sperm and the available literature on microtubule stability in ataxin 1 KO mice, we conclude that HDAC6 may act as a MAP and maintain dynamic instability in sperm flagella, and that dynamic instability may be a pre-requisite for normal sperm motility.

**Keywords:** Acetylated Alpha-tubulin, Deacetylase Activity, HDAC6, HDAC Inhibitor, Sperm Motility

## **O-13: Evaluation of Leptin Concentration in Seminal Plasma and Its Correlation with Reactive Oxygen Species, Lipid Peroxidation and Pronuclear Formation in Candidates for ICSI Treatment**

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**Background:** Leptin, as a key hormone in energy homeostasis, plays an important role in reproduction and male fertility. However, its negative or positive effect on sperm quality and assisted reproduction technique is still controversial. The aim of this study was the comparison of seminal leptin concentration in selected couples with male and female factor for intracytoplasmic microinjection (ICSI) and its correlation with semen quality, oxidative stress, and pronuclear formation.

**Materials and Methods:** Semen samples were obtained from 94 infertile couples (74 male factor and 20 female factor) who were candidate for ICSI treatment in Royan institute. Following classical semen analysis, seminal plasma was separated for assessment of leptin concentration and lipid peroxidation (LPO). Chemiluminescence was employed for reactive oxygen species (ROS) measurement. The formations about pronuclear (PN) and cleavage rate were also recorded after ICSI.

**Results:** Although, seminal leptin concentration of female factor patients was higher than male factor ( $P < 0.01$ ), lower amount of ROS ( $P < 0.01$ ) significantly was observed in this group. In spite of these outcomes, seminal leptin concentration indicated a positive correlation with LPO in male factor group ( $P < 0.01$ ) and motility in female factor group ( $P = 0.04$ ). Moreover, no correlation was found between seminal leptin concentration and pronuclear formation after ICSI.

**Conclusion:** This study indicates that seminal leptin leads to sperm lipid peroxidation in infertile male and it could be used as a marker of oxidative stress assessment in semen.

**Keywords:** Human Sperm, Leptin, Reactive Oxygen Species, Lipid Peroxidation

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## Ethics and Reproductive Health

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### O-14: Assisted Reproductive Technique History in Iran

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**Background:** Infertility is a major problem with a prevalence around 9% in the world and a life time prevalence of about 25% in Iran. Assisted reproductive technique (ART), came into the world on 1978 with the birth of Luise Brown, but early days of ART in Iran was not clear. The aim of this study is to investigate the history of ART in Iran and its time line in this Islamic society.

**Materials and Methods:** The pioneers in ART were recognized and recruited through snow ball sampling and introduction by Iranian Society of Reproductive Medicine (ISRM). Deep interview was arranged with each pioneer who accepted to participate in the research and written texts of the interviews were certified by them with the permission of publication. Cross referencing was used to make a perspective of launching the ART in Iran as there was not adequate published documents and papers in this regard. For the first achievements only those with a reference was accepted even if it is a newspaper or magazine

**Results:** Although planning and challenges to establish ART centers was started in 1983, this plan was practically formed in 1985. Efforts were continued till the first Iranian ART baby was born in 1990. The economic ravages of the war between Iran and Iraq, followed by religiously driven mistrust of advanced fertility modalities such as IVF, were main reasons for this tardiness. Since permission for ART was issued through an Islamic edict by the Iranian supreme leader in 1984. Facilitators of progress of the ART achievements in Iran were the acceptance of clergy scholars by issuing decrees (FATWAs) which is considered as important as law in our country. Currently, more than 80 ART centers are working in Iran along with acceptable success rate.

**Conclusion:** Although the time difference between the first pregnancy via ART overseas and that in Iran is 17 years; be that as it may, the rapid development in the use of various ART methods in Iran has been remarkable.

**Keywords:** ART, History, Iran, IVF, Infertility

### O-15: Factors Associated with Poor Quality of Life among Infertile Women Undergoing IVF

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**Background:** Infertility is a medical and social condition and has a considerable impact on a person's quality of life (QoL). Due to this impact, nowadays health-systems not only focus on morbidity and mortality but also on quality of life and well-being. The aim of the study was to determine the QoL of women with fertility problem, and identify factors associated with poor QoL.

**Materials and Methods:** This cross-sectional study included 155 women with fertility problems undergoing IVF in a referral fertility center in Tehran, Iran from December 2013 to March 2014. The Fertility Quality of Life (FertiQoL), the Hospital Anxiety and Depression Scale (HADS) and demographic and fertility information questionnaire were administered to all participants. The relationship of anxiety, depression, demographic and clinical variables with the FertiQoL score was assessed using multiple linear regression analysis.

All data analyses were carried out using SPSS version 16 and a  $P < 0.05$  was considered statistically significant.

**Results:** The mean total QoL score was  $62.57 \pm 16.89$ . Multiple regression analysis showed that the anxiety ( $\beta = -1.59$ ,  $P < 0.001$ ) and depression ( $\beta = -2.09$ ,  $P < 0.001$ ) had a negative impact on QoL, moreover, both cause of infertility and failure in previous treatment were significant factors of poorer QoL.

**Conclusion:** QoL in infertile women is negatively affected by their anxiety and depression levels. Thus, healthcare professionals should consider infertile women's psychological distress when examining and treating them; the evidence from this study suggests that more support might be needed for those women who their source of infertility involves both male and female and also unknown.

**Keywords:** Infertility, Quality of Life, Depression, Anxiety

### **O-16: Psychometric Properties of The Persian Version of Prenatal Attachment Inventory (PAI) in Iranian Pregnant Women**

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**Background:** Prenatal attachment inventory (PAI) was developed by Muller in 1993, and has been used widely in many studies and translated into several languages. This study aimed to translate PAI into Persian, and assess the underlying structure of the PAI and the appropriateness of the one-factor solution proposed by Muller

**Materials and Methods:** In this cross-sectional study, a total of 322 primigravidas in 27<sup>th</sup> to 34<sup>th</sup> gestational weeks referring to private and governmental prenatal clinic in Tehran, Iran, were recruited. The Persian versions of the PAI and a demographic questionnaire were administered to all participants, and the participants were re-tested 2 weeks after the initial testing. The psychometric properties of the PAI were investigated: construct validity using confirmatory factor analysis (CFA), internal consistency reliability using Cronbach's alpha, and test-retest reliability using Intra class correlation coefficient (ICC).

**Results:** Results of the CFA indicated that a single-factor model provides a good fit to the data, confirming an original model by its developer. The Cronbach's alpha coefficient for PAI was 0.856 and the test-retest reliability with ICC was 0.784. Considering duration between marriage and pregnancy, women with low duration scored significantly higher than women with high on PAI ( $P = 0.043$ ).

**Conclusion:** The Persian version of PAI showed that one factor structure is adequate, and can be used for measuring psychological affectionate attachment between Iranian mothers and their fetus.

**Keywords:** Prenatal Attachment, Pregnancy, Psychometric Properties

### **O-17: Development A Questionnaire**

### **Measuring Attitude toward Oocyte Donation**

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**Background:** Since there was not a valid and pervasive questionnaire regarding attitude toward oocyte donation, this study aimed to design and develop a tool to measure attitude toward donated oocyte.

**Materials and Methods:** In this methodological research, qualitative research was done on 15 infertile. Also, literature review and search in various data bases were carried out. Validity of this questionnaire was conducted by knowledgeable experts, who determined indexes such as relevancy, clarity, and comprehensiveness. Reliability of the questionnaire was assessed based on the opinion of experts and infertile couples referred to a infertility clinic.

**Results:** The questionnaire was designed in 52 statements in various issues such as oocyte donation process, donor and recipient characteristics, and family, emotional, psychological, legal, religious, and socio – economic dimensions as five scoring points (1 – strongly disagree, 2 – disagree, 3 – partly, 4 – agree, and 5 – strongly agree). The overall relevancy and clarity of questionnaire were respectively 97 and 96%. The overall comprehensiveness was 100%.

**Conclusion:** The findings of this preliminary validation study indicates that questionnaire is a valid measure for measuring and assessing attitude toward donated oocyte, and now it can be used in studies on different groups of a society.

**Keywords:** Oocyte Donation, Attitude, Questionnaire, Infertility

### **O-18: Framework of Informed Consent and Ethical Codes for Clinical Trials Especially Designed for Assisted Reproduction**

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Clinical trials are known as the most valid medical research. It is able to promote medical evidences on prevention, diagnosis, screening, treatment, and quality of life because in this type of study, an intervention is intentionally performed on subjects. Therefore, it encompasses ethical concerns and considerations, especially when human subjects are studied. In other words, clinical trials are considered valuable as they give the best possible response to public health problems in human societies, and do not damage to human's health and



wellbeing. The World Medical Association (WMA) (64<sup>th</sup> WMA General Assembly, Fortaleza, Brazil, October 2013) has developed the declaration of Helsinki as a statement of ethical principles for medical research involving human subjects. There were no dedicated ethical statements only for a certain type of study, especially for clinical trials, in WMA. All principles were designed as cover medical researches. At present, large clinical trials are conducting on various fields. One of the most important existing clinical trials is researches on infertility treatment and Assisted Reproductive Techniques (ARTs). The trials are distinct and different from other trials. Several reasons make such a distinction. First, trials on infertility treatments generally involve multiple participants. If the intervention succeeds, there is a pregnancy that may or may not lead to an infant. Therefore, at a minimum, a successful treatment involves three individuals, that is, a newborn, and potential mother and father. In some reproduction techniques, it is required to use gametes or fetus of others to achieve pregnancy or live birth. In the trials, it is possible to include third party. Of the participants who have the target of reproduction intervention, there is a newborn of infant does not exist at the start of the trial. Most often one is ignored as subjects who affected by such interventions. Hence, there is a gap in ethical statements of WMA, especially for trials on infertility treatments. It needs to modify WMA declaration of Helsinki. to improve the ethical statements of last version of WMA to be applicable for clinical trials regarding infertility treatments.

## Female Infertility

### O-19: A Comparison of Pelvic Magnetic Resonance Imaging, Trans-Vaginal and Trans-Rectal Sonography with Laparoscopic Findings in The Diagnosis of Deep Infiltrating Endometriosis

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**Background:** The performance of TVS, TRS and MRI for the diagnosis of DIE have been separately reported in earlier studies. However, the three methods have not been concurrently compared in terms of their overall performance and classified results as per different anatomical locations of DIE lesions in a large study population. This study was an attempt to compare pelvic magnetic resonance imaging, trans-vaginal and trans-rectal sonography with laparoscopic findings in the

diagnosis of deep infiltrating endometriosis.

**Materials and Methods:** 317 patients with endometriosis undergoing operative laparoscopy from March 2010 to December 2014. Women (mean age 31± 5.4 years) who presented with signs and symptoms of endometriosis and scheduled for operative laparoscopy, underwent pre-operative assessment using pelvic MRI, TVS and TRS. Results were compared with laparoscopy findings. Sensitivity, specificity and accuracy of the three modalities in the diagnosis of DIE lesions were evaluated.

**Results:** Regardless of anatomical location, TRS possessed a marginally higher sensitivity for the diagnosis of DIE lesions than TVS and MRI (81.12% vs. 80.14% and 77.87%, respectively). However, specificity was slightly higher for MRI as compared to TVS and TRS (97.14% vs 96.65% and 95.77%, respectively). In addition TRS held a similar accuracy compared to TVS and MRI (93.28% vs 93.14% and 92.79%, respectively).

**Conclusion:** While TVS is amongst the preferred imaging modalities for the pre-operative assessment of DIE lesions, TRS, can be considered as an alternative modality for the diagnosis of DIE. TRS is an alternative imaging in virgin individuals who may not undergo TVS. MRI should be considered as a complementary method when ovarian fossa and ureter are suspected to be involved with DIE.

**Keywords:** Deep Infiltrating Endometriosis, Laparoscopy, Magnetic Resonance Imaging, Trans-Rectal Sonography, Trans-Vaginal Sonography

### O-20: The Combination of Basic Fibroblast Growth Factor and Follicular Stimulating Hormone Promotes Human Follicle Development *In Vitro* Culture

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**Background:** Fertility preservation is an important part of scientific study in the field of reproductive medicine. Ovarian cryopreservation and in vitro follicles culture provide option for fertility conservation in older women or cancer patients. Basic fibroblast growth factor (bFGF) or FGF-2, is member of fibroblast growth factors family which play critical roles in cell migration proliferation, and differentiation during embryonic development. We planned this study to investigate the effect of Basic fibroblast growth factor in combination with follicular stimulating hormone on the follicular activation, maturation, viability and steroid production in human ovarian tissue culture after vitrification

**Materials and Methods:** Ovarian tissue was obtained from (n=8) women undergoing gynecological operations. Then cortical tissue strips were vitrified for two weeks. After thawing cortical strip were divided to three groups including:

FSH, FSH + bFGF (200 ng) and control (without FSH). Cortical strip were cultured in a ( $\alpha$ -MEM) medium for 14 days. Then ovarian fragments were processed for histological and apoptosis status using Hematoxylin and eosin (H & E) staining and TUNEL kit respectively. Estradiol (E2), Anti mullerian hormone (AMH) and progesterone concentration in culture medium were calculated using ELISA kit and viability were evaluated using Calcein-AM Ethidium homodimer-1.

**Results:** The percentage and diameter of primary and secondary follicles significantly increased in bFGF+ FSH group compared with other groups. Also Estradiol, progesterone and AMH production were higher in the bFGF + FSH group compared with other groups. In addition, the apoptosis status significantly decreased in bFGF + FSH group. Finally in FSH group the diameter of follicle increased and the apoptosis rate decreased compared with control group but it was not significant.

**Conclusion:** The bFGF-FSH combination activated primordial follicles and promotes growth of primordial and primary follicles *in vitro*. On the other hand bFGF-FSH decreases the rate of apoptosis during *in vitro* culture of human cortical strip.

**Keywords:** bFGF, *In Vitro* Culture, Vitrification, Primordial Follicle, FSH

### **O-21: Differential Expression and Epigenetic Pattern of HOX Family Genes in Cumulus Cells of Mature MII Oocytes from Patients with Polycystic Ovary Syndrome**

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**Background:** Polycystic ovary syndrome (PCOS) is the leading cause of infertility in women of reproductive age. HOX genes are important for development of müllerian tract in the embryonic period and adult function. In the current study, cumulus cells (CCs) which have critical roles in successful folliculogenesis and oogenesis, were aimed to monitor epigenetic alterations on histone codes (acetylation/methylation of histone3) in PCOS patients vs. control group.

**Materials and Methods:** CCs were collected from 20 PCOS patients and 20 fertile women (18-36 year) referred to the Royan Institute to have IVF-ICSI under GnRH antagonist protocol. Informed consents were obtained from the participants. After cDNA synthesis, qRT-PCR was performed using specific primers for HOXA1-5, HOXB1-5, HOXC4-5 and HOXD1-4 genes. ChIP-Real Time PCR was performed to quantify the epigenetic marks of H3K9ac/me on regulatory regions of mentioned HOX genes.

**Results:** Expression profile of HOX family genes of CCs,

revealed significant decrease in mRNA levels of HOXA1 and HOXC4 ( $P<0.01$ ) and significant increase in HOXA2, HOXB2 and HOXD4 ( $P<0.01$ ) in PCOS patients versus control group.

Obtained ChIP data showed significant increase of H3K9ac on regulatory regions of HOXA1, HOXB2, HOXC4, HOXD1, HOXD3 and HOXD4 ( $P<0.01$ ), and HOXC5 ( $P<0.05$ ). A significant decrease of H3K9ac was observed for HOXA2, HOXA4, HOXA5, HOXB1 and HOXB5 ( $P<0.01$ ), and HOXB3 ( $P<0.05$ ) in PCOS patients vs. control group. On the other side, the methylation level of H3K9 was significantly decreased on promoters of HOXA2, HOXA3, HOXA4, HOXA5, HOXB3 and HOXC4 ( $P\leq 0.01$ ), and HOXB5 ( $P<0.05$ ) in PCOS patients vs. control group. However, these data showed a significant increase for HOXB1, HOXB2, HOXC5, HOXD1, HOXD3 and HOXD4 ( $P\leq 0.01$ ) and HOXB4 ( $P<0.05$ ).

**Conclusion:** Current study implies a strong correlation between expression and epigenetics marks of HOX family genes and PCOS disorder. This finding might propose further definitions of PCOS, and eventually provides insights to understand the pathogenesis of this disorder.

**Keywords:** Epigenetic, Gene Expression, HOX Genes, PCOS, Female Infertility

### **O-22: Bioenergy/Oxidative Status of Human Ovarian Tissue Cryopreserved by Slow Freezing or Vitrification**

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**Background:** Ovarian tissue cryopreservation represents a promising strategy to preserve the ovarian function in cancer patients. It is usually performed by slow freezing/rapid thawing (SF/RT). Recent studies emphasize an ultrarapid cryopreservation procedure, vitrification/warming (V/W), since it might prevent damages due to ice crystal formation. Comparative studies between the cryopreservation procedures are primarily based on morphological evaluation of ovarian tissue. This study aims to investigate the bioenergy/oxidative status using Confocal Laser Scanning Microscopy (CLSM) of ovarian tissue cryopreserved by SF/RT and V/W, in association with a morpho-ultrastructural analysis by Light (LM) and Transmission Electron Microscopy (TEM).

**Materials and Methods:** Six ovarian biopsies of consent cancer patients were cryopreserved by SF/RT and V/W. Fresh

and cryopreserved tissues were processed for CLSM using MitoTracker Orange CMTM Ros (mitochondria activity) and DCHFDA (intracellular reactive oxygen species levels-ROS) fluorescent probes and for ematossilin/eosin staining (LM) and TEM. Mitochondria activity and ROS levels of fresh, SF/RT and V/W samples were compared by ANOVA.

**Results:** Bioenergy/oxidative status resulted significantly different in fresh and cryopreserved ovarian tissues. Fresh samples presented higher mitochondria activity and ROS levels than SF/RT ( $P=0.002$  and  $P<0.001$ , respectively) and V/W ( $P<0.001$ ) samples. The cryopreservation protocols also differed for mitochondria activity and ROS levels ( $P<0.001$ ). Morpho-ultrastructural analysis showed a well-preserved fresh tissue and stromal and follicle damages in cryopreserved tissues. The most alterations were interstitial oedema and oocytes cytoplasm vacuolization in SF/RT samples, and slight stromal vacuolization and swelling of oocyte mitochondria in V/W samples.

**Conclusion:** CLSM analysis of bioenergy/oxidative status revealed cryopreservation-induced functional ovarian damages, as demonstrated by the reduction of mitochondrial oxidative phosphorylation activity and by the decrease of ROS levels. The application of CLSM, in association with LM and TEM, can be used as an efficient test to evaluate the effectiveness of cryopreservation procedures.

**Keywords:** Cryopreservation of Human Ovarian Tissue, Confocal Laser Scanning Microscopy, Mitochondria Activity, Intracellular Reactive Oxygen Species Levels.

### **O-23: Hysteroscopic Polypectomy without Cycle Cancellation in IVF/ICSI Cycles: A Matched Case-Control Study**

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**Background:** The effect of hysteroscopic polypectomy for endometrial polyps during treatment cycles is still debated and further studies are necessary to evaluate beneficial effect of this procedure.

**Materials and Methods:** This matched case-control study compared retrospectively 86 women with and without hysteroscopic polypectomy who underwent in vitro fertilization and / or Intracytoplasmic sperm injection (IVF/ICSI) cycles during January 2011 to December 2013. We evaluated the recorded data of one hundred sixty women were diagnosed with endometrial polyps less than 2 cm by hysteroscopy during the period of the study in Royan Institute. In fifty-eight patients, polyp resection was performed through hysteroscopic polypectomy during ovarian stimulation in the standard treatment cycles. The interval between polypectomy and embryo transfer was 3–17 days. The hysteroscopy polypectomy during stimulation were approved by the Royan Institute Review Board and Ethics committee. All the women were treated by hysteroscopic polypectomy after informed consent had been taken. The control group

was selected among 102 women who were not treated by hysteroscopic polypectomy by matching for age and polyp size. The outcomes of IVF/ICSI cycles were compared between case and control groups using Student t and chi-square tests.

**Results:** Finally 43 patients in each group were matched for age and polyp size. Results showed the two groups were comparable in terms of body mass index, duration and cause of infertility, number of previous cycles, type and duration of ovarian stimulation, number of retrieved oocytes, number and quality of transferred embryos, fertilization and implantation rates. The clinical pregnancy and live birth rates were similar between case and control groups (34.9 vs. 32.5 and 30.2 vs. 27.9%,  $P=0.9$  and  $P=0.8$  respectively).

**Conclusion:** In present study, we found no beneficial or disadvantage effects of hysteroscopic polypectomy during ovarian stimulation on IVF/ICSI outcomes. We suggest further clinical randomized trials to confirm these results.

**Keywords:** Hysteroscopic Polypectomy, IVF/ICSI Cycles, Case-Control Study

### **O-24: Single Oocyte Secretoma Mapping by NMR-Metabolomics Technology: A Non-Invasive Strategy to Select The Best Oocytes to Fertilize Avoiding Supernumerary Embryos and Increasing Take-Home-Baby-Rate after IVF**

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**Background:** Current strategies based on random selection of MII-oocytes to fertilize appear unsatisfactory in selecting the best number and the most vital oocytes to fertilize especially in poor responder women in which “chronological age” does not mismatch with “biological age”. The metabolomics-profiling approach, evaluating the final products of cell regulatory process (genome/transcriptome/proteome), may represent a non-invasive, cost-effective tool to improve oocyte selection. The aim of the project was to increase the take-home-baby-rate after ARTs by generating an algorithmic and predictive model able to discriminate real time which and how many oocyte should be fertilized to reduce the number of frozen supernumerary embryos, to avoid multiple embryo-transfer policy and to optimize the cost-effectiveness of IVF cycle by increasing the cumulative pregnancy rate.

**Materials and Methods:** We performed 35 ART cycles by standard GnRH antagonist flexible short-protocol using rFSH plus rLH for controlled ovarian stimulation and rhCG for ovulation induction in women expected poor responders since biological age does not mismatch with chronological age. Once all oocytes were retrieved, they were denuded by standard procedure with hyaluronidase solution and incubated in individual drops [containing N-2-hydroxyethylpiperazine-N0-2-ethanesulfonic acid-(HEPES)-buffered human tubal fluid (HTF)] at 37°C in 5%-CO<sub>2</sub>, 5%-O<sub>2</sub>, 90%-N<sub>2</sub> and 98% humidity for 4-hours prior to ICSI. After incubation, oocyte medium

(5µl) was collected from all drops and analyzed by Nuclear-Magnetic-Resonance-Spectroscopy after a standard preparation of samples. Biostatistics evaluation of obtained spectral analysis of oocytes secretoma was performed by univariate and multivariate analysis of Principal Components Analysis and Partial Least Square fitting. Statistical analysis of general features, clinical parameters and biostatistics information was performed by SPSS software.

**Results:** Metabolomic information from 128 oocytes secretoma showed strong significant differences in metabolites of glucoma (glucose, pyruvate, lactate) lipidoma (endocannabinoids, lysophosphatidic acid, and prostaglandins) and proteoma (histidine, tyrosine, alanine, creatine) between fertilized oocytes from those not fertilized. In addition, significant differences in metabolites were also collected from the comparison of fertilized oocytes secretome between ones generating blastocyst and evolutive pregnancy and those generating blastocyst and non-evolutive pregnancy.

**Conclusion:** Metabolic screening of collected oocytes may allow biologist and clinicians to discriminate between the pool of retrieved oocytes those with high fertilization rate, potentially generating high viable embryos and subsequently ongoing pregnancy. The developing technologies based on predictive metabolic screening may improve the success of single embryo-transfer policy (avoiding multiple pregnancy and increasing the cumulative pregnancy rate) starting from the single oocyte fertilization (minimizing the embryos frozen policy and increasing the gamete frozen policy). These innovations may finally result in cost-effectiveness improvement of IVF treatments in poor responders women (increasing the overall take home baby rate and reducing costs).

**Keywords:** Poor Responders, Metabolomics, Nuclear Magnetic Resonance Technology, Bioengineering, Predictive Models

### **O-25: Serum Stem-Cell-Factor Assay in Elderly Poor Responder Patients Undergoing IVF: A New Biomarker to Customize Follicle Aspiration Cycle by Cycle.**

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**Background:** Since the success rate in older poor-responder (POR) infertile women is expected to be extremely low irrespective of the treatment protocol, clinicians continue the search for biomarkers which may predict cases in which proceeding with follicle aspiration may be appropriate. Experimental studies on murine model suggest that SCF(Stem-Cell-Factor) produced by ovarian cumulus granulosa cell is a critical factor involved in the promotion of follicular growth and development of oocytes. In humans, SCF produced during follicular phase may reflect a successful stimulation and oocyte maturation, and so, it may be a predictor of IVF(in-vitro-fertilization) outcome. The aim of our research project is to understand if in POR infertile women undergoing IVF, the serum SCF (s-SCF) concentrations may correlate to in-

tra-follicular SCF (f-SCF) concentrations according to different COH (controlled-ovarian-hyperstimulation) protocols. Moreover we evaluate if s-SCF concentrations before ovulation induction may represent a new tool to establish whether or not to perform follicle aspiration in elderly POR patients undergoing IVF.

**Materials and Methods:** We recruited 37 elderly (43-50 years old) infertile women scheduled for their first fresh non-donor IVF treatment. All eligible patients underwent COH with standard long-protocol (rFSH starting dose 300IU) and, in the event of treatment failure (35 patients), repeat a cycle with LH-protocol (300 IU rFSH+150 IU rLH) within 6 months from the first treatment. When at least 3 follicles exceeded 16mm in diameter we administered rhCG for ovulation induction. Oocyte retrieval took place 35 hours after hCG administration. When obtained, 1 or 2 embryos were transferred 3 days after pick-up. From 144 samples collected at pick-up day, s-SCF and f-SCF levels were measured by ELISA-Kit to evaluate whether different protocols of COH may be associated with different levels of f-SCF and s-SCF, whether a correlation exists between f-SCF and s-SCF and whether a cut off value of s-SCF at ovulation induction might be associated with number and quality of oocytes and embryos obtained.

**Results:** No differences were observed between the two protocols in terms of both f-SCF and s-SCF levels. The comparison between f-SCF and s-SCF levels showed a strong linear correlation ( $P<0.001$ ). The comparison between s-SCF levels and clinical outcomes showed a statistically significant correlation between both the number of MII oocytes retrieved ( $P<0.001$ ) and embryos obtained after fertilization ( $P<0.001$ ). Cases with at least 3 MII oocytes showed s-SCF values  $>800$  pg/mL, 2 MII oocytes  $>600$  pg/mL and 1 MII oocytes  $>400$  pg/mL. In 100% of cases with s-SCF  $<400$  pg/mL no MII oocytes were recovered. All 5 pregnancies occurred in patients with s-SCF values  $>1000$  pg/mL.

**Conclusion:** S-SCF, strongly correlating with f-SCF, demonstrated a good accuracy in predicting both cycles in which no oocytes and cycles in which at least one, two or three oocytes will be collected.

**Keywords:** Stem-Cell-Factor, Poor Ovarian Response, Assisted Reproduction, Predictive Biomarker, Stimulation Protocol

### **O-26: Effects of Recombinant-LH Supplementation on The Proteomic Profile of Follicular Fluid from Poor Responder Patients: Focus on Follicular Growth Factors and Oocyte Maturity Markers**

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**Background:** Poor and fragmentary data was available regarding the effects of recombinant-LH (r-LH) in in-vitro granulosa and theca cells of human origin. No data was available from *in vivo* studies regarding the effects of r-LH supplementation on SCF,EGF,ERK-1/2 and AKT-1 pathways in the fol-

licular fluid of older-poor-responder women undergoing IVF cycle. Evidence from in-vitro and animal studies seems to confirm that r-LH, in addition increasing cAMP levels (involved in steroidogenesis), activates the ERK-1/2 (proliferation) and AKT-1 (anti-apoptotic) pathways. The aim of the study project is to understand if the r-LH supplementation during IVF (in-vitro-fertilization) cycles in poor responder patients may influence the pathways involved in follicular growth and oocyte maturity. To understand which follicular signaling pathways are influenced by r-LH supplementation and finally to understand if these factors may explain the clinical advantages of r-LH supplementation in older poor-responder-patients.

**Materials and Methods:** We recruited 28 poor responder patients older than 42 years. All patients underwent COH (controlled-ovarian-hyperstimulation) using r-FSH 300 IU/day (recombinant-FSH) alone in the first cycle (s-COH group) and using r-FSH (300 IU/day) with r-LH (150 IU/day) supplementation (ex-COH group) in the second cycle. We compared follicular concentrations of SCF, EGF, Erk 1-2, p-Erk 1-2, Akt-1 and p-Akt-1 between the two groups. Follicular levels of SCF, EGF, Erk 1-2, p-Erk-1/2, Akt-1, p-Akt-1 were detected using appropriate ELISA-Kit and reported in pg/mL, ng/mL or Unit/mL, according to the manufacturer's indications.

**Results:** Follicular levels (56 samples) of EGF, Erk 1-2, p-Erk-1/2, Akt-1, p-Akt-1 were significantly different between s-COH versus ex-COH, with the exception of SCF. In detail, mean value of EGF was  $9.40 \pm 2.92$  vs.  $11.75 \pm 3.95$  pg/mL ( $P < 0.05$ ), ERK 1-2  $184.82 \pm 50.15$  vs.  $332.14 \pm 111.35$  pg/mL ( $P < 0.001$ ), p-ERK-1/2  $20.89 \pm 3.41$  vs.  $40.18 \pm 10.37$  U/mL ( $P < 0.001$ ), AKT-1  $5.35 \pm 2.45$  vs.  $10.42 \pm 2.08$  ng/mL ( $P < 0.001$ ), p-AKT-1  $28.07 \pm 8.98$  vs.  $42.36 \pm 10.06$  U/mL ( $P < 0.001$ ), SCF  $830.25 \pm 364.09$  vs.  $735.43 \pm 300.39$  pg/mL (p: n. s.). The increasing intra-follicular levels of proteins (particularly in their active conformation) involved in cellular proliferation and anti-apoptotic pathways may explain the better clinical outcome observed after r-LH supplementation. The absence of significant variations in SCF levels confirmed both that this pathway is activated only by FSH stimulation and that differences collected in other pathways is generated by r-LH signaling.

**Conclusion:** In poor-responder patients, r-LH supplementation during IVF influences the pathways involved in follicular growth and oocyte maturity. The treatment significantly increases follicular levels of EGF, ERK-1/2 and AKT-1 and particularly the availability of the phosphorylated forms (active forms). These evidences explain the improvements in qualitative and quantitative ovarian response. This is the first *in vivo* study reporting these evidences, partially demonstrated by *in-vitro*/experimental studies on animals.

**Keywords:** ART, Poor Responder, Follicular Growth Factors, Oocyte Maturity Markers, Recombinant-LH

### **O-27: Low Dose Aspirin Administration During IVF: Molecular Changes Potentially Involved in Detrimental Effects on Oocytes Maturation and Fertilization Rate**

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**Background:** The hypothesis that the LDA (low-dose-aspirin) could improve ovarian and uterine perfusion induced clinicians to administer it in women undergoing IVF. Unfortunately, no studies have shown significant differences in terms of pregnancy rate among patients treated or untreated with LDA. The absence of proven clinical benefits of LDA supplementation, led us to wonder whether aspirin, administered during COH could worsen the ovarian response and the oocyte fertilization rate. During the peri-ovulatory period and LH trigger, different intracellular signaling pathways (including SCF, EGF and ERK-1/2, AKT-1 phosphorylation) have been demonstrated to be involved in both follicular growth and oocytes maturation. At the same time, the local production of prostaglandins derived by LH surge is considered essential for oocytes maturation and ovulation process. The aim of this study project is to understand if LDA administration during COH may be associated to alterations in ovarian follicle intracellular signaling pathways by the analysis of follicular fluid concentrations of several growth factors such as SCF, EGF, ERK-1/2, p-ERK-1/2, AKT-1, p-AKT-1.

**Materials and Methods:** We recruited 55 normo-responder women (25-45 years) undergoing 2 fresh non-donor IVF cycle for idiopathic primary infertility. All patients underwent long COH protocol using r (recombinant)-FSH at starting dose of 250 IU daily after the check of correct hypothalamic inhibition. All eligible patients were treated in the first cycle with 100 mg/daily LDA starting from the first gonadotropin stimulation day until hCG administration (LDA-Group). In the following cycle (within 6 months from the initial cycle) the same patients repeated COH using the same protocols and gonadotropin drugs without LDA supplementation (control-Group). When an adequate number of follicles was found at transvaginal sonography (TVS), we administrated rhCG. Oocyte retrieval took place 35h after hCG administration and all oocytes were fertilized by ICSI technique. We compared the follicular concentrations of SCF, EGF, Erk 1-2, p-Erk 1-2, Akt-1 and p-Akt-1 in both groups. Follicular levels of SCF, EGF, Erk 1-2, p-Erk-1/2, Akt-1, p-Akt-1 were detected using appropriate ELISA-Kit and reported in pg/mL, ng/mL or Unit/mL, according to the manufacturer's indications.

**Results:** Follicular levels (110 samples) of EGF, Erk 1-2, p-Erk-1/2, Akt-1, p-Akt-1 were significantly different between LDA-Group versus control-Group, except for SCF concentration. Mean value of EGF was  $8.12 \pm 1.81$  vs.  $12.73 \pm 3.25$  pg/mL ( $P < 0.01$ ), ERK 1-2 was  $248.73 \pm 49.16$  vs.  $345.21 \pm 115.12$  pg/mL ( $P < 0.001$ ), p-ERK-1/2  $13.48 \pm 4.02$  vs.  $37.33 \pm 12.42$  U/mL ( $P < 0.001$ ), AKT-1  $7.99 \pm 3.25$  vs.  $12.18 \pm 3.64$  ng/mL ( $P < 0.001$ ), p-AKT-1  $19.12 \pm 10.17$  vs.  $44.18 \pm 12.81$  U/mL ( $P < 0.001$ ), SCF  $769.32 \pm 361.02$  vs.  $802.64 \pm 298.35$  pg/mL (p: n. s.).

**Conclusion:** The increasing of intracellular and intra-follicular levels of several growth factors represent one of the most reported mechanism through rLH improve the oocytes quality during IVF in poor responders patients. During spontaneous ovulation cycle, the physiological LH raise increases the oxidative status of follicular environment via COX-2 induction as effects of post-receptor signaling. LDA acting as an non

selective COX 1-2 inhibitor may potentially reduce the expression of several growth factors involved in oocytes maturation. Our data clearly showed that follicular levels of EGF, ERK-1/2, AKT-1, and their phosphorylated forms p-ERK-1/2 and p-AKT-1 were significantly reduced in patients receiving LDA as opposite was demonstrated in other our research project in which rLH supplementation increased the levels of the above mentioned growth factors.

**Keywords:** ART, Follicular Growth Factors, Oocyte Maturity Markers, LH Activities, Low Dose Aspirin.

### **O-28: Role of HOX Family Related Genes in Pain Generation of Endometriosis Patients**

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**Background:** Endometriosis is a common gynecological disease, can cause severe pelvic pain. Studies demonstrated the presence of sensory nerve fibers in endometrium of endometriosis patient.

Nevertheless, no information is available on mechanisms of sensory nerve formation in eutopic or ectopic lesions. Since HOX genes have important roles in both reproductive tract and nerve growth, we decided to determine role of them in formation of new nerve fibers in endometriotic lesions.

**Materials and Methods:** Samples obtained from fifteen patients (15 with and 15 without endometriosis) of reproductive age with normal menstrual cycles, where the same patient provided both eutopic and ectopic tissues and control samples were surgically checked for the absence of endometriosis. The expression profile of 84 genes of HOX family related to various aspects of nerve fibers formation (formation of nociceptors, dopaminergic neurons and signal transduction) was investigated using qRT-PCR array.

**Results:** Our data showed significant over-expression of some genes which are involved in various aspect of development of sensory neurons in the tissue and signal transduction such as genes that involved in relay pain, touch and GABAergic neurons formation (VAX1 , LBX1, LBX2 and PITX2), dopaminergic neurons (EN1 and PITX3), committed to a cutaneous sensory neuron fate (PAX3), chemosensory integration (PHOX2b), promote nerve formation (DRGX, EMX1, PHOX2B and OTP), various aspects of somatic motor neuron (ISL1, 2), neuronal migration (HLX) and touch/mechanosensory neurons (SHOX2) in eutopic and ectopic tissue versus control group. All of these genes have significant differences more than 30-1800 times in fold regulation between groups and P value for each of them is less than 0.001.

**Conclusion:** Overexpression (up to 1800 times) of some HOX family related genes that play critical roles in sensory, adrenergic and cholinergic neurons formation, lead to aberrant innervations of ectopic and eutopic tissues. So, formation of new plexus of neuron and secretion of many inflammatory mediators released from endometriotic lesions can stimulate sensory nerve fibers to cause pain symptoms.

**Keywords:** Endometriosis, Pain, Nerve Fiber, HOX

### **O-29: Aberrant Methylation of Lysine 9 on Histone 3 in PII Promoter of CYP19A1 Gene in Women with Endometriosis**

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**Background:** Cytochrome aromatase p450, encoded by the gene CYP19A1, is a key enzyme for estrogen biosynthesis. Among the multiple promoters of CYP19A1, the proximal promoter PII is the most active ones in ovary and endometrium. Endometriosis is a chronic estrogen dependent gynecological condition characterized by the presence of ectopic glands and stroma outside the uterine cavity. Recently, evidence has emerged that endometriosis is an epigenetic disease. Thus, in the current work, the expression and epigenetic alteration of CYP19A1 were aimed to study.

**Materials and Methods:** Epigenetic analysis of PII promoter of CYP19A1 was assayed by chromatin immunoprecipitation (CHIP), using anti-methylated histone 3 (H3K9Me2) antibody. Also, quantitative expression analysis of this gene was performed by real-time PCR technique. To this end, ectopic lesions and eutopic endometrium samples were collected using laparoscopy from 10 women with documented endometriosis. As a control group, endometrial tissues were collected from 10 healthy fertile women who underwent laparoscopy for tubal ligation surgery during menstrual cycle. Informed consent was collected from each participant.

**Results:** Our finding showed that the methylation level of H3K9me2 in promoter PII decreased in eutopic endometrium in proliferative phase and increased in secretory phase in patients vs. control group. Furthermore, the gene expression profile was the inverse of this suppressive epigenetic mark. However, incorporation of H3K9me2 and mRNA expression of CYP19A1 in ectopic endometrial tissues was higher in both phases versus control group.

**Conclusion:** These results show an epigenetic switch between PII promoter of CYP19A1 gene in endometrial and endometriotic tissue of patients with endometriosis. Aberrant

histone methylation may play a role in progression of endometriosis and support the opinion that epigenetic abnormalities have causative function in endometriosis. Therefore, it is suggested to investigate the epigenetic alteration of other promoters correlated with this gene in patients with endometriosis.

**Keywords:** CYP19A1, Epigenetic, Menstrual Cycle, Endometriosis

### **O-30: The Correlation between Myometrial Thickness and *In Vitro* Fertilization/ Intracyto- Plasmic Sperm Injection (IVF/ ICSI) Outcomes**

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**Background:** This study was designed to evaluate the relationship between baseline myometrial thickness and IVF-ET/ICSI outcomes and to assess the myometrial thickness changes after ovarian stimulation.

**Materials and Methods:** In this prospective cohort study, 453 infertile women undergoing IVF-ET/ICSI cycles without any obvious pathology of uterine were enrolled. Trans-vaginal ultrasounds were conducted on days 2-4 of the cycle (menstrual phase) preceding ovarian stimulation and the day of human chorionic gonadotropin (hCG) injection for measurement of myometrial thickness in anterior and posterior of uterine. The summation of baseline myometrial thickness in anterior and posterior (anterior-posterior diameter), preceding ovarian stimulation was divided into three following main groups: (A) <25 mm, (B) 25-29.9 mm and (C) ≥30 mm.

**Results:** The clinical pregnancy and implantation rates were significantly lower in group A as compared to groups B and C (P=0.027 and P=0.006, respectively). The mean of myometrial thickness in anterior and posterior of uterine on the day of injection of hCG decreased significantly compared to the previous ovarian stimulation (P<0.001).

**Conclusion:** In IVF-ET/ICSI cycles, the baseline myometrial thickness (anterior-posterior diameter) <25 mm is associated with lower implantation and pregnancy rates compared with a myometrial thickness ≥25 mm.

**Keywords:** Myometrial Thickness, Trans-Vaginal Ultrasound, IVF-ET/ ICSI Outcome

### **O-31: AMH and AMHR2 Genetic Variants in Chinese Women with Primary Ovarian Insufficiency and Normal Age at Natural**

### **Menopause**

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**Background:** To investigate the role of the anti-Müllerian hormone (AMH) signalling pathway in the pathophysiology of idiopathic primary ovarian insufficiency (POI) and age at natural menopause (ANM) using a genetic approach

**Materials and Methods:** DNA sequencing was used to detect the genotype distribution and allele frequency of the genes AMH and AMH receptor II (AMHR2) in 120 cases of idiopathic POI and 120 normal-ANM women

**Results:** Fourteen sequence variants of AMHR2, including 10 novel variants, were identified. Two novel exonic missense variants were p.I209N and p.L354F. The missense variant p.I209N, which is conserved in different species, was predicted to have functional and structural impacts on the AMHR2 protein. The clinical significance of one additional variant (p.L354F) remains arguable pending functional studies. The genotype frequencies of AMH and AMHR2 were similar in distribution for POI patients and normal-ANM women

**Conclusion:** These findings suggest that POI patients and normal-ANM women in China share AMH and AMHR2 genetic variants. The AMH signalling pathway associated with ANM also may contribute to POI

**Keywords:** Age at Natural Menopause, AMH Receptor, Anti-Müllerian Hormone, Primary Ovarian Insufficiency

### **O-32: Endometrial Secretome and Its Role in Uterine Functions**

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**Background:** Earlier it was believed that uterine fluid (uf) or secretions are not relevant after the embryo implantation in humans. However, recent reports suggest that uterine secretions continue to play important role till the first trimester of human pregnancy. Evidences also suggest that uf mirrors

endometrial functions or dysfunctions. Considering the relevance of uf in endometrial functions, studies were undertaken 1. to develop human uf proteomes and 2. to identify uf proteins that display differential abundance during the receptive phase and also 3. to assess the functional relevance of differentially abundant proteins using an animal model.

**Materials and Methods:** 2D-PAGE and gel-free Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) were employed. Samples were collected in the pre-receptive (i.e. day two post-ovulation, n=7) or receptive phase (i.e. day six post-ovulation, n=7) of the menstrual cycles, from regularly cycling healthy fertile women. Receptive phase samples were also collected from women with unexplained infertility. Further, to test the functional significance, rats (*Rattus norvegicus*) were used as an experimental model. Samples were collected in the proestrous (n=8) and metestrous (n=8) phases of estrous cycles and also from pregnant rats (n=18) during day 3-5 post-coitum (p.c.).

**Results:** Our studies demonstrated higher abundance of uf alpha-1 antitrypsin precursor and apolipoprotein A-1 in the receptive phase, than in the nonreceptive phase, in regularly cycling women (Parmar et al, 2008). iTRAQ revealed identities of 127 proteins in the human uf. Of these, 27 proteins displayed differential abundance in the receptive (R) phase, compared to the pre-receptive (PR) phase. High Mobility Group Binding Protein 1 (HMGB1), one of the differentially abundant proteins displayed less abundance in the R phase than in the PR phase; in secretions as well as in endometrial tissues. Interestingly rats also revealed a lesser abundance of HMGB1 in the receptive phase, compared to that in the nonreceptive phase uf (Bhutada et al. 2013). Thus, human and rat data indicated an association of endometrial receptivity with a decline in the levels of uf HMGB1. A significant decline was also observed in the expression of endometrial HMGB1 on the day of implantation in pregnant rats. Further, recombinant HMGB1 (0.25-1.5 ug/horn) was administered on day three p.c. in mated rats. The horns administered with HMGB1 showed pregnancy failure, whereas those with saline remained unaffected. This indicated the detrimental effect of an excess of extracellular HMGB1 on pregnancy in rats. Also, morphological changes in the endometrium, an increase in the expression of luminal epithelial NF $\kappa$ B; and also various inflammatory molecules such as Receptor for Advanced Glycation End Products, Tumor necrosis factor-alpha and interleukin-6; were observed in HMGB1 treated rats, when compared with untreated rats (Bhutada et al. 2014). Significantly higher expression of endometrial HMGB1 was also observed during the receptive phase in the women with unexplained infertility, compared with healthy proven fertile women.

**Conclusion:** Our study, for the first time, employed iTRAQ, a gel-free approach to characterize human uf proteome. The study also demonstrated that an excess of extracellular HMGB1 in the receptive phase induces inflammatory changes in the endometrium which interfere with pregnancy.

**Keywords:** Uterine Fluid, Proteomics, HMGB1, Inflammation, Pregnancy

### **O-33: Usage, Development and Effectiveness of A New Surgical Technique for The Treatment of Severe Adenomyosis**

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**Background:** Advised treatment of the severe forms of Adenomyosis is Hysterectomy but for the patients that want to preserve their uterus, conservative surgery named "Adenomyomectomy" for removal of endometrial tissue from the myometrium can be performed. This technique must be developed for reduction of the spontaneous uterine rupture, adhesions and recurrence rate. So, this study is to investigate the safety and therapeutic outcomes of different and novel adenomyomectomy technique.

**Materials and Methods:** Prospectively, 103 Iranian patients with documented severe adenomyosis were candidates for adenomyomectomy over a period of 7 years (April 2004 -March 2011). The surgical procedure is the resection of adenomatous lesions with the thin (0.5 cm) margin, wedge shaped in two sides of the uterus wall, with sagittal incision on the body of the uterine and reconstruction of the layers and inverted suture for the serosa layer ends.

**Results:** Out of 103 patients, 57 cases (55.34%) were presented with infertility, 17 cases (16.5%) with IVF failure, 9 cases (8.74%) with recurrent abortion and 20 cases (19.42%) with Abnormal Vaginal Bleeding (AUB). Out of 70 patients that wish to bear child, 21 persons (30%) become pregnant; spontaneously (7 cases) or by ART technique (14 cases). 16 pregnancies became full term and candidates for cesarean section (C/S). There was a significant reduction in both dysmenorrhoea and hypermenorrhoea. Only one case had relapsed adenomyosis.

**Conclusion:** Adenomyomectomy is the conservative and effective option on treatment of adenomyosis. The described procedure in this study can be an efficient procedure for the treatment of severe adenomyosis.

**Keywords:** Adenomyomectomy, Severe Adenomyosis, Surgical Procedure, Treatment Outcome, IVF Failure.

### **O-34: Cytoplasmic Transplantation in Oocytes Obtained from Ovarian Tissue Xenotransplantation Lead to Reduced Apoptosis and Higher Fertilization Rate**

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**Background:** Although ovarian tissue cryopreservation and transplantation is used for fertility preservation in patient undergoing cytotoxic treatments but obtaining good quality oocyte is still a debate. Xenotransplantation has been introduced as a reliable technique for better understanding of the transplantation conditions, but to achieve healthy embryos, the quality of obtained oocytes need to be improved.

**Materials and Methods:** Mature oocyte were obtained from xenotransplanted human ovarian tissue which were cryopreserved for about 3 years. Cytoplasmic transplantation was done in experimental group amongst good oocytes and those which had impaired quality. Mechanisms involved after cytoplasmic transplantation in improving the oocyte quality in recipient oocytes were studied (n=138).

**Results:** Cytoplasm transfer from healthy donor oocyte significantly improved the mitochondrial activity leading to reanimation of the recipient oocyte and improving the quality, fertilization rate (P<0.01) and blastocyst formation rate (P<0.01). Our findings also showed that ATM mediated double strand DNA break repair was significantly improved after cytoplasmic transplantation.

**Conclusion:** Cytoplasmic transplantation after grafting human ovarian tissue can improve the outcome of the treatment and can be used for further exploration of the mechanisms involved in oocyte aging and poor developmental capacity.

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## Genetics

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### O-35: Assessment of Genetic Variations of *DPY19L2* in Total Globozoospermic Patients Referring to Royan Institute

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**Background:** Globozoospermia is a rare but severe teratozoospermia disorder which causes male infertility. Total globozoospermia is diagnosed by the presence of 100% round-headed spermatozoa lacking an acrosome in semen analysis. Recent studies have shown that in large majority of globozoospermic patients, deletion of a 200 kb segment including the *DPY19L2* gene occurs. Among all the genes in this locus, *DPY19L2* is the only gene which is dominantly expressed in the testis and has been shown to be involved in the cause of this phenotype. The aim of this study was to assess genetic variations among Iranian infertile men with total globozoospermia referred to Royan Institute.

**Materials and Methods:** The DNA of 24 total globozoospermic and 24 men with normal spermogam as the control group, were extracted from their blood samples. After design-

ing primers for the break points and exons 1, 5, 7, 8, 9, 19 and 21, PCR reactions were done for each DNA sample.

**Results:** As it was shown in our previous study, *DPY19L2* was deleted in 70.83% of total globozoospermic patients. Evaluation of some exons of this gene revealed that in 28.57% of patients who had *DPY19L2* gene, exon 7 was deleted.

**Conclusion:** This study confirms that *DPY19L2* is the major gene responsible for total globozoospermia in Iranian men, which is in accordance with previous reports for patients from different ethnicities. Moreover, deletion of exon 7 is also one of the possible causes of this disease.

**Keywords:** *DPY19L2* Gene, Total Globozoospermia, Male Infertility

### O-36: Evaluation of Genetic Variations in Intron 4 and Exon 5 of *RABL2B* Gene in Infertile Men with Oligoasthenoteratospermia and Immotile Short Tail Sperm Defects

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**Background:** One of the main causes of male infertility is defect in structure and function of sperm cells. Infertile men with oligoasthenoteratospermia (OAT) defect, have sperms with abnormalities in count, motility and morphology. Patients with immotile short tail sperm (ISTS) disorder have immotile short-tailed sperm with disorganized axonem, and a significant decrease in sperm counts. Numerous proteins are involved in sperm formation. One of these proteins is RAB Like 2B (*RABL2B*), which recently its essential role in fertility in male mouse has been demonstrated. So its gene, which called RAB Like 2B (*RABL2B*), is an appropriate candidate gene in human studies. Exon 5 of *RABL2B* gene, codes one of the main domains of the protein and intron 4 is the location for binding of some important transcription factors. The purpose of this study was to evaluate the genetic variations of exon 5 and intron 4 of *RABL2B* gene in infertile men with OAT and ISTS defects.

**Materials and Methods:** In this study, 30 infertile men with OAT, 30 patients with ISTS and 30 normozoospermic men as controls were recruited. To study the genetic variations, DNA was extracted from peripheral blood, and then PCR sequencing was done.

**Results:** Sequence analysis results did not identify any mutations or single-nucleotide polymorphisms (SNPs) in exon 5, but an intronic variant (rs:144944885), was found in heterozygote form in 5 patients with OAT and one patient with ISTS. No mutation or SNP was identified in controls. Bioinformatics analysis suggested that SRSF7, which is a splicing factor, binds to this part of intron.

**Conclusion:** Due to the high expression of *RABL2B* gene in testis, and considering the fact that not many studies have been conducted about the role of this gene in human male fertility, evaluation of other exons and regulatory areas of this gene is recommended.

**Keywords:** OAT, ISTS, *RABL2B* Gene

### **O-37: Pseudomalignant Nature of Placenta during Normal and Pathological Gestation Is Regulated by Epigenetic Mechanisms which Can be Exploited To Design Non-Invasive Fetal Dna Markers**

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**Background:** Placentation shares many analogues with the development of tumors such as rapid proliferation, invasiveness, gene expression profiles especially the expression of tumor suppressor genes, oncogenes and matrix metallo proteinases (MMPs). Thus, a placenta has been described as a pseudomalignant tissue. However, placentation is tightly regulated and any deregulation of this pseudomalignant nature leads to the development of gestational trophoblastic diseases (GTDs) and preeclampsia. Therefore, this study was designed to analyze the role of epigenetic mechanisms like DNA methylation and histone3 trimethylation at promoter regions of tumor suppressor genes (RASSF1A, APC, P16, RB1 and PRKCDBP), oncogenes (c-myc, c-jun, VEGF, EGFR and hTERT) and MMPs (MMP-2 & -9) and tissue inhibitors of MMPs (TIMP-2 & -1) in maintaining normal pregnancy and pathogenesis of pregnancy related disorders. Further, the methylation data was utilized to search for novel fetal DNA epigenetic markers in maternal plasma.

**Materials and Methods:** Epigenetic profiling was done in five subject groups, which included first, second and third trimester with normal pregnancy and preeclampsia and hydatidiform mole complicated pregnancies (n=30 for each group) and a choriocarcinoma cell line (JEG-3). Placental villi sample and maternal blood were collected from each pregnant woman. DNA methylation and Histone3K9/27me3 were estimated via Methylation-Specific High Resolution Melting (MS-HRM) and chromatin immunoprecipitation assay (ChIP) respectively, while mRNA expression was quantified by qRT-PCR.

**Results:** Our study revealed the association of advancing normal gestation with decreasing expression of RASSF1A and APC mediated by DNA methylation and H3K9/27me3, while increasing expression of P16, RB1 and PRKCDBP regulated by H3K9/27me3 only for P16 and RB1, while by H3K9/27me3 and DNA methylation for PRKCDBP within normal placental villi samples. CpG methylation independent and H3K9/27me3 mediated reverse trend was observed in the differential expression of MMP-2 & -9 and their inhibitors (TIMP-2 & -1 respectively) in regulating the placental inva-

sion during normal pregnancy. Decreasing expression levels of VEGF, EGFR, c-myc, c-jun and hTERT with advancing normal gestation, was independent of DNA methylation, while mediated by H3 K9/K27 trimethylations. Development of preeclampsia was observed to be associated with decreased expression of all studied oncogenes, MMPs, RASSF1A and P16 and increased expression of TIMPs and PRKCDBP. However, development of GTDs were observed to be associated with increased expression of oncogenes, MMPs and decreased expression of tumor suppressor genes and TIMPs. These changes were associated with abnormal changes in DNA methylation and H3K9/27me3. Further, based on difference in methylation of these genes of placental and maternal origin, we have been able to report few fetal DNA epigenetic markers in maternal plasma, to be utilized for prenatal diagnosis like APC, PRKCDBP and for specific diagnosis of pregnancy related disorders like c-myc and VEGF.

**Conclusion:** Thus, our study is the first of its kind to report the role of DNA and H3 methylation in regulating the pseudomalignancy during normal gestation and dysregulation of these mechanisms as contributing factors for development of placental disorders. Further, we have reported a few novel fetal DNA markers for non-invasive prenatal diagnosis and specific diagnosis of pregnancy related disorders.

**Keywords:** Placenta, DNA Methylation, Histone3 Trimethylation, Preeclampsia, Gestational Trophoblastic Diseases

### **O-38: Concurrent Whole-Genome Haplotyping and Copy-Number Profiling of Single Cells**

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**Background:** Methods for haplotyping and DNA copy-number typing of single cells are paramount for studying genomic heterogeneity and enabling genetic diagnosis. Before analyzing the DNA of a single cell by microarray or next-generation sequencing, a whole-genome amplification (WGA) process is required, but it substantially distorts the frequency and composition of the cell's alleles. As a consequence, haplotyping methods suffer from error-prone discrete SNP genotypes (AA, AB, BB) and DNA copy-number profiling remains

difficult because true DNA copy-number aberrations have to be discriminated from WGA artifacts.

**Materials and Methods:** Here, we developed a single-cell genome analysis method that reconstructs genome-wide haplotype architectures as well as the copy-number and segregational origin of those haplotypes by employing phased parental genotypes and deciphering WGA-distorted SNP B-allele fractions via a process we coin haplarithmisis.

**Results:** Our approach proved accurate on 55 embryos from 12 couples carrying either autosomal dominant, recessive or X-linked Mendelian disorders, or simple or complex translocations. The method allowed diagnosing an embryo for multiple monogenic disorders at once, and, in contrast to current PGD for translocation cases, it enabled distinguishing embryos that inherited normal chromosomes from embryos that inherited a balanced configuration of the rearranged derivative chromosomes.

**Conclusion:** We demonstrate that the method can be applied as a generic method for preimplantation genetic diagnosis (PGD) on single cells biopsied from human embryos, enabling diagnosis of disease alleles genome wide as well as numerical and structural chromosomal anomalies. Moreover, meiotic segregation errors can be distinguished from mitotic ones. The method, therefore, facilitates genetic selection of embryos, and broadens the range of classic PGD.

**Keywords:** Single-Cell Genomics, Haplotyping, Copy Number Typing, Reproductive Genomics, Embryo Genomics.

organ can be modeled employing a tensor pre-computation approach. The interface between the two can be designed to comply with the finite element method (FEM)-type method. The displacement of the common nodes shared by both models are hence imposed by the mass-tensor model, which, in turn, receives the resulting forces as its input.

**Results:** Analogous to the relationship between robotics and simulation, the present innovation of simulation can be applied to the simulation of ultrasound guided surgeries.

**Conclusion:** The goals of laparoscopic surgery will only be achieved if gynecologic surgeons choosing this less invasive approach have the requisite expertise. We believe that the use of this ultrasound deformable model for virtual surgery, will contribute to the attainment of these skills and improved clinical outcomes.

**Keywords:** Simulation, Oocyte Retrieval, Ultrasound Guided Surgeries, Deformable Display Models, Infertility

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## Reproductive Imaging

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### **O-39: Ultrasound Deformable Model for Virtual Surgery Simulation of Oocyte Retrieval in Infertility Programs**

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**Background:** The use of a medical simulator should enhance the goals of minimally invasive surgery:

patient safety, cosmesis, shortening the length of hospital admissions, and reducing cost. Using an innovative approach to the handling of ultrasound images in virtual reality simulation, this article describes a process that employs a hybrid model of deformable models that can be applied in the teaching and practice of oocyte retrieval in infertility programs.

**Materials and Methods:** Oocyte aspiration is modeled using a spline mass model by which the collapse of tissue during needle insertion can be modeled using tensor mass models. Organ incision can be modeled using a mass-tensor type model in the incision area and the remainder of the

## Poster Presentations

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### Andrology

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#### **P-1: Morphine Has Detrimental Effects on Sperm Parameters, Chromatin Quality and DNA Integrity in Mice**

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**Background:** Morphine as a natural alkaloid (opiate) is considered as one of the problems associated with poor semen production and sperm quality. For the first time, this experimental study was carried out to evaluate the impact of intraperitoneal injection of morphine on sperm parameters, chromatin quality and DNA integrity of spermatozoa aspirated from cauda epididymis of mice.

**Materials and Methods:** Totally 24 adult male balb/c mice (8 weeks old, 30 g) were equally divided into 3 groups (n=8). Mice of group 1 served as control fed on basal diet, group 2 received basal diet and normal saline and group 3 received basal diet and morphine (15 mg/kg/daily, intraperitoneal) for 35 days. Finally, the right tail of epididymis of each animal was removed and placed in Ham's F10 for 30 min. Released spermatozoa were used to analyze count, motility, viability (eosin-nigrosin staining), morphology (Papanicolaou stain), chromatin quality with Toluidine blue (TB) and apoptosis via TUNEL assay.

**Results:** In morphine-treated mice a significant decrease was found in sperm viability, normal morphology, count and motility compared to other groups (P<0.05). With regard to chromatin quality the rates of TB-reacted spermatozoa were significantly higher in morphine group (P<0.05). In addition, in morphine mice there was a significant increase in apoptosis compared to other groups (P<0.05).

**Conclusion:** Morphine abuse disturbs sperm parameters and DNA integrity and also causes the production of spermatozoa with less condensed chromatin. Thus, the above mentioned anomalies may reduce the fertility potential of spermatozoa in mice as an experimental model.

**Keywords:** Mice, Morphine, Sperm Parameters, Chromatin Quality, Apoptosis

#### **P-2: Protective Effects of Ethyl Pyruvate on Serum Antioxidant Capacity and Testis Germinal Epithelium Morphometry in Cyclophosphamide Treated Mice**

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**Background:** Cyclophosphamide (CP) is a chemotherapeutic drug which causes deleterious effects on tissue of testicle and increases free radicals in the body. The aim of this study is to investigate protective effects of ethyl pyruvate (EP) on improvement of testicle in CP treated animals.

**Materials and Methods:** In this study, 15 male mice (6-8 weeks) were divided into 3 groups. Control group received normal saline (0.1ml/day, IP), CP group received CP (15 mg/kg/week, IP), and CP+EP group treated with EP (40 mg/kg/day, IP) along with CP. After 35 days, serum total antioxidant capacity (TAC) and samples of testis were used for measurement of the germinal epithelium thickness and malondialdehyde (MDA) assaying.

**Results:** This study showed that mean thickness of germinal epithelium in the CP+EP group were higher than those of the CP group (P<0.05). TAC in the CP group was significantly reduced compared with other groups (P<0.05). The MDA levels in the CP group was higher than the two other groups, whereas this increasing was only significant with control group (P<0.05).

**Conclusion:** The results of this study showed the role of EP in increasing of serum antioxidants and improved germinal epithelium thickness and reduced testicular damage and level of MDA.

**Keywords:** Testis, Cyclophosphamide, Ethyl Pyruvate, Malondialdehyde (MDA)

#### **P-3: The Role of Varicocele in Determining The Serum Testosterone Levels in Infertile Men with Varicocele**

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**Background:** Varicocele generally are found in 15% of men with up to 35% with primary infertility and 70 to 81% with secondary infertility. To determine whether infertile men with varicoceles have lower testosterone levels than those fertile men and to ascertain if testosterone levels increase after varicocelectomy.

**Materials and Methods:** We measured preoperative testosterone levels in 85 men with palpable varicoceles (grade II and III) and in 85 fertile men as a comparison group. Patients were categorized into two age groups (Less than 35 years, More than 35 years). The testosterone levels between groups

were compared by age.

**Results:** The mean of testosterone level before surgery in infertile men with varicocele and fertile men were 592(233) and 596(323) ng/dl ( $P=0.93$ ), respectively. No statistically significant changes were noted in testosterone levels for any of the groups. Moreover, there was no correlation between age and testosterone level in two groups, also after varicocelectomy, the testosterone levels no significantly increased from 592(233) to 646(250) ng/dl ( $P=0.076$ ). These groups of patients were categorized into two age groups. Group I, less than 35 years ( $\leq 35$ ), group II, more than 35 years ( $>35$ ). This difference persisted when analyzed by age. After increased serum testosterone level 26 patient of 85 infertile patient by varicocele, now they have a child.

**Conclusion:** Varicocelectomy resulted in significant increases in the serum testosterone level in infertile men with varicocele. These findings suggest that repair may increase testosterone levels in men with varicocele and low testosterone levels.

**Keywords:** Androgen, Testosterone, Varicocele, Varicocelectomy, Infertility

#### **P-4: Effect of Chronic Stress on Stereological Properties of Seminiferous Tubules in Adult Male Rats**

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**Background:** Male fertility and reproduction can be affected by stress. The means by which stress influences reproduction is not clearly understood. The aim of the present study was to examine, using stereological methods, the role of glucocorticoids during chronic restraint stress on spermatogenesis.

**Materials and Methods:** Twenty four male adult Sprague-Dawley rats were allocated to four equal groups; stress, RU486, stress/RU486, and control groups. In stress group, the rats were restrained, 1 h/day, for 12 days. In RU486 group, the rats were injected RU486 at a dose of 2.5 mg/kg for 12 days. In stress/RU486 group, the rats were injected RU486 1 h before the stress for 12 days. The testes of the all groups were removed and in 10 circular transverse sections of tubules stained with hematoxylin-eosin, stereological parameters were measured including cellular (germinal epithelium) diameter and area of the seminiferous tubules, total diameter and cross sectional area, number of seminiferous tubules per unit area, and numerical density of the tubules of the seminiferous tubules.

The testes were also rated for its spermatogenic potential on a modified spermatogenic scale of 0 to 6. Data were analyzed by one-way ANOVA and LSD post hoc test ( $P<0.05$ ).

**Results:** Restraint stress significantly reduced lumen diameter, thickness of germinal epithelium and numerical density of seminiferous tubules. This reduction was reversed by subcutaneously injection of the anti-glucocorticoid, RU486 prior to stress session.

**Conclusion:** During chronic stress, increases in glucocorticoids in male rats act via glucocorticoid receptors on testicular tissue to suppress spermatogenesis.

**Keywords:** Stereology, Spermatogenesis, Glucocorticoid Receptor, Restraint Stress, Rats

#### **P-5: Effect of Chronic Stress on Kiss-1 mRNA Expression in Male Rat Arcuate Hypothalamic Nucleus**

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**Background:** Kisspeptin is a potent stimulator of the hypothalamic-pituitary-gonadal axis and may play a critical role in modulating of stress-induced suppression of reproduction. The aim of the present study was to investigate the effect of glucocorticoid receptor antagonist (RU486) and stress on expression of Kiss-1 mRNA in arcuate nucleus (ARC) of rat hypothalamus.

**Materials and Methods:** Twenty four male rats of the Sprague-Dawley strain were randomly allocated into stress, RU-486, stress/RU-486, and control groups ( $n=6$ ). The stress group rats were immobilized 1 h/day, for 12 days through wrapping of their upper and lower limbs by packing tape with holes for ventilation. RU-486 (2.5 mg/kg, subcutaneously) for 12 days was injected into the RU-486 group rats. The stress/RU-486 group rats were injected with the same dose of RU-486 1 hour before the stress process for 12 days. The control group was allowed to freely move in the cages without receiving any stress or drug. All rats were decapitated and their ARC was collected. Moreover, five rats were ethically killed 2 weeks after castration and served as real time PCR control group. Relative expression of Kiss-1 mRNAs (compared to the castrated group) in ARC was determined using qualitative real-time PCR. Mean of data in four groups were compared by

one-way ANOVA and LSD post-hoc test (SPSS 22;  $P < 0.05$ ).

**Results:** Mean and SE of relative expression of Kiss-1 mRNA in ARC in the stress, RU-486, and stress/RU-486 groups ( $8.9 \pm 2.5$ ,  $7.7 \pm 1.5$  and  $7.9 \pm 2.3\%$ , respectively) were less than that of the control rats ( $27.9 \pm 9.1\%$ ,  $P < 0.05$ ). Relative expression of Kiss-1 mRNA was not different between the stress, RU-486, and stress/RU-486 groups ( $P > 0.05$ ).

**Conclusion:** Chronic 12 days stress decreased Kiss-1 mRNA expression in the ARC nucleus of male rats. Therefore, decrease of male fertility by the chronic stress can be caused via the decrease of Kiss-1 mRNA expression.

**Keywords:** Kiss-1 mRNA, Chronic Stress, Arcuate Nucleus, Rats

### **P-6: Effects of Green Biologic Rodenticide, Biorotox®, on Fertility of Male Rats**

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**Background:** Biorotox® is an invented green biologic rodenticide. The present study was designed to determine whether this toxin could cause infertility in male rats.

**Materials and Methods:** Sixteen Sprague-Dawley adult male rats were randomly divided into control and treatment groups. Treatment group was received diets containing Biorotox® for 35 days. Control group was given standard rat food. Weight of body and volume of testis were measured. Furthermore, epididymal semen samples were collected and incubated for 45 min at 37 °C in 1 mL of PBS, to evaluate sperm counts, motility and viability. Data of indices were evaluated using the independent sample t test.  $P < 0.05$  was selected significant.

**Results:** In the sperm quality evaluation, mean and SE of sperm motility in the treatment rats ( $66.3 \pm 4.9\%$ ) was lower than that of control group ( $22.5 \pm 3.6\%$ ,  $P = 0.001$ ). However, there was no significant different in the whole body weight ( $342.1 \pm 12.5$  vs.  $316.6 \pm 10.9$  g), testes volume ( $1.6 \pm 0.05$  vs.  $1.5 \pm 0.04$  ml), sperm concentration ( $2.8 \pm 0.4$  vs.  $1.9 \pm 0.3 \times 10^6/\text{ml}$ ) and viability ( $44.9 \pm 11.1$  vs.  $28.6 \pm 7.0\%$ ) between control and treatment groups, respectively ( $P > 0.05$ ).

**Conclusion:** Biorotox® in the rat diet decreased motility of spermatozoa which may have an adverse effect on rat reproduction. And it could be an efficient product for control the population of these rodents.

**Keywords:** Biorotox®, Rat, Sperm Quality, Fertility

### **P-7: Effects of Long-Term Exposure to Radiofrequency Radiations Emitted by Common Mobile Jammers on Sperm Quality Parameters in Rat**

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**Background:** Exposure to electromagnetic fields (EMFs) by using electrical machines, tools, industrial instruments, power lines, and communications devices has occurred as a result of technological developments and is causing a threat to normal lives. The aim of this study was to evaluate the effects of long-term exposure to radiofrequency (RF) radiations emitted by mobile jammers on sperm quality.

**Materials and Methods:** Thirty adult male rats were randomly divided into control, sham and experimental groups. Experimental rats were exposed in the restraint tube to radiofrequency radiations emitted by mobile jammers at a distance of 100 cm, for 40 days, 5 days/week, 7 h/day. Sham group was placed in restraint tubes same as experimental group, without radiations. The control group was allowed to freely move in the cages, without radiations. Weight (before and after experiment) and volume of testis ( $\text{cm}^3$ ) of the animals were measured. Furthermore, to evaluate sperm counts, motility and viability, epididymal semen samples were collected and incubated for 40 min at 37 °C in 1 mL of PBS. Data were analyzed by using one-way ANOVA.  $P < 0.05$  was selected significant.

**Results:** In the sperm quality evaluation, percentage of sperm viability in experimental group exposed to jammer RF radiation was significantly lower than that of sham-exposed rats ( $P < 0.05$ ). Moreover, the motility of sperm in sham group after subjected to restraint stress was significantly lower than that of control groups ( $P < 0.05$ ). However, there were no significant different in sperm concentration, weight and volume of testis of the animals between groups ( $P > 0.05$ ).

**Conclusion:** These findings lead to the conclusion that restraint stress or long-term exposure to radiofrequency radiations emitted by mobile jammers may significantly decrease sperm quality.

**Keywords:** Electromagnetic Fields, Mobile Jammers, Radiofrequency, Sperm Motility

### **P-8: The Protective Effect of Ascorbic Acid on The Germ Cells Population and Daily Sperm Production in Mice Treated with Sodium Arsenite**

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**Background:** Oxidative stress has been suggested to be a major cause of reproductive failure. Arsenic as an environmental toxicant can exert malformation in the reproductive system by inducing oxidative stress. Ascorbic acid, a potent antioxidant, is able to restrict oxidative stress. The aim of this study was to investigate the adverse effect of sodium arsenite

on the germ cells population and daily sperm production as well as to examine whether vitamin C is able to ameliorate these undesired effects.

**Materials and Methods:** Twenty-four adult male NMRI mice were randomly allocated into four groups including control, Sodium arsenite (7 mg/kg/day, orally), ascorbic acid (150 mg/kg/day, orally) and finally Sodium arsenite plus ascorbic acid. Mice were treated for 35 days. At the end, mice were sacrificed and their right testis were taken out, fixed, processed and stained with heidenhain azan method. The number of germ and sertoli cells were examined with the optical dissector method. Daily sperm production (DSP) was measured using the homogenization technique. Data were analyzed using one way ANOVA and means were considered significantly different at  $P < 0.05$ .

**Results:** A significant decrease in the mean total number of spermatocyte, spermatid and sertoli cells and daily sperm production was found in sodium arsenite group compared to the control ones ( $P < 0.002$ ). The above parameters significantly increased in the sodium arsenite plus ascorbic acid group compared to the sodium arsenite group ( $P < 0.004$ ). In addition the total number of spermatocyte, spermatid, sertoli cells and DSP significantly increased in mice treated with ascorbic acid only compared to the control group ( $P < 0.002$ ).

**Conclusion:** Our results indicate that ascorbic acid may be useful in reducing sodium arsenite- induced toxic effects on the reproductive system.

**Keywords:** Sodium Arsenite, Ascorbic Acid, Germ Cells Population, Daily Sperm Production

### **P-9: Antioxidant Effects of Vitamin E on Sperm Parameters in Adult Male Mice Treated with Nonyl Phenol**

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**Background:** Nonyl phenol (NP) has been considered an endocrine disrupting substance causing reproductive dysfunction and increase in reactive oxygen species production in different organs including testis. vitamin E (VitE) is a potent scavenger of free radicals and is able to prevent the membrane damage mediated by free radicals. The aim of this study was to elucidate the protective effects of VitE against reproductive toxicity induced by NP in adult male mice.

**Materials and Methods:** In this study, 32 adult male mice (8-12 weeks) divided in to four experimental groups, control group received corn oil only, NP group received NP (250 mg/kgbw), VitE group received VitE (100 mg/kgbw), NP plus VitE group received 250 mg/kg NP + 100 mg/kg VitE for 35 days by gavaged. Finally, body and testes weight were recorded and analyzed caudal epididymal spermatozoa.

**Results:** NP caused a decrease in body and testes relative weights, sperm number and sperm motility compared to control and p-NP+VitE groups. A significant increase was also found in sperm viability in VitE group compared to both NP and control groups. Administration of VitE combined with NP ameliorated these toxic effects.

**Conclusion:** VitE reduced the oxidative stress toxicity induced by NP in the reproductive system of adult male mice. VitE not only is able to compensate the toxic effects of p-NP on testis weight, sperm number, sperm motility, but also increases sperm viability in adult male mice.

**Keywords:** Epididymis, Viability, Oxidative Stress, Testes

### **P-10: Protective Effects of Satureja Khuzestanica Extract on Nonyl Phenol Induced Cytotoxicity in Sperm of Adult Mice**

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**Background:** This study aimed to assess the protective effects of saturejakhuzistanica (SJKH) on sperm quality parameters in the Nonyl phenol (NP) - treated mice.

**Materials and Methods:** In present experimental study, 32 young adult male mice (8-12 weeks) were randomly divided into four groups including control and test groups. The control group received corn oil orally, and the test groups were treated NP (250 mg/kg), SJKH (250 mg/kg), NP+SJKH (500 mg/kg) for 35 consecutive days orally by gavage. On day 35, after euthanasia the epididymal sperms were isolated. Then the total mean sperm count, sperm viability and motility were determined the total antioxidant capacity (TAOC) of all experimental groups were also evaluated.

**Results:** The NP-treated animals showed a significant changes in all parameters of sperm quality assessment compared to the control group. SJKH was able to protect from NP-induced effects on sperm maturity and DNA damage. Co-administration of NP and SJKH resulted in protection from NP reduced TAOC. **Conclusion:** These data suggested that NP administration could adversely affect the sperm quality. Moreover, the protective effect of SJKH on NP-induced sperm toxicity was also documented.

**Keywords:** Nonyl Phenol, Antioxidant, Sperm Quality, DNA Damage

### **P-11: Differential Expression of mRNA Aromatase in Ejaculated Spermatozoa from Infertile Men in Relation to either Asthenozoospermia or Teratozoospermia** Said L<sup>1,2</sup>

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**Background:** Estrogens biosynthesis in ejaculated spermatozoa is an autonomous process that may influence sperm functions. The purpose of this study was to evaluate the relationship between the expression of aromatase, sperm quality and seminal neutral  $\alpha$ -glucosidase marker in semen of Tunisian infertile men.

**Materials and Methods:** Fifty seven men were investigated: they were composed of two groups: normospermic (n=18) and infertile men divided into : asthenozoospermia (n=16), teratozoospermia (n=12) and asthenoteratozoospermia (n=11). All the patients underwent semen analysis and measurements of neutral-alpha glucosidase. The aromatase mRNA levels transcription was estimated by Real time PCR.

**Results:** Aromatase mRNA levels were reduced in groups T (52%) and AT (67%) compared to control one and inversely correlated with the percentage of normal forms. A higher coefficient of correlation was noted in presence of microcephaly or acrosome malformations ( $r=-0.64$ ). Asthenozoospermic group was divided in two subgroups according to the relative amount of aromatase. The subgroup (A2) with higher aromatase transcript level was associated with an increased seminal pH, a decreased sperm viability, low sperm percentage motility and low neutral  $\alpha$ -glucosidase semen levels.

**Conclusion:** Our data highlights the involvement of aromatase in motility and morphology of spermatozoa. Thus, this enzyme could bring new insights about quality and fertilizing capacity of human spermatozoa.

**Keywords:** Aromatase, Fertility, Morphology, Motility, Spermatozoa

**P-12: Long-Term Follow-Up (18–35 Years) of Male Patients with History of Bladder Exstrophy (BE) Repair in Childhood: Erectile Function and Fertility Potential Outcome**

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**Background:** Bladder exstrophy is a rare condition that may lead to severe psychosexual malformation and require a lifelong follow-up. Aim. We describe the long-term sexual outcome of patients with bladder exstrophy treated at our institution at early stage.

**Materials and Methods:** Thirty patients with mean age of 26 years (range 18–35 years) were included in the study. Fifteen patients underwent staged primary reconstruction, five patients underwent complete primary repair, and 10 patients underwent primary or secondary ureterosigmoidostomy. Main Outcome Measures evaluation consisted of pediatric medical records, interview questionnaire including the psychosexual history, International Index of Erectile Function (IIEF), and semen analysis.

**Results:** Erectile functions were maintained in 28 patients based on IIEF domain score. In all cases, penile length was objectively less than average (mean 7.65 cm). Seven patients were not satisfied with their penile length, and four cases complained of slight curvature. Ten patients were married (33%), of whom four patients had children (after normal conception in three, and after assisted reproductive technique in one). The remaining 20 patients were not married because of the feeling of sexual inadequacy to be able to engage in sexual intercourse (six patients), afraid of the cosmetic appearance of the genitalia (10 patients), and incontinence (four patients). Retrograde ejaculation was documented in 16 cases (53.5%), low volume ejaculate in eight cases (26.5%), and anejaculation in six cases (20%). Cosmetic outcome was considered satisfactory by 50% of the patients. Sixteen patients voided per urethra, four performed clean intermittent catheterization, and 10 patients had ureterosigmoidostomy diversion. Urinary tract infection was documented in 20% of the cases, and recurrent attacks of pyelonephritis in 10% of the cases

**Conclusion:** Long-term outcome of bladder exstrophy repair in male patients showed fair results with respect to sexual function with more or less stable sexual relationship. We should do our best to solve the problem of those with restricted sexual lives.

**Keywords:** Bladder Exstrophy, Male; Erectile Function, Penile Length Concerns

**P-13: The Expression of SOX3 Gene in Human Sertoli Cells of Azoospermic Patient**

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**Background:** Sertoli cells located in seminiferous tubular basement membrane and surrounds different types of germ cell and they were only somatic cell type that directly contacts germ cells. These cells have several role like support activities, nutrition of germ cells. During spermatogenesis, expression of SOX3 is necessary for differentiation of spermatogonia A. SOX3 is a transcription factor, expressed in sertoli cells and cause their differentiation. The purpose of this study was to separation and purification of sertoli cells from human testis and to investigate the expression of SOX3 gene in sertoli cells of azoospermic patients

**Materials and Methods:** Biopsies were obtained from 10 men who referred to Royan Institute and underwent testicular sperm extraction (TESE). Tissue samples were transferred to lectin coated petri dishes after enzymatic dissociation and isolation. After few passages, all the cells were harvested and



the cell type was confirmed by immunocytochemistry. SOX3 gene expression level was determined by Real time RT-PCR.

**Results:** Isolation, purification and culture of the human sertoli cells were performed successfully. It was shown that SOX3 gene are expressed in these cells and there is a significant difference in the expression of SOX3 in sertoli cells derived from tissues with successful sperm extraction (TESE+) compared to samples without sperm (TESE-).

**Conclusion:** The higher expression of SOX3 gene in (TESE) than that of (TESE+), indicates negative effect of SOX3 gene over expression in spermatogenesis

**Keywords:** Sertoli Cells, SOX3, Spermatogenesis, TESE

#### **P-14: Effects of Gamma-Ray and Silver Nano Particles on Treatment of Human Prostate Cancer Cell line DU145**

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**Background:** Prostate cancer is the second most common cancers in the world which causing harms and waste cost. Furthermore more prostate treatments are in effective and lead to male infertility. Purpose of this study was to evaluate the role gamma radiation with silver nano particles in treatment of human prostate cancer cell line in vitro.

**Materials and Methods:** Human prostate cancer cell line DU145 was purchased from Pasture institute. The cells were incubated with DMEM medium and 15% FBS serum over a period of 3-5 days. Cells was put on 96 well plates and divided into experimental and control groups. The first experimental with treatment doses of 2, 6, 10 Gray (Gy) Gamma ray and the second experimental group simultaneous treatment of Gamma ray and 53 µg/ml silver nano particles. All groups were stained with trypan blue as well as by MTT assay and by ELISA reader was studied

**Results:** The results showed that using of Gamma ray and silver nano particles caused a significant decreased in the number of cells in experimental groups compare to control cells. Survival rates of cells with Anova test was significant in experimental group of 6 Gy Gamma-ray with nano silver treatment to other experimental and control groups

**Conclusion:** Gamma radiation with silver nano particles treatment of DU145 cell line leads to a process of effects of secondary electrons (Auger electron) which induce DNA damage strands and cell death. These findings may suggest as a new strategy for treatment of male reproductive cancers

**Keywords:** Gamma-Ray, Nano Particles, Prostate, Cancer, Cell Line

#### **P-15: Effect of Consumption of Korean Red Ginseng and Sodium Valproate on**

#### **Apoptosis of Spermatogenic Cells and Sperm Quality in Pilocarpin-Induced Epilepsy Rat Model**

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**Background:** Reproductive dysfunction and endocrine disorders are common among men with complex partial seizures of temporal lobe origin. Diminished sexual desire and responsiveness along with decreased libido and less orgasm are frequently described in men with temporal lobe epilepsy (TLE). It appears that the reduced fertility in epileptic men is accompanied by erectile dysfunction and abnormal sperm quality. Sexual dysfunctions in epileptic cases may be a result of psychosocial stress, antiepileptic drugs and also epileptiform activity in the temporal lobe. The aim of this study is considering the adverse effects of epilepsy on reproductive system and also undesirable effects of antiepileptic drugs such as sodium valproate on fertility on one side and protective effects of Ginseng on reproductive system on the other side. Antioxidant property of Ginseng may delay apoptosis process of spermatogenic cells which accelerated through epilepsy and sodium valproate.

**Materials and Methods:** In this experimental study, 56 male rat of wistar species were divided into seven groups and 8 rats were placed in each group such as; control group, epileptic group treated by normal saline (Vehicle), epileptic group treated by sodium valproate and ginseng, epileptic group treated by ginseng, the recipient group of sodium valproate and ginseng, epileptic group treated by sodium valproate and the recipient group of sodium valproate. Animals after induction of epilepsy with pilocarpin, was treated intraperitoneally by ginseng (150 mg /kg /day) and sodium valproate (200 mg/kg/day) for one month period. Sperm parameters (motility, morphology and count) were examined by optic microscope at the end of study. Tunnel diagnostic kit was used for studying amount of apoptosis of spermatogenesis cells group.

**Results:** Count sperm in seminiferous tubules of epileptic and recipient animals of sodium valproate was reduced. There is observed no significant change in sperm count in other groups. The most sperm morphological abnormalities observed in Vehicle group and epileptic animals treated by sodium valproate in comparison with recipient animals of ginseng. There was a significant difference between epileptic animals treated by sodium valproate and epileptic animals treated by ginseng from total motility point of view in comparison with epileptic animals treated by sodium valproate and vehicle. Number of seminiferous tubules containing apoptotic cells in recipient animals of sodium valproate, vehicle and epileptic animals treated by sodium valproate were very much in comparison with control group and recipient animals of ginseng.

**Conclusion:** Therefore, according to achieve results, ginseng prescription to patients suffering epilepsy that treated by sodium valproate can be very effective in improving such parameters. Ginseng may prevent oxidative stress effects of sodium valproate and complications from epilepsy on the reproductive system of male animals.

**Keywords:** Epilepsy, Sodium Valproate, Reproductive System, Ginseng, Sperm Parameters

### **P-16: Antiapoptotic and Antioxidant Effect of Insulin Like Factor-3 on Sperm of Fertile Men After Cryopreservation**

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**Background:** Freezing process has destructive effect on different functions of sperm. Therefore, sperm motility was reduced and apoptosis and DNA damage and also levels of reactive oxygen species (ROS) were increased. Insulin-like factor-3 (INSL3) is a member of Relaxin family and directly affects on spermatogenesis and also plays a protective role against apoptosis. Therefore, with due regard to harmful effects of freezing on sperm and antiapoptotic role of INSL3 in male reproductive system, this study was designed to study the effects of this hormone in reducing complications from freezing.

**Materials and Methods:** Semen samples were collected from 15 fertile men. Each sample was divided into six parts. One part of sample was kept as control group and insulin-like 3 (INSL3) hormone with concentrations of 10, 100, 250, 500 and 1000 ng/ml was added to other five parts. We assayed the level of DNA damage through TUNEL method, activated Caspase enzymes with FLICA marker and reactive oxygen species in mitochondria and cytosol with MSR and DHE markers through flowcytometry method, respectively. And, also motility and morphology of sperm were studied after two weeks freezing.

**Results:** The level of DNA damage in 250 ng/ml concentration in compare with control group has significant reduction and also activation level of Caspases in 100 ng/ml concentration in compare with 1000 ng/ml concentration has significant increase ( $P \leq 0.05$ ). However, significant differences were not observed in different concentrations of hormone concerning motility, morphology and ROS level in mitochondria and cytosol.

**Conclusion:** This study demonstrated that the effect of INSL3 was completely dose-dependent. But, generally, 250 ng/ml concentration caused decrease in DNA damage level in comparison to the control group and it seems that 250 ng/ml concentration is a suitable concentration for protecting sperm in freezing medium.

**Keywords:** INSL3, Cryopreservation, Sperm, Antiapoptotic, Antioxidant

### **P-17: Effect of Calcium Ionophore A23187 on Sperm Hyper-Active Motility Patterns**

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**Background:** The role of calcium in physiological phenomena such as sperm acrosome reaction and hyper-activation has been well known. Calcium ionophore A23187 is routinely used for induction of acrosome reaction in mammalian sperm. Therefore, we decided to evaluate the effect of calcium ionophore A23187 on the sperm hyper-active motility patterns.

**Materials and Methods:** Ten semen samples from individuals referring to andrology unit of Isfahan Fertility and Infertility Center (IFIC) were collected for this study. All the samples were processed with DGC (Density Gradient Centrifuging). After induction of acrosome reaction with calcium ionophore A23187, percentage of hyper-activated sperm was assessed using CASA (Computer-Aided Sperm Analysis) system and compared before and after induction of acrosome reaction.

**Results:** The results of this study showed that percentage of hyper-activated sperm were significantly lower after induction of acrosome reaction compared to before induction of acrosome reaction ( $P < 0.05$ ).

**Conclusion:** Ca<sup>2+</sup> ionophore A23187 increases intracellular Ca<sup>2+</sup> concentration. Therefore, sperm are immobilized in the acrosome reaction condition.

**Keywords:** Hyper-Active Motility, Acrosome Reaction, Calcium Ionophore

### **P-18: Protective Effect of Selenium-Enriched Saccharomyces Cerevisiae Cytoplasm and Cell Wall on Chronic Immobilization Stress-Induced Damages in Testis; Evidence for Apoptosis**

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**Background:** Previous reports showed that immobilization stress (IMS) results in severe damages at spermatogenesis level. Present study was performed in order to evaluate

the protective effect of selenium-enriched yeast fragments on IMS-induced derangements.

**Materials and Methods:** For this purpose, 42 mature male Wister rats were assigned into 6 groups (7 rats in each group) including; control, stress-induced and SE-enriched *Saccharomyces cerevisiae* fragments-treated groups. The animals in treatment groups subdivided into 4 groups as; SE-enriched yeast cytoplasm (SEC)+stress, SE-enriched *Saccharomyces cerevisiae* cell wall+stress (SECW), SEC alone-treated and SECW alone-treated groups. In order to induce stress, the rats were immobilized by keeping them into transparent plastic jars with 5 holes for 2 hours a day. All animals received the chemicals ( $5 \times 10^8$  CFU/ml, in one mL for day) orally for 42 days. At the end of day 42. The testicles were dissected out, fixed in formaldehyde. The expression of bcl-2 and p53 were investigated by using immunohistochemical and RT-PCR techniques. Moreover, the TUNEL staining was performed in order to evaluate apoptosis.

**Results:** Observations demonstrated that co-administrating cytoplasm+cell wall in SECW group significantly ( $P < 0.05$ ) reduced p53 at both protein and mRNA levels and remarkably ( $P < 0.05$ ) enhanced bcl-2 protein and mRNA. Estimating apoptotic cells distribution between experimental groups illustrated a significant reduction more significantly in SEC and SECW-treated groups.

**Conclusion:** Our data suggested that co-administrating SE-enriched yeast cytoplasm with SE-enriched yeast cell wall reduces IMS-induced apoptosis by up-regulating bcl-2 mRNA and protein contents and by down-regulating p52 expression.

**Keywords:** Immobilization Stress, *Saccharomyces Cerevisiae*, Selenium, p53, bcl-2

### **P-19: The Effect of Mice Maternal Diet Supplemented with Omega-3 Fatty Acids on The Testicular Structure of Offspring: Stereological Study**

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**Background:** Despite the bulk of studies on fatty acid sup-

plementation in maternal diet and confirmed the positive effects on brain and vision, these effects on offspring reproductive organs have not been tested. The aim of the present study was to stereological evaluate the effect of feeding Fish Oil (FO) for mothers on the testis structure of offspring.

**Materials and Methods:** Sixty mature female NMRI mice were divided into 3 groups (n=6): I. mothers fed control diet (CTR; standard diet pre and postnatal period); II. mothers gavages 0.01 ml/d Fish Oil (FO) + CTR diet during prenatal period and III. mothers gavages 0.01 ml/d FO + CTR diet during Postnatal period till weaning of offspring. Male offspring were sacrificed and their right testis was fixed, processed, stained with H & E. The morphological and changes of testicular tissue were estimated using stereological methods. Data were analyzed using SPSS.

**Results:** The testis weight was the highest in FO postnatal group. The volume of testis dramatically affects by treatments (80, 58.5 and 111 mm<sup>3</sup> for CTR, FO Prenatal and FO postnatal, respectively;  $P < 0.05$ ). The volume of seminiferous tubules in FO postnatal (89 mm<sup>3</sup>) higher than other groups (39 and 49 mm<sup>3</sup> for FO Prenatal and CTR;  $P < 0.05$ ) as well as total length of seminiferous tubule in FO postnatal (2.8 m) compared with others (1.8 and 1.9 m for FO prenatal and CTR;  $P < 0.05$ ).

**Conclusion:** For the first time we demonstrated that maternal dietary fatty acid supplementation by omega-3 fatty acids affect mature offspring testis tissue. Although fed mothers by FO during milking period improved testis structure of offspring, maternal diet which supplemented by FO during prenatal may be destroy the offspring testis tissue, which warrants further studies.

**Keywords:** Maternal Nutrition, Fatty Acids, Offspring Testes, Stereology

### **P-20: Efficiency of Magnetic-Activated Cell Sorting System in Separation of Normal Sperm**

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**Background:** Sperm preparation before assisted reproductive techniques is very important for obtaining successful fertilization and pregnancy. During ICSI technique, sperms are selected based on viability and morphology. But this selection did not guarantee integrity of sperm DNA. Therefore, novel sperm selection procedures are introduced for selection of sperm with low DNA damage. One of these procedures is magnetic-activated cell sorting system (MACS) method based on surface marker. In this study, we compared sperm chromatin, protamine deficiency and morphology between density

gradient centrifugation (DGC) and MACS procedures.

**Materials and Methods:** Semen samples were obtained from 20 infertile individuals with male factor. Each semen sample was divided into three fractions; first fraction was washed with PBS as unprocessed sample. The second and third fractions were processed by MACS and DGC, respectively. After each fraction, percentage of sperm DNA fragmentation, protamine deficiency and morphology were assessed using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, chromomycin A3 (CMA3) staining and papanicolaou respectively.

**Results:** Percentages of sperm DNA fragmentation, protamine deficiency and abnormal morphology have significantly decreased after MACS and DGC procedures compared to unprocessed sample ( $P < 0.05$ ). It is noticeable that MACS procedure can separate non-apoptotic sperm from apoptotic sperm population.

**Conclusion:** Sperm selection based on surface marker (MACS) could be useful for separation of sperm with low DNA damage, low protamine deficiency, low abnormal morphology with intact acrosome and low apoptotic sperm. Therefore, we suggest that MACS procedure could be sufficient for clinical aims in the future.

**Keywords:** Sperm Selection, MACS, DGC, DNA Fragmentation, Protamine Deficiency

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## Animal Biotechnology

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### P-21: RF-Amide Related Peptide mRNA Expression in Male Rat Dorsomedial Hypothalamic Nucleus during Chronic Stress

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**Background:** Chronic stress induces male reproductive dysfunction; however, the involved mechanisms are not clear. It has been suggested that the gonadotrophin-inhibitory hormone, known as RF-amide related peptide (RFRP) in mammalian species, may mediate this inhibitory effect of stress. The objective of the present study was to compare the effect of chronic stress and glucocorticoids receptor antagonist

(RU486) on expression of RFRP mRNA in dorsomedial hypothalamic nucleus (DMH) of rats.

**Materials and Methods:** Twenty four male rats of the Sprague-Dawley strain were randomly allotted into four equal groups (n=6); stress, RU-486, stress/RU-486, and control groups. In stress group, the rats were immobilized, 1 h/day, for 12 days. In RU-486 group, the rats were injected subcutaneously with RU-486 (2.5 mg/kg) for 12 days. In stress/RU-486 group, the rats were injected subcutaneously with the same dose of RU-486 1 hours before the stress process for 12 days. The control group was allowed to freely move in the cages and did not receive any stress or drug. DMH of all rats were collected after 12 days. Moreover, five rats were ethically killed 2 weeks after castration and served as real time PCR control group. Relative expression of RFRP mRNAs (compared to the castrated group) in DMH was determined using real-time PCR. Mean of data in four groups were compared by one-way ANOVA and LSD post-hoc test (SPSS 22;  $P < 0.05$ ).

**Results:** Mean and SE of relative expression of RFRP mRNA in DMH in the chronic stress group ( $106.2 \pm 24.1\%$ ) was higher than that of the RU-486 and control rats ( $57.8 \pm 3.9\%$  and  $43.6 \pm 10.5\%$ , respectively;  $P < 0.05$ ). Relative expression of RFRP mRNA was not different between the stress group ( $106.2 \pm 24.1\%$ ) and stress/RU-486 rats ( $83.8 \pm 13.9\%$ ,  $P > 0.05$ ).

**Conclusion:** Chronic stress (12 days) increased RFRP-3 mRNA expression in DMH of male rat which may one of pathways of fertility dysfunction in males.

**Keywords:** Chronic Stress, RFRP mRNA, Hypothalamus, Rats

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## Embryology

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### P-22: Codon Optimization of Coagulation Factor IX and Cloning in to The Chinese Hamster Ovary Cells

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**Background:** Human coagulation factor IX is a 57kDa plasma serine protease made in Liver which plays a vital role in the blood coagulation cascade. FIX deficiency causes severe disorder Hemophilia B or Christmas disease. Nowadays, recombinant proteins have important roles in treatment of diseases. Although, cultivated mammalian cells because of their ability for producing properly folded protein molecules, have become a dominant system for the production of recombinant

proteins with medical application. There are several kinds of methods to optimization of gene expression but one of the most useful method is codon optimization. Codon optimization is adaptation of nucleic acid sequences according to host cells codon usage. The replacement of rare codons with the frequent codons of host cells could enhance the expression of interested protein. Altering the coding sequence to increase protein expression is highly cost-effective. The aim of this study is expression and production of clotting FIX in CHO cells and eventually increasing the expression level based on codon usage host cells.

**Materials and Methods:** The DNA sequences of the open reading frame (ORF) for the FIX protein have been well adapted to the codon usage of CHO cells. First of all this sequence has been cloned into pCDNA3.1+ expression vector, then the construct was transfected into the CHO cells with electroporation method. Later, the protein production was confirmed by SDS-PAGE, Western blotting.

**Results:** The exact bond of FIX has not been detected in SDS-PAGE due to the abundance of albumin, in contrast the Western blotting results of the cells supernatant confirmed the expression and secretion of FIX protein from cells.

**Conclusion:** According to the above-mentioned, it has showed acceptable level of protein expression in SDS-PAGE and western blotting. So we will analysis data by ELISA technique.

**Keywords:** Codon Optimization, Coagulation Factor IX, Gene Expression, Chinese Hamster Ovary Cells, Codon Usage

### **P-23: The Effect of LIF on *In Vitro* Growth and Apoptosis Incidence in Vitrified Human Ovarian Tissue after 14 days culture**

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**Background:** The high effectiveness of vitrification, as a cryopreservation protocol, for human oocytes and embryos is shown, whereas data on human ovarian tissue are limited.

This study aimed to assessment of follicular growth, ultrastructure, and apoptosis incidence in human ovarian tissue following vitrification/warming and after culture in the presence of LIF.

**Materials and Methods:** Biopsies of ovarian cortex from normal pregnant women divided to 2 main groups: vitrified and non-vitrified and some of fragments in both groups culture in presence and absence of LIF. Then, the morphology, ultrastructure and incidence of apoptosis using TUNEL and DNA Laddering and caspase 3/7 assay and analysis of apoptosis related genes expression in ovarian tissue fragments were evaluated before and after 2 weeks culture.

**Results:** Morphology and ultrastructure of vitrified human ovarian tissue were similar to vitrified group and were well preserved. Apoptosis evaluation assessments (DNA Laddering, TUNEL, Caspase-3/7 activity, apoptotic genes expression) in both non-vitrified and vitrified groups showed no significant differences.

Morphological studies of ovarian tissue in LIF treated groups showed better conservation of ovarian follicles (P<0.05). But, there were no significant differences between non-vitrified and vitrified ovarian tissue in LIF treated groups. The levels of 17-β estradiol and progesterone were higher and DHEA was lower than other cultured groups. Apoptosis evaluation techniques showed that apoptosis incidence in LIF treated groups were lower than non-treated cultured groups and non-cultured ovarian tissue (P<0.05).

**Conclusion:** We concluded that vitrification of human ovarian tissue has not increased the incidence of apoptosis and LIF as an antiapoptotic factor could improve survival and development of cultured follicles and reduce incidence of apoptosis in ovarian tissue.

**Keywords:** Vitrification, Human Ovarian Tissue Culture, LIF, Apoptosis Related Genes

### **P-24: *In Vitro* Growth and Apoptosis Incidence, valuation of in Vitrified Human Ovarian Tissue Following Treatment with Growth Differentiating Factor 9B (GDF-9B) and Leukemia Inhibitory Factor (LIF)**

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**Background:** The conventional freezing and vitrification are different cryopreservation protocols for fertility preservation in cancer patients. The high effectiveness of vitrification for human oocytes and embryos is shown, whereas data on human ovarian tissue are limited.

Follicular growth, ultrastructure, and apoptosis incidence in human ovarian tissue following vitrification/warming and after culture in the presence of GDF-9B and or LIF evaluated.

**Material and Methods:** Ovarian cortex biopsies from normal pregnant women fragmented and then divided to 2 main groups: vitrified and non-vitrified. Ovarian fragments in each main group cultured in 3 groups: in presence GDF-9B or LIF or absence any factor. Then the morphology, ultrastructure and incidence of apoptosis using biochemical analyses and analysis of apoptosis related genes expression in ovarian tissue fragments in all 6 experimental groups were evaluated before and after 2 weeks culture.

**Results:** Morphology and ultrastructure of vitrified human ovarian tissue were better preserved in vitrified group in presence of LIF compared to GDF-treated and non-treated groups.

Apoptosis biochemical evaluations in all non-vitrified and vitrified treated groups were same. Apoptotic genes expression in both non-vitrified and vitrified groups at the end of culture period showed significant differences compared to non-treated groups.

Morphological studies of ovarian tissue in all groups showed better conservation of ovarian follicles in presence of GDF-9B and LIF treated groups ( $P < 0.05$ ). But there were no significant differences between non-vitrified and vitrified ovarian tissue in all treated groups.

The levels of hormones at the end of culture period in treated groups were higher compared to non-treated groups.

Apoptosis evaluation techniques showed that apoptosis incidence in treated groups were lower than non-treated cultured groups and non-cultured ovarian tissue ( $P < 0.05$ ).

**Conclusion:** This study showed that culture of vitrified human ovarian tissue has not increased the incidence of apoptosis and GDF-9B and LIF could improve survival and development of cultured vitrified ovarian fragments and reduce incidence of apoptosis in human ovarian tissue.

**Keywords:** Vitrication, Human Ovarian Tissue Culture, GDF 9B, LIF, Apoptosis Related Genes

### **P-25: Characterization of Sheep Ovarian Multipotent Theca Stem Cells**

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**Background:** Theca cells play important roles during folliculogenesis. They synthesize androgens, provide crosstalk with granulosa cells and oocytes during development, and provide structural support of the growing follicle to produce a mature and fertilizable oocyte. In children patients who are going to be ovariectomy, folliculogenesis is at early stage and thus the follicles need to be mature in vitro. However, maturation of primordial follicles is almost impossible in vitro. Co-culture of early stage follicles with naturally surrounding cells like theca cells may significantly improve the current methods toward mature follicles.

**Materials and Methods:** In this study, we successfully isolated and characterized theca stem cells (TSCs) from sheep ovary. Adherent TSCs were morphologically similar to those of sheep adult fibroblast cells (AFs)

**Results:** Cell cycle analysis indicated that the TSCs have a higher proliferative capacity than AFs. TSCs were positive for the mesenchymal stromal cells (MSCs) markers including CD29, CD44, CD73 and CD105. The isolated TSCs also expressed stemness markers including OCT4 and KLF4. Nanog was expressed at very low level and SOX2 was not expressed by TSCs. To further confirmation, TSCs were induced to differentiate into osteocytes and adipocytes. Differentiation of osteocytes and adipocytes were validated by staining with

alizarin red and oil red respectively. Moreover, real time PCR was investigated for analysis of specific markers of the differentiated cells. Osteocalcin and *coll* and were induced in osteocytes. Adipocytes were positive for LPL and PPAR  $\alpha$ . TSCs resembled those of ovarian stromal cells and showed only moderate potential to differentiate towards lineages of mesenchymal origin

**Conclusion:** Result of this study can be used for obtaining theca cells which further can be used for co-culture with primary stage follicles in order to gain mature follicles in vitro.

**Keywords:** Theca Stem Cells, Folliculogenesis, Granulosa

### **P-26: Impact of The Preconception Diet and Life Style on Fertility Success in Women Undergoing IVF/ICSI Treatment**

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**Background:** Infertility is a significant public health problem and diagnosis and treatment are tense, invasive, and costly. Preconception interventions in infertile couples can increase the chance of pregnancy and lower its complications. The main goal to do this study is determine effect of the preconception diet and life style on fertility success in women undergoing IVF/ICSI treatment.

**Materials and Methods:** Information of this survey with numerous articles from 2000 to 2015 in internet different sites and books collected and evaluated.

**Results:** Nutrition and lifestyle factors, diet, exercise, stress, alcohol- and drug use, smoking and obesity affect reproductive performance, also during assisted reproduction. Screening on lifestyle factors, such as smoking and obesity, is relatively right, compared with that based on nutritional kind. High BMI associates with an elevate in serum and follicular fluid leptin condensation and decline in serum adiponectin levels. Studies performed on the relation between (micro)nutrients and fertility also provide evidence that nutrition changes fertility in both women and men. Vitamin D may play a function in human spermatogenesis. Some studies have characterized the prevalence of vitamin D deficiency in women undergo ART procedures. many patients perceive advantage to rigorous exercise during the IVF cycle and full bedrest following transfer. The role of antioxidant intake in pregnancy and birth outcome is a subject of emerging interest. In adult women, use of antioxidant-rich fruit and vegetables is negatively related with oxidative stress. Zinc has been the widest nutritious element that has been studied on couple's fertility improvement. Health care increases the chance of infertility treatment and promotes pregnancy outcome through changing risk factors and conducting the preconception interventions.

**Conclusion:** Preconception counseling can correct mater-

nalfetal health, both during pregnancy and afterwards. Life style, diet, sexual health, substance abuse, and psychosocial factors influence on outcome of fertility treatment and pregnancy health, which is central to preconception care.

**Keywords:** Diet, Lifestyle, Fertility Success

### **P-27: Study The Effect of Venlafaxine on Oogenesis in Adult Female Rats**

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**Background:** Venlafaxine is an anti depression medicine, a part of inhibitor group serotonin and norepinephrine . this medicine is well absorbed through digestive tube. The most important purpose of this survey is to conduct research on the effect of Venlafaxine on Oogenesis in female rats.

**Materials and Methods:** The rats with approximate weight of 250 ± 20 were divided into three groups. The rats received dissolved Venlafaxine in physiological serum in the form of gavage with the concentration of 200, 400 and 600 for the period of 21 days. The control group didn't receive medicine. After the completion of test period, the slices of Ovary were considered after being stained by using H & E method.

**Results:** Results reveal that the number of primary follicles and Secondary follicles and corpus luteum in the experimental group, which received venlafaxine, show considerable reduction in comparison with those of control group (P<0.001).

**Conclusion:** The results indicate that Venlafaxine probably reduces the capability of reproduction and has negative impact on Oogenesis in wistar female rats. Some of the ripple effects gradually disappear when the medicine intake discontinued.

**Keywords:** Venlafaxine, Ogenesis, Rat

### **P-28: The Examination of Cranberry Extract on Liver Tissue of Mouse Embryos**

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**Background:** The aim of this study is to examine the effects of cranberry extract prescription in sensitive days of pregnancy on development and histomorphometric changes in liver of mouse embryo.

**Materials and Methods:** A total of 24 pregnant mice were divided into equal 4 groups. The control group received no injection, while sham group was injected with saline and experimental groups were given cranberry extract at a dose of 8 and 80 mg/kg respectively by intra peritoneally injections on 7<sup>th</sup>, 8<sup>th</sup> and 9<sup>th</sup> day of gestation. Female mice were killed in 18<sup>th</sup> day of gestation and not only did embryos examine in terms of apparent abnormalities, but also they were histologically subjected about their liver tissue.

**Results:** No apparent abnormality in control, sham and 8 mg/kg group was seen at all, whereas in 80 mg/kg group some abnormalities such as micromelia, fetal and placental atrophy and subcutaneous bleeding were observed. The mean of crown- rump length of embryos an placenta diameter in 80 mg/kg group showed a significant decrease in comparison with that of control group. In histomorphometric study of embryos liver in 80 mg/kg group, there were significant increase and decrease in the average of hepatocyte occupancy rate and sinusoids extent percentage rather than that of control group respectively. Also about measurement of hepatocyte nuclei in 80mg/kg group, there was a significant decrease in comparison with that of control group.

**Conclusion:** Considering the obtained results, it is supposed that prescription of 80 mg/kg cranberry extract to pregnant mouse can cause abnormalities in histological changes in liver tissue and fetal development procedure.

**Keywords:** Abnormality, Cranberry, Embryo Liver, Mouse

### **P-29: Antioxidants Improve The Quality of Cryopreserved SSCs**

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**Background:** Radiotherapy and chemotherapy could affect spermatogenesis process, so it is important to preserve fertility of patients who suffer from cancer disease. Cryopreservation of SSCs is considered as a helpful procedure for preserving male fertility in cancer patients. It seems that antioxidants can decrease ROS formation and reduce side-effects of cryopreservation on cells. In this study, we added catalase and alpha-tocopherol to the basic freezing medium in order to evaluate the effects of these two antioxidants on SSCs.

**Materials and Methods:** SSCs were isolated from testes of 3 to 6 days old mice using enzymatic digestion. The purity of isolated cells was evaluated by flow cytometry. Catalase (20 and 40 µl/ml) or alpha-tocopherol (100 and 200 µl/ml) was added to the basic freezing medium. The cell viability, ROS formation and expression of Bax and Bcl2 were evaluated in these conditions.

**Results:** The survival rate of the frozen cells in the presence of catalase or alpha-tocopherol was significantly more than control group ( $P \leq 0.05$ ). In addition, the Bax expression level in the catalase and alpha-tocopherol groups was significantly ( $P \leq 0.05$ ) lower than the control group and a significant rise of Bcl2 expression was detected in the catalase and alpha-tocopherol groups ( $P \leq 0.05$ ). However, the ROS production in catalase and alpha-tocopherol was significantly ( $P \leq 0.05$ ) lower compared with the control group.

**Conclusion:** This cryopreservation method could help to increase the number of SSCs and improve the quality and viability of these cells after cryopreservation

**Keywords:** Spermatogonial Stem Cell, Cryopreservation, Catalase, Alpha-Tocopherol

### **P-30: The Investigation of Transcript Expression Level of Mitochondrial Transcription Factor A (TFAM) during *In Vitro* Maturation (IVM) in Single Human Oocytes**

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**Background:** *In vitro* maturation (IVM) of human oocytes has acquired increasing attention in infertility treatment with great promise. This technique is an alternative conventional *in vitro* fertilization-embryo transfer (IVF-ET), and can be reduced the side effects of gonadotropin stimulation such as ovarian hyperstimulation (OHSS). Oocyte maturation is a complex process including cytoplasmic and nuclear maturation which essential for the acquisition of oocyte competence. Mitochondria are the most predominant organelle in human oocytes. Mitochondria are maternally inherited organelles that

supply ATP to the cell by oxidative phosphorylation. Mitochondria have their own genomes. Little is known about the transcript expression of mitochondrial related genomes during oocyte maturation. This study was to identify mitochondrial transcription factor A (TFAM) during IVM in single human oocytes.

**Materials and Methods:** Oocytes at various stages of germinal vesicle (GV) and metaphase I (MI) maturation obtained from 27 consenting women (age 21–35 years), with male factors who were selected for ovarian stimulation and ICSI procedures. Matured oocytes (MII) were generated following IVM. The mRNA level of TFAM identified using single cell taqman Real time-PCR.

**Results:** The expression level of the target TFAM gene was low at the germinal vesicle (GV) and MI stages ( $P > 0.05$ ). Although, the mRNA level of TFAM gene remained stable in metaphase II (MII) following IVM, the mRNA level of TFAM increased significantly at the stage of MII in *in vivo* ( $P < 0.05$ ).

**Conclusion:** Key regulators of cytoplasmic maturation including mitochondrial gene expression were explored to characterize the important components of IVM so that the culture medium with mitochondrial supplemented may be further optimized in oocyte competence.

**Keywords:** TFAM, Human Oocyte, Real-Time PCR, Mitochondria

### **P-31: Effect of Body Mass Index (BMI) and The Level of Plasma Concentrations of Biomarkers in Semen and Blood Serum in Infertile Men**

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**Background:** The present study aimed to shed light focus on the level of certain hormones such as AMH, FSH, LH, Inhibin B, alpha-glucosidase, and testosterone in the seminal plasma and blood serum to patients infertile men groups (azoospermia, oligospermia and normospermia) as well as calculate the body mass index and find some relationships between these biomarkers and BMI for the same patients.

**Materials and Methods:** When the plasma semen examination and blood serum to 41 Iraqi men infertile (azoospermia (n=18), oligospermia (n=18), and normospermia (n=5)) in Sadr Teaching / Najaf Hospital laboratories of Fertility Center and using the ELISA method as well as the measurement of body mass index for these patients, for the period from 01/11/2014 to 01/02/2015.

**Results:** The results of the current study showed a significant decrease  $P < 0.05$  in all hormonal parameters studied when the two groups azoospermia and oligospermia compared with the control group normospermia. As well as the BMI effect is obvious in these vital signs when examined relations where the current study showed significantly inverse relationship between BMI and FSH, LH, and testosterone and the relationship is a positive but not significantly between BMI and inhibin B, alpha-glycosidase and AMH



These patients when compared with group control (normospermia). There was no significant difference in the level of concentration of these hormones in semen and serum except testosterone was significantly higher in semen than in plasma serum.

**Conclusion:** The present study concluded that BMI has an important impact on the level of concentration of hormones FSH, LH, and testosterone, which plays an important role in spermatogenesis and the emergence of cases of patients Azospermia and Oligospermia.

**Keywords:** BMI, Biomarkers Hormones, Azospermia, Oligospermia

### **P-32: The Study of Morphological Abnormalities in The Sperm of Men Referred to The Center of Infertility ACECR-Arak in The First 6 Months of 1393**

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**Background:** Infertility and fertility decline are quite a conundrum for medicine, rampant among 10-15 percent of couples. In about 40 percent of cases, men are the main culprit. Since sperm production disorders are the focal cause of man infertility, insights into these disorders and taking action to ameliorate the situation is quite vital. The current study concentrates on infertility due to sperm malformation.

**Materials and Methods:** In this cross sectional-descriptive analysis, natural morphology and sperm malformation are the elements which are material to the investigation. Over a span of six months, the semen of 478 patients who referred to the center of infertility ACECR-Arak is meticulously assessed. The analyses were used applying Papanicolaou stains method and in compliance with WHO canons.

**Results:** In this study  $3.83 \pm 3.21$  percent of sperms are morphologically natural. From among  $96.17 \pm 3.27$  percent of malformed sperms,  $5.65 \pm 3.2$  percent have anomaly in their heads,  $4.4 \pm 3.5$  percent in their torso and  $5.21 \pm 3.7$  percent have anomalies in their tails.  $80.9 \pm 14.5$  percent of sperms are also classified as amorphous.

**Conclusion:** Current study clearly demonstrated that the higher the levels of morphological disorders, the less the chances of a fecund pregnancy. To show sound judgment, in-depth analysis of all related characteristics of semen is a must. Factors like genetic background, different diseases and pollutants can devastatingly affect morphological parameters of the sperm. Stress Oxidative is just one of the mechanism leading to this anomaly.

**Keywords:** Infertility, Sperm Morphology, Semen

### **P-33: Ameliorative Effects of Ethyl Pyruvate on Testis Histochemistry, Serum Superoxide Dismutase and Testosterone in Chemotherapeutic Mice**

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**Background:** Many of the drugs such as CP which are used for chemotherapy inneoplastic patientsleaves toxic side effects in various systems of the body including male reproductive system.

**Materials and Methods:** In this experimental study, 15 adult male mice (6-8 weeks) were randomly divided into 3 groups: control group received normal saline (0.2 ml/day, IP), CP group received CP (15 mg/kg/week, IP), the CP+EP group received EP (40 mg/kg/day, IP) along with CP, were treated for 35 days. Superoxide dismutase (SOD) and serum testosterone concentration in the animals were measured and samples of testis were used for histochemical studies

**Results:** This study showed that alkaline phosphatase (ALP), PAS positive reactions and lipid granules in cytoplasm of the Leydig cells in the CP group increased compared with other groups ( $P < 0.05$ ). The SOD and testosterone levels in EP group were higher than the other groups ( $P < 0.05$ ).

**Conclusion:** This study showed the ability of ethyl pyruvate in ameliorating of testis histochemistic parameters and SOD.

**Keywords:** Testis, Cyclophosphamide, Ethyl Pyruvate, Histochemistry, Superoxide Dismutase (SOD)

### **P-34: Crocin Ameliorate The *In Vitro* Fertilization (IVF) in Mice Treated with Cyclophosphamide**

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**Background:** Oxidative stress induced by cyclophosphamide (CP), as a chemotherapeutic agent, causes vulnerability of sperm and declining the fertility capacity. Then in this study the effect of crocin (saffron extract) on amelioration of these side effects was investigated.

**Materials and Methods:** In this study 24 mice were divided in three groups (n=8). Control group received normal saline (0.2 ml/day, IP), CP group received cyclophosphamide (15 mg/kg/week, IP), and CP+Cr group received crocin (200 mg/kg/day, IP) along with CP for 35 days. Then, the animals were euthanized for obtaining sperm from the tail of epididymis. After stimulation of ovulation in 72 female adult mice, oocytes were collected and transferred in HTF medium that contained BSA. Then, *in vitro* fertilization (IVF) was done with capacitated sperms. The rate of fertilization and primitive embryonic growth were evaluated for 120 hours.

**Results:** The results showed increase in fertilization, two cell embryos and blastocysts in CP+Cr Group in comparison to those of CP group ( $P < 0.05$ ). Also, the crocin in CP+Cr group prevented the increase of total number of arrested embryos and percentage of arrested embryos type I, II, and III ( $P < 0.05$ ).

**Conclusion:** This study showed the efficiency of crocin in amelioration of fertility and growth of primitive embryo in animals that received CP.

**Keywords:** Cyclophosphamide, Crocin, Mice, *In Vitro* Fertilization (IVF)

### **P-35: Andrological Anomalies of Chromium and Ameliorations by Morus Nigra and Syzygium Cumini in Mice**

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**Background:** Chromium (Cr) supplements used as “magic lift” without authentications and Cr contaminated sewage water cultivate vegetables and crops around the vicinity of big cities. It is scientific responsibility to find out the toxic effects and preventive measures against such pollutants by local cheapest sources. Testicular histopathology of hexavalent chromium ( $Cr^{+6}$ ) was investigated in adult male albino mice (*Mus musculus*) as mammalian model and the ameliorative effects of Mulberry (*Morus nigra*) fruit extract (MFE) and Jambul (*Syzygium cumini*) were studied against  $Cr^{+6}$  exposure.

**Materials and Methods:** Fifty mice were grouped ( $n=10$ ) as: Control, Cr-free drinking water (15 days); Cr, 50 ppm in drinking water (10 days) followed by no treatment (5 days); Cr-M, Cr-J, Cr-MJ treated as Cr group but followed by MFE, JFE and co-treatment (0.25 mL/12 h) respectively for next 5 days. Animal recovered at 16<sup>th</sup> day to collect blood and testes for biochemical, hematological, histological and micrometric observations.

**Results:** The  $Cr^{+6}$  treated animals showed atrophy and necrosis of seminiferous tubules (ST) to create inter-tubular spaces of testes and increased the testosterone level about 8 folds. The cross sectional area (CSA) of the ST was increased ( $29975.0 \pm 2373.1 \mu^2$ ) as compare to control ( $27184.48 \pm 488.4 \mu^2$ ) with dislodged parrot beak headed (PBH) spermatozoa as compared to club headed (CH) spermatozoa. The accumulation of debris along with tail-less spermatozoa within ST may cause lyse of ST at vacuolated fragile margin. The micrometric studies indicated that spermatogonia and primary spermatocytes were significantly ( $P \leq 0.001$ ) decreased. The irregular distorted sperm heads with alteration of sperm tail

length and mid-piece thickness indicated the anomalies of gametes and the possible intimations of infertility, which can be recovered by fruit extracts.

**Conclusion:** The histological, micrometric, lipid profile findings, enzymatic evaluations and liver function assessments clearly revealed that Mulberry Fruit Extract (MFE) and Jambul Fruit Extract (JFE) have ameliorative aptitude against andro-hepatic anomalies of hexavalent chromium.

**Keywords:** Atrophy, Steatosis, Cirrhosis, Necrosis, Seminiferous Tubules

### **P-36: Addition of Zinc in The Vitrification Medium Improves *In Vitro* Maturation of Oocytes Derived from Vitrified-Warmed Follicles**

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**Background:** Follicle cryopreservation has been proposed as an alternative fertility preservation option. Follicles can be cryopreserved in intact ovarian tissue pieces or after isolation of individual follicles from the fresh ovarian tissue using enzymatic or mechanical techniques. Researchers have used different cryoprotectants and various techniques to improve cryopreservation. It is plausible that inclusion of an antioxidant in the cryopreservation solutions may help in maintaining embryo viability after cryopreservation by reducing the harmful effects of oxygen radicals during the cryopreservation procedure. Zinc is an essential trace element. It has been known as a non adequate level of zinc can alter not only gene expression but also a variety of cellular functions. The present study was designed to determine the effects of different zinc concentrations in the follicle vitrification solutions on incidence of follicles viability and *in vitro* maturation of oocytes derived from vitrified-warmed follicles.

**Materials and Methods:** Ovaries of 2-4 week-old NMRI mice were removed from the animals after being killed and placed in alpha-minimum essential medium ( $\alpha$ -MEM; supplemented with 10% (FBS) and 100 IU/ml penicillin +100  $\mu$ g/ml streptomycin). The ovaries were mechanically dissected using fine hypodermic needles and follicles randomly assigned to following groups: V0, V1, V2, V3 (vitrified-warmed follicles with 0, 100, 150 and 200  $\mu$ g/dl zinc concentration in vitrification solution). Follicles were vitrified sequentially by immersion into two vitrification solutions VS1: 7.5% ethylene glycol (EG) and 7.5% DMSO in  $\alpha$ -MEM supplemented with 20% FBS for 3 minutes and VS2: 15% EG and 15% DMSO in  $\alpha$ -MEM supplemented with 20% FBS and 0.5 M sucrose for 30 seconds. They were placed in the pulled straw with a minimum volume of vitrification medium and then plunged into liquid nitrogen for 1 week. Warming was performed in  $\alpha$ -MEM including 20% FBS that supplemented with descending concentrations of sucrose (1, 0.5, 0.25 M) at room temperature for 3 minutes. After 2 hours of culture, in each experiment 20 preantral follicles per group were stained with

0.4% trypan blue for 1 min at room temperature. The follicular viability was assessed under an inverted microscope. 1.5 IU/ml recombinant human chorionic gonadotrophin (rhCG) and 5 ng/ml recombinant epidermal growth factor (rEGF) were added to the cultures as in vitro ovulatory stimulus. Optimal nuclear maturation rate was achieved after 18-20 hours of induction.

**Results:** Our results showed that the rate of viability and metaphase II increased in presence of zinc in vitrification medium. The viability rate of follicles after vitrification-warming was 70.3, 81.2, 88.5 and 98.0 % also percentage rates of oocytes reaching to MII stage were 35.5, 40.12, 47 and 58.3 % respectively in groups V0, 1, 2, 3 (ANOVA, P value<0.05).

**Conclusion:** The high percentage of survival and maturation rates proves the success of the vitrification procedure. The results also demonstrated zinc supplementation of vitrification medium has a positive effects on the the viability of follicles and maturation rate.

**Keywords:** Vitrification, Zinc, Follicle, *In Vitro* Maturation, Mouse

### **P-37: Vitrification of Mouse Ovary in Presence of Zinc in Vitrification Medium: Histological Evaluation**

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**Background:** Whole ovary cryopreservation has been proved to be a feasible technique in fertility preservation. The normal ovarian cortex contains a large population of primordial follicles, this characteristic make them far more tolerant to cryoinjury. Researchers have used different cryoprotectants and various techniques to improve cryopreservation. It is plausible that inclusion of an antioxidant in the cryopreservation solutions may help in maintaining embryo viability after cryopreservation by reducing the effects of harmful oxygen radicals during the cryopreservation procedure. Zinc is an essential trace element. It has been known that a non adequate level of zinc can alter not only gene expression but also a variety of cellular functions. The present study was designed to determine the effects of different zinc concentrations in the ovary vitrification solutions on follicle morphology as assessed by light microscopy.

**Materials and Methods:** In present study, the ovaries of 2-4 week-old NMRI mice dissected and randomly assigned to following groups :V0,V1,V2 ,V3 (vitrified warmed ovaries with 0, 100,150 and 200 µg/dl zinc concentration in vitrification solution). Ovaries were vitrified sequentially by immersion into two vitrification solutions VS1: 7.5% ethylene glycol (EG) and 7.5% DMSO in  $\alpha$ -MEM supplemented with FBS 20% for 7 minutes and VS2: 15% EG + 15% DMSO in  $\alpha$ -MEM supplemented with 20% FBS and 0.5 M sucrose for 3 minutes. They were placed in the 0.25 ml straw with a minimum volume of vitrification medium and then plunged into liquid nitrogen for 1 week. Warming was performed in

$\alpha$ -MEM including 20% FBS that supplemented with descending concentrations of sucrose (1, 0.5, 0.25 M) at room temperature for 5 minutes. The recovered vitrified ovaries were fixed in 10% buffered formalin, embedded in paraffin wax, serially sectioned at 5 µm, stained with haematoxylin and eosin and analyzed under light microscope.

**Results:** Most histological features of vitrified samples were normal. The cytoplasm of the oocytes was clear and all granulosa layers and theca were intact and firmly attached to the related basement membranes. Stromal cells were normal and with distinct boundaries, prominent nucleus, and pinkish cytoplasm. However in some follicles, stromal cells had distinct margins with foamy cytoplasm, detachment of granulosa layers from the basement membrane and deformity of oocytes were also seen. After vitrification-warming the least damage was observed in the small follicles. The rate of normal preantral follicles in the group V3 was statistically higher than V0 group ( $94.61 \pm 50\%$  vs.  $77.42 \pm 17\%$  ANOVA, P-value<0.05). The proportions of normal follicles in cryopreserved groups V0, 1, 2, 3 were  $83.36 \pm 3.6\%$ ,  $94.35 \pm 48\%$ ,  $92.55 \pm 11\%$  and  $94.52 \pm 05\%$  respectively (ANOVA, P value<0.05).

**Conclusion:** Our study showed ovary vitrification with optimal cryoprotectant solutions such as EG plus DMSO is the most effective for preserving the structural efficiency of ovarian follicles. Vitrification didnt not cause significant changes to the morphology of follicles. Also results demonstrate that supplementation of vitrification medium in a dose manner improved the rate of normal follicles.

**Keywords:** Vitrification, Zinc, Ovary, Histology, Mouse

### **P-38: Evaluation of Sperm Characteristics in Men Referred to The Center of Infertility ACECR-Arak during The First 6 Months of 1393**

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**Background:** Fertility disorder is a multifaceted physiopathological condition comprising men's or women's impotence in fertility or a combination of both. Men's debility in fertilization is the main culprit in 40-50 percent of the studied cases. The first step in scrutinizing infertility is to analyze semen. So, the assessment hinges on analyzing factors like sperm density, total count and motility.

**Materials and Methods:** In this cross sectional-descriptive study, the semen of 478 patients who referred to the center of infertility ACECR-Arak is analyzed. Parameters like sperm density and quantity, and grades of sperm motility are the main foci of interest. All analyses are conducted using optical microscope and in compliance with WHO set standards.

**Results:** The average of sperm density and total number of sperms found in semen are  $58.72 \pm 33.3 \times$  and  $246.11 \pm 166.8$

×, respectively. In the samples tested motility is about  $49.48 \pm 18.3$  percent in which  $11.92 \pm 10.2$  percent of sperms demonstrated grade A of motility,  $23.84 \pm 12.8$  percent grade B and grade C is assigned to  $13.71 \pm 7.2$  percent of sperm motility.  $50.6 \pm 18.1$  percent was also motionless perms.

**Conclusion:** According to the data obtained, judging about men's potential to fertilize necessitates attention to other characteristics of semen as well as simultaneous analysis of influential factors in women.

**Keywords:** Infertility, Sperm Motility, Semen

### **P-39: Protective Effect of Silymarin, Celecoxib and Testosterone on Varicocele-Induced Damages in Testis of Rats**

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**Background:** Varicocele (VCL) is characterized by a progressive disorder which is defined by tortuosity of the pampiniform plexus veins. Although elevated oxidative stress, diminished endocrine status and severe inflammation are considered as main reasons for VCL-induced apoptosis, the role of estrogen receptors, testicular aromatization and proto-oncogenes under these conditions are remained unknown. Present study was done to uncover the therapeutic effect of silymarin (SMN, potent antioxidant), Celecoxib (CCB, COXII-inhibitor) and testosterone co-administration on mentioned parameters.

**Materials and Methods:** Forty two mature male Wistar rats (2 months old) were randomly divided into 7 groups (N=6) as control-sham and experimental. VCL-induced animals in control-sham group received no chemical. The animals in experimental groups subdivided into 6 groups as; SMN alone-received (100 mg/kg), CCB alone-received (10 mg/kg), testosterone alone-received (40 µg/kg), SMN + CCB-received, SMN + testosterone-received, CCB + testosterone-received and SMN + testosterone+CCB-received groups. All chemicals were administrated orally, for 60 days. After 60 days testes were dissected out. The ER $\alpha$ , ER $\beta$ , p53 and bcl-2 expression was evaluated by using immunohistochemical and RT-PCR techniques. The mRNA level of cytochrome CYP19 was assessed by using RT-PCR.

**Results:** Co-administration of SMN+CCB+testosterone significantly (P<0.05) up-regulated VCL-reduced ER $\alpha$ , bcl-2 (both at mRNA and protein levels) and CYP19 expression, and down-regulated VCL-increased ER $\beta$ , p53 expression. Simultaneous administration of SMN+CCB+testosterone exerted remarkably better results compared with alone forms of administration.

**Conclusion:** Our data suggested that co-administrating antioxidant, anti-inflammatory and exogenous testosterone promotes the endocrine statuses of the testis by ameliorating aromatization, elevating ERs and bcl-2 expression, and finally

suppressing p53 mRNA level.

**Keywords:** Varicocele, Silymarin, Celecoxib, Spermatogenesis, Apoptosis

### **P-40: The Effect of Methanolic Extract of Avocado (Persea Americana) Seed on Quality and Quantity Index of Sperm in Mice with Diabetes Type II**

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**Background:** Type II diabetes is a metabolic disorder that can gradually cause damage to the function of many organs such as the gonads. It is believed that poor blood glucose control is the main cause of diabetic side effects. Avocado (*Persea americana*) is a native tree of Central and South America that has traditionally been used due to its antioxidant, anti-inflammatory, anti-hyperglycemic, anti-hyperlipidemic and anti-hypertensive effect but there is no scientific information about effect of Avocado seed extract on sperm characteristics in diabetic patients.

**Materials and Methods:** In this study, 40 adult male mice were divided into 8 groups including: control, diabetic, 3 sham and 3 treatment groups. Diabetes type II was induced by high fat diet and low dose of streptozotocin (STZ). One week after STZ injection, sham and treatment groups received 50, 100 and 150 mg/kg methanolic extract of Avocado seed for 40 consecutive days orally via gavage. Finally, animals were euthanized and the epididymis was removed and dissected in Ham's F10 solution, incubated at 37°C and sperm motility was evaluated. Sperm were counted using a hemocytometer. Smears were prepared from the suspension stained with eosin-nigrosin and examined for sperm abnormalities by light microscope.

**Results:** Sperm analysis indicated that however extract did not change total number of sperm but increased quality of sperm in high dosage in sham group compared to control significantly. Results showed that Avocado can significantly reduce malformed, abnormal and dead sperm and increase sperm with grade A and B in treatment group compared to diabetic animals (P<0.05) and bring it to control group in a dose-dependent manner.

**Conclusion:** It can be concluded that administration of methanolic extract of Avocado seed is a suitable protective strategy for side effect of diabetes and can increase fertilization ability in the diabetic males.

**Keywords:** Avocado, *Persea Americana*, Diabetes type II, Sperm

### **P-41: Investigation of Correlation be-**

### **tween Zinc, Copper and Iron Concentrations in Blood Serum, Testicular Tissue and Epididymal Tissue of Rams and their Associations with Serum Luteinizing Hormone Concentration**

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**Background:** Correlation between metals may be useful in predicting toxicity to various biologically important organisms. The purpose of the current analysis was to evaluate the association of levels zinc, copper and iron in the ram blood serum, testicular tissue and epididymal tissue, and correlations of these elements to luteinizing hormone were investigated.

**Materials and Methods:** Testis and blood samples used for this study were collected from forty five healthy adult rams from Hamadan abattoir. Metals concentrations were measured by flame atomic absorption spectrometry (FAAS). Concentration of LH was measured by enzyme linked immunosorbent assay (ELISA). Statistical analysis of data was carried out using the SAS software version 9.2. The level of significance was set at  $P < 0.01$  and  $P < 0.05$ .

**Results:** The mean concentrations of metals in blood serum, epididymal tissue and testicular tissue were: zinc 1.29 ( $\mu\text{g/ml}$ ), 184.11 ( $\mu\text{g/g}$ ) and 212.46 ( $\mu\text{g/g}$ ), respectively, copper 0.36 ( $\mu\text{g/ml}$ ), 18.92 ( $\mu\text{g/g}$ ) and 16.78 ( $\mu\text{g/g}$ ), respectively, iron 2.42 ( $\mu\text{g/ml}$ ), 110.06 ( $\mu\text{g/g}$ ) and 150.53 ( $\mu\text{g/g}$ ), respectively. The mean concentration of LH in blood serum was 58.86 (ng/L).

The analysis showed high positive correlation between serum copper and serum iron ( $r=0.400$ ), serum copper and iron epididymal ( $r=0.484$ ), serum copper and zinc testicular ( $r=0.542$ ), serum iron and copper epididymal ( $r=0.482$ ), zinc epididymal and copper epididymal ( $r=0.434$ ), zinc epididymal and iron epididymal ( $r=0.622$ ), zinc epididymal and zinc testicular ( $r=0.489$ ), copper epididymal and iron epididymal ( $r=0.450$ ), iron epididymal and zinc testicular ( $r=0.457$ ), zinc testicular and copper testicular ( $r=0.464$ ), zinc testicular and iron testicular ( $r=0.783$ ). There was no correlation between the metals and serum luteinizing hormone.

**Conclusion:** In conclusion, our results suggest that there are positive correlation in the concentration of studied elements (zinc, copper and iron) in blood serum, testicular tissue and epididymal tissue. Furthermore, this study suggests that LH with zinc, copper and iron are not correlated.

**Keywords:** Zinc, Copper, Iron, Luteinizing Hormone, Testis

### **P-42: Concentrations of Zinc, Copper, Iron, lead, and Cadmium in Ram Epididymal Tissue and Their Correlations to Serum Testosterone**

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**Background:** With the advent of rapid industrialization, several chemicals are released in the air, water and soil. Chemical elements play a crucial role in male reproduction. Heavy metals exposure can adversely affect male fertility and result in severe impairment of testicular functions including germ cell death and inhibition of testicular steroidogenesis. The aim of this study was to investigate the relationship between the concentrations of Zn, Cu, Fe, Pb, and Cd in the tissue of ram epididymal and serum testosterone.

**Materials and Methods:** Samples were taken from 45 ram in Hamadan. Concentrations of zinc (Zn), copper (Cu), iron (Fe), Cadmium (Cd) and lead (Pb) in epididymal tissue by flame atomic absorption spectrometry (FAAS) were measured. Serum concentration of testosterone was measured by enzyme linked fluorescent assay (ELFA) method. The data were analysed by SAS software. The level of significance was set at  $P < 0.01$  and  $P < 0.05$ .

**Results:** Analysis by FAAS showed that mean concentrations of zinc, copper, iron, cadmium, and lead in samples were 184.11, 18.93, 110.06, 0.05, and 3.53 ( $\mu\text{g/g}$ ), respectively. The mean level of Serum concentrations of testosterone in the study samples was 1.63 ng/ml. In our study, the correlation analysis revealed a high positive correlation between zinc and testosterone concentration ( $r=0.460$ ) and also positive correlation between lead and testosterone concentration ( $r=0.360$ ).

**Conclusion:** Heavy metals disrupt hormone production and action in the mammalian testes. Lead effects on spermatogenesis, steroidogenesis, and red-ox system. It has been shown that Zn supplementation increases serum testosterone levels. According to this study, it was concluded that Zn and Pb have positive correlation with testosterone. It should always consider the impact of metals on reproductive hormones.

**Keywords:** Epididymal, Testosterone, Metals, Ram

### **P-43: Micronutrients and Sperm DNA Quality in Older Men**

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**Background:** Recent systematic reviews of the effects of micronutrients on male fertility have identified clear positive effects on basic sperm characteristics. The vast majority of

studies reviewed found that micronutrients, particularly those that are antioxidants or aid their function, significantly reduce sperm oxidative stress or DNA damage in subfertile males but greater evidence is required to clearly state whether these improvements translate to improved fertility. To investigate whether lifestyle factors such as increased dietary intake of micronutrients reduce the risks of sperm DNA damage, and whether older men benefit more younger men.

**Materials and Methods:** This is a review article.

**Results:** Sociodemographics, occupational exposures, medical and reproductive histories, and lifestyle habits were determined. The average daily dietary and supplement intake of micronutrients (vitamin C, vitamin E,  $\beta$ -carotene, zinc and folate) was determined. Men with the highest intake of vitamin C had approximately 16% less sperm DNA damage (alkaline sperm comet) than men with the lowest intake, with similar findings for vitamin E, folate, and zinc (but not  $\beta$ -carotene). Older men (>44 years) with the highest vitamin C intake had approximately 20% less sperm DNA damage compared with older men with the lowest intake, with similar findings for vitamin E and zinc. The older man with the highest intake of these micronutrients showed levels of sperm damage that were similar to those of the younger men. However, younger men (<44 years) did not benefit from higher intakes of the micronutrients surveyed.

**Conclusion:** Men with higher dietary and supplement intake of certain micronutrients may produce sperm with less DNA damage, especially among older men. This raises the broader question of how lifestyle factors, including higher intakes of antioxidants and micronutrients, might protect somatic as well as germ cells against age-associated genomic damage. Human sperm alkaline and neutral comet assay, vitamin C, vitamin E,  $\beta$ -carotene, zinc, folate.

**Keywords:** Vitamin C, Vitamin E,  $\beta$ -Carotene, Zinc, Folate

#### **P-44: Infertility and Non-Steroidal Anti-Inflammatory Drug Usage (An Experimental Design)**

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**Background:** Unusual effect of celecoxib may be seen in some patients that have used this drug for a long time. Celecoxib is a form of non-steroidal anti-inflammatory drug (NSAID) that directly targets COX-2, an enzyme responsible for inflammation and pain which is the main feature of celecoxib. The goal of this survey is to assess the effect of celecoxib on male-reproductive system's functions.

**Materials and Methods:** The goal of this experimental survey is to determine the effect of celecoxib on rat reproductive system, on spermatogenesis and on the level of blood testosterone hormone.

Histological studies and weight measuring of (testis, prostate, seminal vesicle and epididymis) and the level of blood testosterone are done. Fifty rats with 200-230 g. weight were

selected and compared in 5 groups for 15 days as following: Control group (no drug given), sham group (solvent drug: Di-methyl sulfoxide), 3 cases group (orally celecoxib 10, 20 and 40 mg/kg given daily). In the end of 15 days heart blood sampling for measuring serum testosterone level was accomplished and later the reproductive systems were separated and prepared for histological study.

**Results:** Differences in sertoli cells were seen in the control and the case groups. So that in the case group (40 mg/kg) number of sertoli cells decreased due to decrease testosterone level. This can cause production of abnormal sperms. Significant differences were seen in the mean weight of prostate per body weight in case group (40 mg/kg) in compared to the control group.

**Conclusion:** Decreased testosterone hormone were seen in male rat after using high doses of celecoxib.

**Keywords:** Non-Steroidal Anti-Inflammatory Drug, Male, Infertility, Testis

#### **P-45: Xenotransplantation of Mouse Ovaries into Pseudopregnant Rat Uteri**

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**Background:** Xenotransplantation of ovary can help individuals who may be at sterility risk after chemotherapy or radiotherapy of diseases such as cancer. Moreover, this technique can be used to preserve fertility of valuable animal species. The aim of the present study was to preserve balb/c mice ovarian tissue by xenotransplantation into uterus of rat that is immune suppressed.

**Materials and Methods:** Seven female Sprague-Dawley rats in proestrous stage were mated with male vasectomised rats and after observation of vaginal plaque they were selected as pseudopregnant seven days after mating. Both ovaries from seven mice in diestrous stage were transplanted into the pseudopregnant rat uteri cavity. Fourteen days after xenotransplantation, the ovaries were removed with the rat uterus, and stained with haematoxylin and eosin for histological examination.

**Results:** Four transplantations were successful and mice ovaries were found in uterine cavities of the recipient rats. However, six ovaries were inactive with no significant secondary or tertiary follicles or corpora lutea, but there was no evidence of tissue necrosis. Two ovaries showed evidence of inactive corpora lutea with primordial follicles.

**Conclusion:** Survival of the transplanted ovaries in the rat uterine cavity demonstrates that mice ovaries can be immunologically acceptable to the pseudopregnant uterine cavity.

**Keywords:** Xenotransplantation, Ovary, Pseudopregnancy, Mouse, Rat

#### **P-46: Vitrification of Mouse Ovaries Under Magnetic Field**

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**Background:** The aim of this study was to investigate the effect of applying the 1-mT magnetic field (MF) in the ovarian tissue vitrification.

**Materials and Methods:** Ovaries of 6-8 week-old Naval Medical Research Institute female mice randomly were divided into 5 groups: V1: Ovaries that immediately after leaving of the mice body, were examined histologically (control group), V2: Ovaries that without exposure to MF were frozen and thawed, V3: Ovaries that were frozen by exposure to MF, V4: Ovaries that were melted by exposure to MF and V5: Ovaries that by exposure to MF were frozen and thawed. Vitrified-warmed samples were incubated for 30 minutes and fixed in Bouin's fixative. The samples were serially sectioned and stained either with H&E or immunohistochemistry kit of pro-caspase-3.

**Results:** The difference in the number of primordial, primary and preantral healthy and dead follicles between the control and V4 groups was not significant. Morphologically healthy antral follicles showed significant reductions in all vitrification groups compared to the control. In comparison with healthy follicles, there were significantly more dead follicles in the V2 group than the control group. The apoptotic follicles increased significantly after vitrification, with the exception of the antral follicles.

**Conclusion:** Results confirmed that, MF-exposed follicles exhibited greater resistance to the freezing and thawing. Thus, it is reasonable to suggest that the 1mT MF exposure reduce the damage of the ovarian tissue during vitrification and helpful for ovarian cryopreservation.

**Keywords:** Ovary, Vitrification, Magnetic Field

#### **P-47: Effect of Blastocoelic Fluid Reduction on Quality and Expression of Developmentally Important Genes in Mouse Blastocysts**

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**Background:** Recent researches reveal that manual puncturing of the trophectoderm of blastocyst before vitrification, increase the quality of embryo. However, in any of these studies, the importance of blastocoelic fluid and its impact on the formation of three cell lines is not mentioned. Therefore, in

the present study, the effect of blastocoelic fluid reduction before vitrification on survival and hatching rate, expression of lineage specific genes (Oct4, Nanog, Cdx2, Eomes and Gata6), and apoptosis related gene (P53) in mouse blastocyst was studied.

**Materials and Methods:** For this purpose, two sources of In vitro and In vivo produced mouse embryos were used and randomly divided in to three groups. 1. Vitrified/warmed blastocysts, 2. Vitrified/warmed blastocysts after artificial collapse (AC) and 3. Fresh blastocysts as control group. The survival and hatching rates of embryos were evaluated. After total RNA extraction of three groups, Real time PCR was accomplished.

**Results:** The survival rate of treatments was similar to the control group. The hatching rate of AC-vitrified/warmed blastocysts was significantly higher than that of non-AC group. The expression results of two sources were approximately similar. There was no significant change in expression of pluripotency genes (Oct4, Nanog) between vitrified/warmed and AC-vitrified/warmed blastocyst. The expression of Cdx2, Eomes, Gata6, and P53 in AC-vitrified/warmed was reduced in compared with vitrified/warmed blastocyst (P<0.05).

**Conclusion:** Considering the increase in hatching rate and decrease in the expression of P53 gene (reduction in stress) and Eomes, Cdx2 genes (reduction damage to the trophectoderm cells) in AC-vitrified/warmed group, this method could be an effective way to contribute to the successful blastocyst vitrification.

**Keywords:** Artificial Collapse, Vitrification, Blastocyst

#### **P-48: Evaluation of The Effectiveness of Zinc and Alpha-Tocopherol on Total Sperm Count, Apoptosis, DNA Damage, DNA Breakage and Viability of Sperms Under Cell Phone Radiation and Comparison Between These Protocols**

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**Background:** There has been a growing claim about the stress induced by the modern life and especially by cell phones. It may have detrimental effect on sperms. We examined the relationship of stress caused by cell phone radiation and the effect of Zinc and Alpha-tocopherol on the sperms and its chromatin in this condition.

**Materials and Methods:** Fresh semen has been collected from 20 young men. Each sample was divided into 5 aliquots including: control, exposed, expose + 15µm Zinc, expose +

5 µM alpha-tocopherol, and expose + 15µ M Zinc + 5 µM alpha-tocopherol group. Samples were irradiated by cell phone for 20 minutes. After radiation, total count estimation was done in the first step. For detecting Apoptosis, TUNEL protocol has been used. DNA breakage test was done by Halo Sperm kit and DNA damage was detected by Acridine Orange staining protocol. Viability test was conducted using Trypan Blue staining. Data were analyzed by one way ANOVA using SPSS 19 software.

**Results:** Our results have indicated that exposed group showed a significant increase in Apoptosis ( $90.5 \pm 44.26$  vs.  $42.4 \pm 06.13$ ), DNA damage ( $33.22 \pm 6.81$  vs.  $29.78 \pm 2.18$ ) and DNA breakage ( $17.78 \pm 3.49$  vs.  $13.11 \pm 6.30$ ) compare to the control group. Total count and Viability ( $68.51 \pm 5.18$  vs.  $85.46$ ) were decreased in this group. Adding antioxidants in semen during cell phone exposure showed protection effect compare to the control and the exposure induced group ( $P < 0.05$ ).

**Conclusion:** The addition of Zinc and Alpha-tocopherol in semen could be useful to human sperms in stress conditions.

**Keywords:** Sperm, DNA Breakage, Apoptosis, Chromatin, DNA Damage

#### **P-49: Protective Effect of Grape Seed Extraction on Dexamethason Induced Spermatogenesis Defect in Mice**

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**Background:** Exposure to glucocorticoids such as dexamethasone (Dex) leads to numerous changes in various biological systems including the reproductive system. The present study investigates the efficacy grape seeds, source of the most useful groups of plant flavonoids, phenolic procyanids, to attenuate Dex-induced spermatogenic defects in the mouse testicles.

**Materials and Methods:** A total of 32 male mice were divided into four groups (8 mice each). The first group served as control and received distill water only for 25 days. Mice of the second group received Dex at the dose of 7 mg/kg body weight by i.p. injection for 7 days. Mice of the third group received efficacy grape seeds extract at a dose of 100 ml/kg body weight. The fourth group received efficacy grape seeds extract at the dose of 100 mg/kg for 25 days and Dex at the same dose of the second group was injected during the last 7 (18 to 25) days. Statistical significance was determined us-

ing one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. A P value  $< 0.05$  denoted the presence of a statistically significant difference

**Results:** Dex induced changes in the parameters of spermatozoa and Testicular histopathology in comparison with the control group. The spermatozoa parameters were altered in Dex + grape seeds extract group in a lesser extent than Dex animals ( $P < 0.05$ ). Dex caused epithelial vacuolization, sloughing of germ cells, reduction of seminiferous tubule diameter, and significant maturation arrest ( $P < 0.05$ ). Grape seeds + Dex treatments showed significantly prevented these histopathologic changes ( $P < 0.05$ ).

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

**Keywords:** Grape Seeds, Dexamethasone, Anti-Oxidant, Spermatogenic Cells

#### **P-50: Evolution of Bovine Embryos Cleavage in Co-Cultured Mesenchyme Stem Cells (MSCs) Derived from Adipose Tissues**

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**Background:** Mesenchyme stem cells (MSCs) are one of the widely used sources in tissue engineering and efficacy of cultural environment. In this survey, rat stem cells are used as coculture. Owing to main obstacle of embryo culture in the lab medium, the survival of embryo in lab is low in comparison with that of normal conditions. The purpose of this research is the survey of fertilization and culture of bovine embryos in mesenchymal stem cells derived from adipose tissue (ADMCS).

**Materials and Methods:** Isolated ADMCS were cultured on 4-well dishes. Three days after cell culture, the bovine immature oocytes were transferred on this mono layer system and the in vitro matured oocytes were fertilized by sperms in another MSCs free environment subsequently. Then, in vitro fertilized embryos were co-cultured with the same mono layer of ADMCS for seven days. During oocyte and embryo coculture, the rates of maturation and cleavage were assessed.

**Results:** This study reveals that co-culture of embryos with mesenchymal stem cells could increase the survival and cleavage of embryos significantly ( $P < 0.05$ ). The maturation rate of bovine co-cultured oocytes (82%) in comparison with



that of control group (78%) has increased significantly. Also, the rate of blastocyst formation in co-cultured embryos (33%) was analytically comparable with that of control group (26%).  
**Conclusion:** What bolsters substantially the quality and development of embryo is co-culture systems.

**Keywords:** Co-Cultured, Stem Cell, Rat

### **P-51: Treatment of Sexual Dysfunction in Patients with Spinal Cord Injury**

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**Background:** Regaining arm and hand function was deemed the most important by quadriplegics, while regaining sexual function was given the highest priority for paraplegics. Improving bladder and bowel function was of shared importance to both injury groups. The objective of this study was to conduct a systematic review and meta-analysis of the existing research on patients with spinal cord injury and therapy of sexual dysfunction.

**Materials and Methods:** Science Direct, Pubmed, Cochrane, CINAHL, Embase, ProQuest Dissertations, scopus, (2000 through 2012) were searched for English-language studies using a list of keywords. The books about physical therapy and medical and neurological were studied too.

**Results:** Sexual arousal and libido are determined by psychological factors, general well-being, and hormones like testosterone. Early sexological counselling is supplied in our rehabilitation centre, not only for the needs of the patients but just as much for the partner of the patient, who is facing "a new sexuality, with most of the time a more active role". Partners are confronted with patients with many frustrations, unrealistic expectations, depression, and aggression, which situation does not promote the resumption of their sexual activity. Hormone substitution is rarely needed early on except after testicular trauma or lesions of the hypophysis. Once rehabilitation advances, mild testosterone deficiencies might require therapy not only for increasing libido and arousal but also to ameliorate the sexual dysfunction, general well-being, muscle strength, and bone structure.

**Conclusion:** Therapy for erectile dysfunction and ejaculatory dysfunction related to fertility treatments is well established but not the treatment of dysfunction of sexual performance and/or orgasm. Special attention is given to priapism and its treatment in patients with spinal cord injury.

**Keywords:** Sexual Dysfunction, Patients with Spinal Cord Injury, Infertility.

### **P-52: Brain-Derived Neurotrophic Factor Promotes The Development of Human Ovarian Early Follicles during Growth *In Vitro***

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**Background:** Cryopreservation of ovarian cortex is increasingly used to preserve fertility before cancer therapy. Recently, studies show that Brain-derived neurotrophic factor (BDNF) may be involved in oocyte maturation. Brain-derived neurotrophic factor (BDNF) is member of neurotrophin family that has anti-apoptotic effects on nervous system. Recent researches show that it also plays key role in female reproductive system such as nuclear and cytoplasmic maturation and embryo development. Therefore, the present study was conducted to evaluate the effects of BDNF on morphology, viability and apoptosis of primordial follicles, when it was added to *in vitro* culture media.

**Materials and Methods:** Cortical tissue strips from normal pregnant (n=8) women were vitrified. After two weeks, thawed cortical strip was divided to 3 groups as following: FSH, FSH + BDNF (100ng) and control (without FSH). Subsequently, cortical strip was cultured in a ( $\alpha$ -MEM) medium for two weeks. Then ovarian fragments were analyzed for morphology (Hematoxylin and eosin, H & E), and incidence of apoptosis (TUNEL kit). Hormones (Estradiol, Anti mullerian hormone and progesteron) concentration in culture medium and viability were evaluated using ELISA kit and Calcein-AM Ethidium homodimer-1 respectively.

**Results:** Our data indicates that the ratio of primary and secondary follicles to primordial significantly increased in BDNF+ FSH group compared with the other groups. Estradiol and progesteron concentration were also significantly increased in BDNF+ FSH group compared with the other groups. Moreover, the percentage of apoptosis in BDNF+ FSH group was decreased significantly in comparison to the other groups. Viability was also significantly higher in BDNF+ FSH group compared to the other groups.

**Conclusion:** This data suggests that combination of BDNF and FSH have beneficial effects on human ovarian early follicles growth *in vitro*.

**Keywords:** BDNF, *In Vitro* Culture, Cryopreservation, Follicle, FSH

### **P-53: Maternal Diet, Vaginal pH and Time of Ovulation Effects on Offspring's Sex Ratio**

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**Background:** Although there are different ways to achieve the desired sex of the fetus, most of those are not completely effective. This paper reports a study on human to check the theories which hypothesize that high potassium maternal diet, douching with alkaline substance to change vaginal PH and intercourse around ovulation affects the sex ratio of the progeny.

**Materials and Methods:** A quasi-experimental study of 242 couples wishing to preselect the male sex of their child was conducted in Urmia University of Medical Sciences in 2001-2012. All of the clients used a constant guideline including high potassium diet, douching with soda (alkaline substance) and determination of timing of ovulation using both ultrasound and calendar methods. They recommended having intercourse on the first 24 hour after proposed ovulation time.

**Results:** Records of 242 patients were studied. Pregnancy outcomes included 178 (73.5%) boys and 64 (26.5%) girls, sex ratio calculated 2.8. In 19 cases, ultrasound was used to determine the time of ovulation, in this group 17 boys and 2 girls were borne with sex ratio of 8.5. This ratio showed a significant difference with the group who have not used ultrasound ( $P=0.000$ ). These results show ultra sound have important role to determine ovulation time. Male ratio has increased dramatically among employed mothers with higher educational and vs. house wife mothers with lover education (sex ratio=6.1 male ratio=0.84).

**Conclusion:** Our results support hypothesis predicting male sex of offspring using high potassium diet, vaginal douching with alkaline substance and sexual intercourse around ovulation.

**Keywords:** Sex Ratio, Vaginal pH, Time of Ovulation, Uromia, Iran

#### **P-54: Evaluation of The Acute and Chronic Effects of Different Gold Nanoparticle Doses on Sperm Parameters and Chromatin Structure in Mice**

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**Background:** The solid and colloidal particles in the range of 1-100 nm are called nanoparticles. Recently, one of the most important metal nanoparticles, with wide usage is gold nanoparticle. Based on the previous studies, gold nanoparticles have spermatotoxic effects and can destroy sperm motility. The aim of present study is the effect of gold nanoparticles on chromatin quality and sperm parameters in mice as an experimental model.

**Materials and Methods:** Seventy two bulb-c male mice were divided into nine groups, including four treatment groups, four sham and a control group. Treated group received gold na-

noparticle solution with 40 and 200  $\mu\text{g} / \text{kg} / \text{day}$  doses through intraperitoneal injection for seven and thirty five days. Sham groups was treated with 1.2 mM sodium citrate solution with 40 and 200  $\mu\text{g} / \text{kg} / \text{day}$  doses for the same days and control group didn't receive any materials. One day after the last injection, mice were sacrificed and the cauda epididymis of each animal was removed for sperm morphology, motility, count and viability analysis. Chromatin structure was assessed using aniline blue, toluidine blue, acridin orange and CMA3 staining.

**Results:** The results of the sperm analysis showed that sperm count, viability, motility and morphology especially in the 35-day group had statistically significant differences when compared with control group. It should be noted that reduction was prominent in progressive motility ( $P<0.05$ ). The results of toluidine blue staining was non-significant but regard to aniline blue, acridine orange and CMA3 we saw a significant increase in abnormal spermatozoa of Au-treated group.

**Conclusion:** Gold nanoparticles can create significant changes in sperm parameters and chromatin structure. These effects were more obvious in maximum dose (200  $\mu\text{g}/\text{kg}/\text{day}$ ) and chronic phase (35 days).

**Keywords:** Gold Nanoparticles, Sperm Parameters, Chromatin Structure

#### **P-55: Cryopreservation of Rooster Semen Using Hand-Made Cryopreservation Media Supplemented with Different Cryoprotectants**

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**Background:** Cryopreservation procedure for poultry semen is not reliable due to structural and biochemical damages which can lead to reduction in sperm quality and fertility. Development of cryopreservation medium has crucial effects for successful recovery of sperm after freezing. The objective of this study was assessment of cryopreservation of rooster semen using hand-made cryopreservation media supplemented with different cryoprotectants.

**Materials and Methods:** Two hand-made diluents (Lake and Beltsville media) were assessed for cryopreservation of rooster semen. Moreover, glycerol (3%) and Dimethylacetamide (6%) were applied as cryoprotectants in the diluents for rooster sperm freezing. Experimental treatments were consisting of A: lake with 3% glycerol, B: Lake with 6% DMA, C: Beltsville with 3% glycerol and D: Beltsville with 6% DMA. For qualification of treatments, motion characteristics and viability were measured using computer semen analysis and Eosin-Nigrosi, respectively. Statistical analysis was applied using SPSS software. The average was compared with Tokay Test.

**Results:** In a general point of view, the highest significant

percentage of sperm with motility and viability were obtained in Lake solution. In Lake solution, glycerol had the higher significant effect for motility and viability compare to DMA. In Beltsevil extender, there were not significant effects between glycerol and DMA.

**Conclusion:** From our results, it can be concluded that Rooster semen could be more efficient after freezing-thawing when it cryopreserved with Lake Solution supplemented with glycerol.

**Keywords:** Cryopreservation, Rooster Semen, DMA, Viability

### **P-56: Artificial Oocyte Activation Alone Intra Cytoplasmic Sperm Injection in Men with Severe Semen Parameters**

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**Background:** Failed fertilization occurs 1-3% in the infertile couples. Failure of oocyte activation (OA) is mainly attributed to failed fertilization. ICSI alone artificial oocyte activation (AOA) is only treatment approach for these individuals. Three procedures are introduced for AOA: chemical, mechanical and electrical. Most of the clinical Centers have commonly used ionomycin for activation of oocyte in the human. In this regard, it is reported that ionomycin could be a suitable agent for infertile couples' candidate of ICSI. Therefore, this clinical study was designed to compare clinical outcomes of infertile men who oocytes of their wife were treated with ionomycin and who oocytes of their wife were not treated with ionomycin (control).

**Materials and Methods:** Semen samples were obtained from 30 infertile individuals with severe male factor. 15 couples candidate of AOA were considered as "study group" and treated with 10 M ionomycin. 15 couples were not treated with ionomycin and considered as "control group". Fertilization rate, embryo quality and pregnancy rate were compared between two groups.

**Results:** Percentage of fertilization rate and cleavage rate were significantly higher in study group compared to control group (P<0.05). However, we did not observe any significant difference in percentage of pregnancy between two groups (P>0.05).

**Conclusion:** We suggested that AOA along with ICSI may improve the chance of fertilization and cleavage rate in couples with severe abnormal semen.

**Keywords:** Ionomycin, AOA, ICSI, Failed Fertilization

### **P-57: Evaluation of Nonyl Phenol Toxic Effect on Sperm Quality of The Reproductive System in Adult Male Mice**

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**Background:** Nonyl phenol (NP) is a degradation product of nonylphenol polyethoxylates, which are widely used in the production of industrial and consumer surfactants. NP a Kind of environmental chemical, is thought to imitate endogenous hormones, inhibit the actions of hormones, and induce reproductive abnormalities. In this study, evaluation of Nonyl phenol toxic effects on reproductive system in adult male mouse.

**Materials and Methods:** In this study, 20 adult male mice randomly divided in 2 groups (N=10), control group received only corn oil and Nonyl phenol group received Nonyl phenol (250 mg/kgbw) for 35 days by gavaged. Sperm analysis (motion, count, morphology and viability) was evaluated at the end of the experiments.

**Results:** The epididymal sperm counts in the groups that received NP showed significant decreases compared to the control group. Also dead and abnormal sperms significantly increased following NP treatment compared with control. The motility of caudal sperm was reduced significantly with NP treatment.

**Conclusion:** The present results highly support the idea that NP induces testicular toxicity by adverse effects on the sperm parameters in adult male mice.

**Keywords:** Nonyl Phenol, Testicular Toxicity, Sperm, Viability

### **P-58: Mesenchymal Stromal Cell Cultures from Human Testis Tissue Cryopreservation and Their Potential for The Future Cell Therapy Applications**

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**Background:** Recent studies have shown that human testis derived cell cultures are neither pluripotent nor from germ cell origin whereas they are from mesenchymal stromal origin and multipotent. It would be desirable to set up a cryopreservation technique to preserve these cells for the future cell therapy applications in the case of people with testis cancer and male infertility. Here we have investigated the efficiency of conventional slow freezing cryopreservation method to preserve human testis derived mesenchymal stromal cells (hTMSCs).

**Materials and Methods:** A human testis from 90 years old man was obtained for research after fully consent ethical approval. The whole testis was minced to small pieces (1mm<sup>3</sup>) and frozen in a 10% DMSO cryopreservation solu-

tion and transferred to -80°C. The samples were transferred in the liquid nitrogen, the day after. After one month samples were thawed and cultured in two different culture conditions; DMEM/10%FBS and DMEM/20% FBS.

**Results:** 13 frozen human testis pieces were thawed and cultured. Initially small number of clusters and cells been observed, and after almost two weeks most of the flasks been confluent by hTMSCs. Human TMSCs have been passaged using trypsin/EDTA. From our experience 20% of FBS was more supportive for cell proliferation after cryopreservation.

**Conclusion:** Our data indicates that 10% DMSO cryopreservation solution would support hTMSCs preservation. Moreover, 20% FBS would be more supportive for the hT-MSC cultures after cryopreservation thawing process. These data will lead itself for the potential of the hTMSCs in the future cell therapy applications. Further investigations are in progress for the identification of the hTMSCs before and after cryopreservation using different markers in a modified defined culture device.

**Keywords:** Stem Cells, Human Testis Derived Mesenchymal Stromal Cells, Cryopreservation.

### **P-59: The Association between Levels of Vitamin E in Follicular Fluid with The Morphology of Oocytes and The Quality of Embryos in Patients with IVF**

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**Background:** According to the global increasing trend of infertility, using assisted reproductive technologies to solve this issue is recommended. It seems that vitamin E is an antioxidant with protective role in the body. Therefore, we aimed to investigate the relation between levels of vitamin E in Follicular fluid with the morphology of oocytes and the quality of embryos in patients with IVF.

**Materials and Methods:** This is a cross-sectional analytical study which was done on oocytes and embryos from 50 women with IVF admitted. Inclusion criteria were 20-45 years of age and Infertility caused by male or female factors. Ten thousand units of hCG were injected and after 36 Hours, they were set under the Follicle suction. the amount of vitamin E was measured and consequently the morphology of oocytes and the quality of embryo were evaluated by inverted optical microscope.

**Results:** 583 oocytes and 275 embryos were examined. No significant relation was observed between age, BMI, duration and cause of infertility with vitamin E ( $P>0.05$ ). At 0.5-1 mg/dl level of vitamin E, there were higher MII oocytes in comparison with others ( $P=0.014$ ). Although, the number of 2PN

embryos at 0.5-1 mg/dl level of vitamin E was the highest but this difference was not statistically significant ( $P=0.872$ ).

**Conclusion:** According to results, at specific levels of Vitamin E, oocytes morphology and embryos quality have been improved

**Keywords:** Follicular Fluid, IVF, Vitamin E, Oocytes, Embryo

### **P-60: Survival Assessment of Mouse In Vitro Embryos after Exposure to Cell Phone Radiation**

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**Background:** Using cellular phone among adults, teenagers and children has rapidly increased all over the world. Also, the concern on the possible health hazards of Electromagnetic Fields (EMF) induced from cell phones on reproduction has been growing in many countries.

The aim was to assess the morphological parameters, survival rate and development of mouse in-vitro embryos obtained by natural breeding as consequences of exposure to the cell phone radiation (talk mode) during incubation.

**Materials and Methods:** For control and experimental groups, we used a total of 40 (20 females and 20 males), 2-3 months old BALB/c mice. In the morning after mating, the ovary burses were surgically removed and the oocytes were dissected out from the ampulla region and divided into two groups. They were cultured for 5 days in vitro, which one of them was exposed to RF radiation (30 min/day). Also, PI and H33342 immunostaining was performed for identification of viable blastocysts

**Results:** There was not significant change in the rate of embryo survival to the blastocyst stage in the experimental group after exposure to (900–1800 MHz) of EMF which emitted from cell phone; compared with control cultures (60.6% vs. 69.3%,  $P=0.15$ ). Also, the loss of cell viability was found by significant increase in the percentage of necrotic and/or dead cells within an exposure blastocyst. ( $P=0.002$ )

**Conclusion:** The normal embryonic development up to the blastocyst stage indicates that EMF-exposure commonly did not have direct adverse effect on the early embryo development in mice. But, it may cause loss of cell viability and changes in membrane permeability and late apoptotic.

**Keywords:** Cell Phone Radiation, Cleavage Embryos, Viability, Hoechst 33342, PI

### **P-61: Effect of 830 nm Diode LASER Irradiation on Human Sperm Motility**

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**Background:** Sperm motility is known as an effective parameter in male fertility and it depends on energy consumption. Low level LASER irradiation could increase energy supply to the cell by producing of adenosine triphosphate (ATP). The purpose of this study is to evaluate how the low level LASER irradiation affects the human sperm motility.

**Materials and Methods:** Fresh human semen specimens of asthenospermic patients were divided into 4 equal portions and irradiated by 830nm GaAlAs LASER irradiation with varying doses as: 0 (control), 4, 6 and 10 J/cm<sup>2</sup>. At the times of 0, 30, 45 and 60 minutes following irradiation, sperm motilities are assessed by means of Computer-Aided Sperm Analysis (CASA) in all samples. Two additional tests (HOS and SCD tests) also performed on the control and high irradiated groups as well.

**Results:** Sperm motility of the control groups significantly decreased after 30, 45 and 60 min of irradiation, while those of irradiated groups remained constant or slightly increased by passing of time. Significant increases have been observed in doses of 4 and 6 J/cm<sup>2</sup> at the times of 60 and 45 min, respectively. SCD test also revealed a significant difference.

**Conclusion:** Our results showed that irradiating human sperms with low level 830nm diode LASER can improve their progressive motility depending on both LASER density and post-exposure time.

**Keywords:** Sperm Motility, Low-Level LASER, HOS Test, SCD Test

### **P-62: Expression of Androgen Receptor Gene in Human Sertoli Cells Derived from Testis Tissues with Different Spermatogenesis Status**

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**Background:** Androgen Receptor (AR) is one of the most important transcription factors in sertoli cells, that binds to DNA and influences expression of genes involved in spermatogenesis. The final distinction and separation of spermatid form seminiferous epithelium is dependent on AR. Sertoli cells are located in seminiferous tubular basement membrane and surrounds different types of germ cell. The purpose of this study was separation and purification of sertoli cells from human testis and to investigate the expression of AR gene in sertoli cells of azoospermic patients.

**Materials and Methods:** Biopsies were obtained from 10 men who referred to the Royan Institute and underwent testicular sperm extraction. Tissue samples were transferred to lectin coated petri dishes after enzymatic dissociation and isolation. After few passages, all the cells were harvested and the cell type was confirmed by immunocytochemistry. AR gene expression was determined by Real time RT-PCR.

**Results:** Isolation, purification and culture of the human sertoli cells were performed successfully. It was shown that AR gene are expressed in these cells and there is significant difference in the expression of AR in sertoli cells derived from tissues with successful sperm extraction (TESE+) compared to samples without sperm (TESE-).

**Conclusion:** The result showed that the expression of AR gene in human sertoli cells could play an important role in spermatogenesis and its expression level could be related to the outcome of sperm extraction.

**Keywords:** Sertoli Cells, Androgen Receptor, Spermatogenesis, TESE

### **P-63: Protective Activity of Tacrolimus Contralateral Epididymal Sperm Fertilizing Capacity following Unilateral Vas Deferens Obstruction: A Murine Model**

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**Background:** Auto-immunization was proposed as the mechanism of contralateral testicular damage following unilateral vas deferens injuries. The objective of this experimental in vitro study was to elucidate the effect of tacrolimus (TS) as a powerful immunosuppressant on contralateral epididymal sperm fertilizing capacity following unilateral vas deferens obstruction (UVO) in mice.

**Materials and Methods:** Adult male mice were randomly assigned to four experimental groups. Two groups of mice were undergone to UVO under anesthesia with an intraperitoneal administration of ketamine (45 mg/kg) and xylazine (10 mg/kg). Following caudal abdominal incision, UVO was induced via left vas deferens ligation by a 4/0 silk suture 2

cm from the epididymis. One of these groups received TS (10 mg/kg per day, orally) for 5 days starting from the day of induction of experimental UVO. Corresponding control groups were also included. Contralateral epididymal sperm fertilizing capacity of all animals was evaluated following in vitro fertilization at 5 weeks postoperatively.

**Results:** UVO resulted in significant decreases in fertilization rate and embryonic development along with increased rates of embryo arrest. TS administration noticeably attenuated all UVO-induced negative changes in the above-noted parameters.

**Conclusion:** These findings suggest that TS therapy may have beneficial effects in UVO-induced fertility problems. However, further studies have to be contemplated to anticipate the effects of TS in human cases.

**Keywords:** Tacrolimus, Vas deferens, In vitro fertilization, Mouse

### **P-64: Anthocyanin Effects on Sheep Oocytes In Vitro Maturation in The Presence of cAMP Modulators.**

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**Background:** We have previously reported the effect of Anthocyanin and cAMP modulators on maturation and apoptosis of sheep oocytes. In this study, we investigated the reduction capacity and apoptotic gene expression results at MII sheep oocytes.

**Materials and Methods:** Good ovine Cumulus-oocyte complexes (COCs) were transferred to Pre-IVM medium, then to two type IVM mediums: 1) 0.1 µg/ml Delphinidin Chloride supplemented SPOM medium and 2) Simple SPOM medium for 24 hours. After maturation period, MII oocytes with first polar body were selected. Immunofluorescence microscopy staining was used to evaluate intracellular GSH and ROS content of MII oocytes. In addition, the expression rate of some apoptotic genes (Bcl-2, Bax, Caspase 3, P53, C-myc) was investigated by Real-time PCR in MII oocytes. Statistical analyses were carried out by T test.

**Results:** Anthocyanin treatment during IVM increased intracellular glutathione (GSH) levels and significantly reduced reactive oxygen species (ROS) ( $19.97 \pm 1.29$  and  $26.77 \pm 2.6$  respectively,  $P < 0.05$ ). Real-time PCR results showed that the expression rates of P53 and Caspase 3 genes in group 1 signif-

icantly were lower than group 2 ( $0.43 \pm 0.08$  and  $0.64 \pm 0.02$  respectively,  $P < 0.05$ ). However Bax ( $1.23 \pm 0.02$ ) and C-myc ( $1.43 \pm 0.11$ ,  $P < 0.05$ ) genes were lower in group 1. The highest expression rate of anti-apoptotic gene (Bcl-2) was seen in the group 2 ( $0.65 \pm 0.16$ ,  $P < 0.05$ ).

**Conclusion:** Anthocyanin treatment during IVM improved maturation and viability of sheep oocytes, most likely by increasing intracellular GSH synthesis and reducing ROS level. About apoptotic genes, the five studied genes did not appear to be involved in the control of oocyte death.

**Keywords:** IVM, Anthocyanin, SPOM, sheep, Apoptosis

### **P-65: Low-Density Lipoproteins Extractions on Boar Spermatozoa Quality Following Freezing-Thawing**

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**Background:** Low-density lipoproteins (LDL) is known to protect boar sperm during freezing-thawing, but little information is known about the effects of LDL extracted from different avian egg yolks on post-thaw boar semen quality. The purpose of this study was to compare and analyze the effects of LDL at various concentrations and different species on boar sperm quality after freezing-thawing.

**Materials and Methods:** LDL extracted from the yolk of hen egg, duck egg, quail egg, pigeon egg or ostrich egg was added to the extender at the concentrations of 0.06, 0.07, 0.08, 0.09 and 0.1 g/ml, respectively, and their effects on frozen-thawed boar sperm quality were assessed.

**Results:** According to all measured parameters, the results showed that sperm motility, acrosome integrity and plasma membrane integrity were 43.20, 52.57 and 48.13%, respectively, after being frozen-thawed with 0.09 g/ml LDL extracted from pigeon egg yolk. All these quality parameters were higher than that of other groups ( $P < 0.05$ ).

**Conclusion:** In conclusion, our results confirmed that LDL extracted from pigeon egg yolk had the best cryoprotective effects on frozen-thawed boar sperm among all of the groups supplemented with LDL from five kinds of avian egg in extender. The optimum concentration of LDL extracted from pigeon egg in boar semen freezing extender was 0.09 g/ml.

**Keywords:** Avian Egg Yolk LDL, Boar Semen, Cryopreservation, Spermatozoa Quality Parameters

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## **Ethics and Reproductive Healths**

### **P-66: There Is No Difference between IVF/ICSI Cycle Outcome in Patients With and without PCOS; A Modified Poisson Regression Model**

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**Background:** Polycystic ovary syndrome is a frequent condition in women of reproductive age with prevalence rate of 5-10%. This study purposed to determine the relationship between polycystic ovary syndrome and the outcome of assisted reproductive treatment (ART) cycle in Tehran, Iran.

**Materials and Methods:** In this historical cohort study, 996 infertile women who were referred to Royan Institute (Tehran, Iran) were included in the study between January 2012 and December 2013. Polycystic ovary syndrome as a main variable and other potential confounder variables were gathered. Modified Poisson Regression was used to analyze the data. Stata software, version 13 was used for all statistical analyses.

**Results:** Unadjusted analysis showed that the risk of failure in polycystic ovary syndrome cases was significantly lower than whom did not have polycystic ovary syndrome (risk ratio: 0.79, 95% CI: 0.66-0.95, P=0.014). After adjusting for the confounder variables, there was no difference between risk of fail in women with and without polycystic ovary syndrome (risk ratio: 0.87, 95% CI: 0.72-1.05, P=0.15). The treatment protocol type, the number of embryo transferred grade A and AB, the number of injected ampoule, and age were the significant predictors of ART cycle outcome.

**Conclusion:** After adjusting for the potential confounders and by modified Poisson regression model, there was no difference between IVF/ICSI cycle outcome in patients with and without polycystic ovary syndrome.

**Keywords:** Polycystic Ovary Syndrome, IVF, ICSI, Pregnancy, Modified Poisson Regression

### **P-67: Exploring The Decisions of Iranian Infertile Couples Undergoing Assisted Reproductive Donation Procedures in Relation to Disclosure to Donor Offspring**

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**Background:** Despite considerable advances in reproductive technology, its application is like a double-edged sword, which is associated with numerous challenges. One of the most challenging issues for couples is disclosure of conception circumstances to the offspring. The purpose of this qualitative study was to explore the decisions of infertile couples

undergoing assisted reproductive donation procedures in relation to disclosure to donor offspring.

**Materials and Methods:** In this exploratory qualitative study, 32 infertile couples who were candidate to use donor egg, donor embryo or surrogacy as well as four members of infertility treatment team including gynecologists and midwives (36 in total) were purposively selected from Montaserieh Infertility Research Centre at Mashhad, Iran in 2012. Data were collected through conducting semi-structured in depth interviews and were analyzed using conventional qualitative content analysis with MAXqda software.

**Results:** Most recipient couples decided not to inform the offspring from using of donation procedures. In relation to couple's decision about secrecy towards the child three categories including: 1. protecting child from probable harms, 2. family relationships support, and 3. lack of compelling reasons to disclose were emerged.

**Conclusion:** Although child protection from possible harms is the main reason for secrecy of assisted reproductive donation procedures in infertile couples, but keeping this secret is not always easy and the possible long-term consequences for children born, can cause irreversible psychosocial damages. Raising public awareness towards donation procedures to change their perceptions and resolving infertile couples' concerns to overcome the situation is recommended.

**Keywords:** Decisions Making, Disclosure, Infertile Couples, Assisted Reproductive Donation Procedures, Qualitative Study

### **P-68: Women Attitudes toward The One-Child and Multiple Children Referring to Obstetric Clinics in Jahrom, Shiraz, Isfahan**

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**Background:** The aim of this study is to assess women's attitudes toward the one-child and multiple children who refer to obstetric clinics in Jahrom, shiraz, Isfahan.

**Materials and Methods:** The study is descriptive and was done on 1068 women who referred to the obstetric clinics of Jahrom, shiraz, Isfahan (214 women in Jahrom city, 427 in Shiraz, 427 in Esfahan). Women responded to a questionnaire containing 21 questions. Data was analyzed by SPSS 17 software.

**Results:** Among the 1068 women: 96% married; age of 58.5% (21-30); education: high school diploma (baccalaureate) 40.2%; Occupation: housewife 85.3%; 39% of childless. Families with 2 or 3 children: learn self-defense (84% agree), there is the process of competitiveness and development among children (83.5% agree), affection and kindness are more (78% agree), more companionable, strong social relationships (73.5% agree), strong transaction (78.5% agree),

their priorities are family issues (55.5% agree), less expectation and further efforts (69.5% agree). But, one-child: high expectations (86% agree), false arrogant (66.5% agree), hermit and introverted (55.5% agree), more egoist (63.5% agree), they wish to provide everything in a short time (73.5% agree), not sociable (42.5% agree), individual issues are priorities (53% agree), less self-confidence (44.5% agree), due to indolence to help his family lower in future (50.5% agree).

**Conclusion:** One child encourages individuals high expectations, arrogant, selfish, hasteful which reduces efficiency and development in the Society. It is very difficult to provide cost of living and education by more than 2 or 3 children due to their high expectations for the use of existing facilities and technology. Several problems in each of the family members, causing loss of their comfort and consequently they can't be progressed in higher levels of academic and social condition. But the exchange of ideas improve the development and growth between 2 or 3 children.

**Keywords:** One Child, Multiple Children, Jahrom, Shiraz, Isfahan

### **P-69: Perinatal Loss Is Very Tragic Event for The Parents and Staff who Are Contact with The Bereaved Parents. This Qualitative Study Aimed to Explore Experiences of Women Losing Pregnancy or Baby after Infertility Treatment is Done**

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**Background:** Perinatal loss is very tragic event for the parents and staff who are contact with the bereaved parents. This qualitative study aimed to explore experiences of women losing pregnancy or baby after infertility treatment is done.

**Materials and Methods:** In this qualitative descriptive study was conducted using content analysis approach. 22 women with history about infertility who experience perinatal loss. Women resident in Shahrecord and Tehran were selected purposefully. Data was collected by an in-depth semi structure interview and analyzed by the conventional content analysis method.

**Results:** The data analysis resulted in emerging four themes of women's experiences about perinatal loss after infertility treatment which include feeling and emotion, Hopelessness, stigma, expectation of effective and respectful communication by health care provider. All of the women shocked and confused when fetal death was disclosed. Some participants felt dissatisfied about disregard for the maternal feeling. Some participants were dissatisfied due to lack of provide information on their partners about cause of perinatal loss.

**Conclusion:** According to these study results, health care providers need specific education about the effective communication and support of bereaved women. Perinatal loss Care protocols to reduce pain of women who's suffering from perinatal grief in infertility centers is necessary.

**Keywords:** Infertility Treatment, Perinatal Loss, Maternal Experience

### **P-70: The Use of Acceptance and Commitment Therapy to Reduce Social Stress in Infertile Women**

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**Background:** Infertility Stressors Included Changes in social and family system in couples changes in the stability and quality of their interpersonal relationships and a decrease in marital satisfaction in sexual relationship that the patients with are the major problem is infertility patients compared to patients with cancer of equal levels of depression and anxiety and suffer less marital satisfaction. on the other hand use of psychotherapy based on Acceptance and commitment is effective for infertility treatment and Leads to to reduce social stress in infertile women.

**Materials and Methods:** Of 110 married women due to infertility Referred to counseling and infertility center in the city of Babol over the few years (93-91) is past 1 year to 6 years of their fertility and between 23 to 45 years of age, level of education was high school to Ph.D. A sample size of 30 individuals selected by sampling and random sampling in two groups of 15 people. A control group and a test group with treatment with medication and acceptance and commitment (ACT) were selected and pre-test and post-test questionnaires, self-concept (Williams and Snell) and fertility problems questionnaires was performed. The course of treatment is 12 sessions 1.5 hour once a week with the approach of ACT was performed.

**Results:** The use of acceptance and commitment therapy leads to reduce 38.2 percent the social stress of infertility of infertile women and 23.8 percent increase compared to the control group is sexual self.

**Conclusion:** Given that the stress of infertility include comprehensive stress, social stress, sexual stress, relationship stress, and stress of the need for child and social stress Infertility is the most common cause of stress which couples have experienced Significant changes in the family and society. Although there would be the lack of studies to determine the effectiveness of these interventions but this kind of psychotherapy interventions can take valuable new steps in the treatment of infertility.

**Keywords:** Acceptance and Commitment, Social Stress in Infertile Women, Infertility Stress



## Female Infertility

### P-71: The Relevance of Echo Patterns for The Success of *In Vitro* Fertilization Evaluated in 280 Patients.

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**Background:** The endometrial echopattern on the day of human chorionic gonadotropin (HCG) administration and its relationship to the outcome of pregnancy after assisted reproductive treatment (ART) cycles was analyzed, prospectively.

**Materials and Methods:** This study conducted between 2013 to 2014. The endometrial pattern was evaluated prospectively on the day of administration of HCG in 280 ART cycles. Inclusion criteria included women in first IVF cycle with the age of <40 years, absence of uterine pathologies and absence of any history of uterine surgery. Sagittal thickness of endometrium should be 8-14mm and at least one of the embryo should be grade A. According to its echopattern classified into two groups: Triple line and echogen(non-triple line). Pregnancy rate (PR) was compared between 2 groups.

**Results:** Among 280 patients finally evaluated, the positive pregnancy was 63(54.8%) in triple line group and 52(45.2%) in echogen(non-triple) group. Their average age was 29.53 ± 4.14 years. According to Chi-square test (P value=0.008), there is significant difference between endometrial echo pattern and pregnancy outcome.

**Conclusion:** The present result shows the significant difference between endometrial patterns in terms of pregnancy rate. Analyzing PR with different endometrial patterns in each group revealed that triple line pattern gives higher PR in comparison with other group. As a result of the present study, we propose that the endometrial pattern could have a predictive value for ART outcome.

**Keywords:** Echo Pattern, Triple Line, Echogen

### P-72: Health-Related Quality of Life and Primi-Gravid: A Comparative Study of Natural Conception and Conception by Assisted Reproduction Technologies

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**Background:** Childbearing for the first time is a unique experience. Quality of life is an important indicator in health studies. This study aimed to assess the quality of life of women who were conceived by ARTs and had successful childbirth for the first time and to compare it with quality of life in women who become pregnant naturally and similarly had successful childbirth for the first time.

**Materials and Methods:** This was a cross sectional comparative study. The accessible sample was recruited from patients attending an infertility clinic and two obstetric and gynecology clinics in Tehran, Iran, during March 2010 to March 2011. In all 276 patients were approached. Of these, 162 women (76 women in natural conception group and 86 women in assisted reproduction technologies group) who met the inclusion criteria were entered into the study. Quality of life was assessed using the 36-item Short Form Health Survey (SF-36). Women completed the questionnaire at two time points: i. last trimester and ii. first month after delivery. Comparison was made between two groups using Mann-Whitney U test and paired samples t test.

**Results:** Comparing the SF-36 scores between women in natural conception group and ARTs group before childbirth, it was found that natural group had better condition on physical functioning, role limitation due to physical problems, bodily pain and social functioning, while the ARTs group reported better status on general health, vitality, role limitation due to emotional problems, and mental health. However, after childbirth, the ARTs group reported a better condition almost on all measures, except for physical functioning. Comparing differences in obtained scores between two groups before and after childbirth, the results showed that improvements in health related quality of life measures for the ARTs group were greater in all measures, except for general health.

**Conclusion:** The findings from this study suggest that health-related quality of life was improved in women who became a mother for the first time by either method. Comparing to women who became mother by natural conception, women who received ARTs showed better quality of life from this first successful experience.

**Keywords:** Conception, Assisted Reproduction Technologies (ARTs), Primigravidity, Quality of Life

### P-73: Infertility Is More Prevalent among Cases with Peritoneal Involvement Endometriosis in Comparison to Those with Ovarian Involvement

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**Background:** We intended to determine the incidence of endometriosis at diagnostic laparoscopy for infertility problem and pelvic pain and/or cyst. In addition we aimed to evaluate symptoms, different stages and locations of endometriosis in fertile or infertile endometriosis patients.

**Materials and Methods:** This observational cross-sectional study was performed in the Department of Obstetrics and Gynecology, Vali-e-Asr Hospital, Tehran, Iran and the Surgery Unit of Royan Institute, Tehran, Iran, between January 2012 and January 2013. All cases were categorized as early (stages I and II) or late (stages III and IV) stage into fertile and infertile endometriosis groups. The extent of endometriosis was divided into peritoneal, ovarian, and ovarian+peritoneal. Endometriosis patients' symptoms were assessed and compared among those different groups.

**Results:** Four hundred and thirteen women were referred for diagnostic laparoscopy, among which 383 patients were categorized into infertility and 30 patients into pelvic pain and/or cyst groups. The incidences of endometriosis at the diagnostic laparoscopic for infertility or pelvic pain and/or cyst are comparable to each other (50.3% vs. 46.6%). The frequencies of dysmenorrhea or non-cyclic pelvic pain were similar between end-stage ( $P=0.1$ ) and late stage endometriosis patients ( $P=0.2$ ). Also the peritoneal endometriosis was more significant among infertile women than those in fertile women ( $P=0.01$ ). In addition dysmenorrhea, noncyclic pelvic pain and premenstrual spotting were more prevalent among cases with ovarian+peritoneal endometriosis lesions.

**Conclusion:** Our results demonstrated no correlation between the frequency of dysmenorrhea or non-cyclic pelvic pain and endometriosis stages, although these pain symptoms were significantly prevalent in cases with both ovarian and peritoneal endometriotic implants. Infertility was more prevalent among peritoneal endometriosis cases in compared with ovarian endometriosis. Further studies are needed to clarify the location of endometriotic implants in order to predict the chance of fertility in endometriosis cases.

**Keywords:** Endometriosis Stage, Peritoneal Endometriosis Lesions, Infertility, Ovarian Endometrioma, Dysmenorrhea

### **P-74: Effect of Suffering from Posttraumatic Stress Disorder on Sexual Life for Iraqis Terrorist Attacks Survivors**

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**Background:** After American occupation to Iraq, no day passed without terrorist attacks or explosion in Iraq. The impact of this events extends to interact with all fields of life. The present study was designed to investigate the basal levels of hormones that affect sexual life of men patients with post-traumatic stress disorder (PTSD) and control group.

**Materials and Methods:** Eighty two males witnessed on explosion occurred at 10th June 2010 in Hilla city in Iraq, as well as thirty five males apparently healthy persons as a control groups. Participants were grouped to four groups according to PTSD Checklist (PCL) scores. The PCL is a self-report questionnaire consisting of 17 Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) PTSD symptoms. Total testosterone, free testosterone, and prolactin (PRL) were determined using ELISA.

**Results:** Total testosterone, free testosterone, and PRL were trend toward decreased in all groups of PTSD patients, when compared to control group. There is negative correlation between each of total testosterone, free testosterone, and PRL with severity of PTSD. PTSD patients showed no interest in having children.

**Conclusion:** In spite of the results of present study are non-significant, and there are only trend toward negative correlation between each hormones and severity of PTSD, but most of the participants in this study shown no interest in having children.

**Keywords:** Posttraumatic Stress Disorder, Sexual Life, Testosterone, Prolactin

### **P-75: The Association of Antiphospholipid Syndrome and Recurrent Miscarriages**

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**Background:** Antiphospholipid syndrome is a coagulation disorder that causes recurrent miscarriages and fetal deaths. In the present review we summarize current knowledge about Antiphospholipid syndrome that associated with recurrent miscarriages. In addition, on the basis of these comprehensive data, possible pathophysiologic mechanisms of this disorder are discussed.

**Materials and Methods:** We searched PubMed using keywords such as "Antiphospholipid Syndrome" and "Recurrent Miscarriages" for articles with significantly high citation that published from 2005 to 2014 and totally 23 articles were used.

**Results:** Sixty percent of recurrent spontaneous abortions are unexplained. Antiphospholipid (Hughes) syndrome (APS) is a

multisystem disease with the predominant features of venous and arterial thrombosis, recurrent pregnancy loss, fetal death and the presence of antiphospholipid antibodies. Many epidemiological studies focus on antiphospholipid syndrome as a cause of recurrent spontaneous abortion. Researchers have found that having antiphospholipid syndrome can increase women's chances of recurrent miscarriages. They believe that antiphospholipid syndrome causes blood clots to block the blood supply to the placenta. Others believe that having antiphospholipid syndrome may interfere with the fertilized egg's ability to implant in the lining of the uterus.

**Conclusion:** There is accumulating evidence that recurrent miscarriages are associated with APS. Also, many studies demonstrate that thrombosis is observed frequently in the placentas of patients with antiphospholipid syndrome. However, published data showed no correlation between the severity of the disease (the number of previous miscarriages) and the prevalence of antiphospholipid antibodies and that these antibodies were also present in normal women. This review explains that women with APS are at increased risk of Recurrent pregnancy loss.

**Keywords:** Antiphospholipid Syndrome, Recurrent Miscarriages, Fetal Deaths

### **P-76: The Effect of Maternal and Paternal Body Mass Index on Live Births after Intracytoplasmic Sperm Injection (ICSI) Cycles**

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**Background:** The present study was designed to investigate the effect of couple's obesity simultaneously on ART outcomes

**Materials and Methods:** This cross-sectional study was performed in Royan institute between September 2012 and January 2014. The recorded data of all patients during this time period were evaluated. The study population was limited to ICSI or IVF/ICSI cycles with autologous oocytes and fresh embryos transfer, which height and weight in both gender recorded. Moreover multilevel logistic regression analysis was used to determine the odds of live birth following ICSI cycles. The analysis was performed according to female and male body mass index (BMI) groups whereas normal-weight patients were considered as reference group. Multilevel logistic regression was performed as couple analyses.

**Results:** In total, 990 women and their husbands went through 927 treatment ICSI and 63 IVF/ICSI cycles during study period. Among ovulatory women, a significant difference between BMI groups was found, with 60% (95% CI: 0.11-0.83) and 84% (CI: 0.02-0.99) decrease in odds of live

birth among overweight and obese, respectively. Among anovulatory women, the association between BMI and live birth presented no clear tendencies. About men, the results presented no significant relationship between BMI and live birth. The results revealed that there is no significant association between couples' BMI and live birth rate.

**Conclusion:** Increased female BMI independently and negatively influenced live birth after ICSI treatments, but increased male BMI both independently and combined had no impact on live birth after ICSI treatments.

**Keywords:** Maternal, Paternal, Body Mass Index, Live Birth Rate, ICSI Cycles

### **P-77: Follicular Fluid Concentrations of Interleukin-6, Interleukin-8, TNF- $\alpha$ and Interleukin 10 in Polycystic Ovarian Syndrome Women**

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**Background:** While the etiology of PCOS has continued to remain a mystery, there can be found some evidence in support of the presence of chronic low-grade inflammation in women with this syndrome. One of the most significant manifestations of chronic inflammation is Pro-inflammatory cytokines. Therefore, the aim of this prospective study was to investigate the pattern of proinflammatory and anti-inflammatory cytokines in the follicular fluid (FF) of PCOS women undergoing *in vitro* fertilization (IVF).

**Materials and Methods:** Eighty women who met the selection criteria were recruited, and the FF was obtained during the follicular aspiration. Hence, we classify them as follows: (A) PCOS women; (B) Control women with normal ovulatory function who underwent IVF for treatment of tubal and/or male infertility.

Obtained FF from each patient was centrifuged at 300 g for 10 min. The supernatant was used to determine the concentration of a quantification of pro inflammatory cytokines interleukin (IL)-8, IL-6, IL-10, and tumor necrosis factor-alpha (TNF- $\alpha$ ) which was measured by the sandwich enzyme immunoassay

ELISA based on a monoclonal antibody specific for them (Boster Biological Technology Ltd, USA), according to the manufacturer's instructions.

**Results:** After BMI was controlled, there was a significant increment in the concentration of inflammatory cytokines IL-8 ( $187 \pm 43.01$  pg/ml), IL-6 ( $29.39 \pm 2.75$  pg/ml) and TNF- $\alpha$  ( $6.35 \pm 1.7$  pg/ml) in the FF of PCOS women undergoing controlled ovarian hyperstimulation versus control women ( $141.92 \pm 28.87$ ,  $7.90 \pm 0.28$  and  $4.02 \pm 0.94$  pg/ml respectively,  $P < 0.05$ ).

In addition, comparing PCOS and control group, we observed significantly lower levels of IL-10 concentration in PCOS women ( $4.02 \pm 0.26$  vs.  $8.69 \pm 1.10$  pg/ml,  $P < 0.001$ ).

**Conclusion:** This result confirms the point that we can have the presence of a chronic low-grade inflammation and an involvement of the immune system in women with polycystic ovarian syndrome. Therefore, it seems that targeting this inflammatory process by means of anti-inflammatory agents might be a good solution to the current treatment.

**Keywords:** Interleukin (IL)-8, IL-6, IL-10, Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ); Polycystic Ovary Syndrome (PCOS)

### **P-78: Endometriosis and Physical Exercises among Infertile Iranian Women**

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**Background:** Endometriosis, defined by overgrowth of endometrial implants outside the uterine cavity, is commonly found on the ovaries. The role of exercise in this disease is not well understood. Regular exercise appears to have protective effects against endometriosis that involve inflammatory processes since it induces elevated systemic levels of inflammatory cytokines production with antioxidant properties and anti-inflammatory and also acts by decreasing estrogen concentrations. Evidence has suggested that the symptoms associated with endometriosis result from a local inflammatory peritoneal reaction caused by ectopic endometrial tissue. Thus, the purpose of this study is to assess the correlation between physical exercise and risk of an endometriosis diagnosis in infertile women.

**Materials and Methods:** A case-control study in infertility Institute, Iran between 2014 and 2015. There were 150 infertile women with endometriosis (cases) and 150 infertile women a normal pelvis (controls) who were evaluated by laparoscopy. A questionnaire was completed for each patient.

Women were also asked about exercise. Exercisers were defined as women who answered 1-2 times a week were referred to as irregular exercisers and  $\geq 3$  times a week was defined as regular exercisers. All statistical analyses were carried by SPSS program. Chi-square and t-tests were used to compare the two groups. Logistic regression was done to build a prediction model in endometriosis.

**Results:** Irregular physical exercise (OR: 2.07; CI: 1.13-3.81;  $P=0.01$ ) was associated with a risk of endometriosis. In the logistic regression model, age ( $P=0.02$ ), level of education ( $P=0.02$ ), BMI ( $P < 0.001$ ), and exercise ( $P=0.01$ ) were the main variables that significantly associated with endometriosis.

**Conclusion:** We find evidence of a beneficial association between regular physical activity and laparoscopically confirmed endometriosis.

**Keywords:** Case - Control Studies, Endometriosis, Infertility, Exercise

### **P-79: Diet and Risk of Endometriosis among Infertile Iranian Women**

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**Background:** Endometriosis is defined as overgrowth of endometrial tissue outside the uterine cavity. Diet may play a role in the aetiology of endometriosis through effects on oxidative stress, prostaglandin metabolism, and steroid hormone levels. The objective of this paper is to assess the role of diet on risk of endometriosis among infertile Iranian women.

**Materials and Methods:** In this case control study in Royan Institute, Iran between 2014 and 2015. There were 150 infertile women with endometriosis (cases) and 150 infertile women without endometriosis (controls) who were evaluated by laparoscopy. A questionnaire was completed for each patient. Women were also asked about their frequency of consumption per week of portions of selected dietary items in the Iranian diet in the year before interview.

**Results:** Women with endometriosis, a significant increase in risk emerged for low intake of milk (OR = 4.39), and cheese (OR = 3.06), green vegetables (OR = 14.52), fresh fruit (OR = 2.10), grain (OR = 2.65), red meat (OR = 2.61). Consumption of fish, egg, carrots, and green tea were not significantly related to endometriosis. In the logistic regression model, age ( $P=0.01$ ), level of education ( $P=0.01$ ), BMI ( $P=0.03$ ), green vegetable ( $P=0.03$ ), grain ( $P=0.001$ ), and cheese ( $P < 0.001$ ) were the main variables that significantly associated with en-

dometriosis. The AUCs for these models were 0.79 (95% CI 0.74-0.84), showing a good predictive performance for the fitted logistic regression model.

**Conclusion:** The present study suggests that specific types of dietary components are associated with endometriosis risk.

**Keywords:** Endometriosis, Case–Control Studies, Infertility, Risk Factors, Diet

### **P-80: The Effects of Rosa Damascene Aqueous Extract on Reproductive System of Female Mice Following Formaldehyde Treatment**

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**Background:** Formaldehyde is a chemical precursor, flammable and colorless which is widely used in different industries. Previous studies, have reported that Formaldehyde has destructive effects on reproductive system of female mice. In the present study we aimed at evaluating the protective effects of Rosa damascene on the toxic effects of Formaldehyde on sex hormones, ovary parameters and folliculogenesis.

**Materials and Methods:** Forty-two healthy NMRI female mice were randomly divided into six groups (n = 7). Group I was control which received distilled water, group II received oral administration 40 mg/kg extract, group III received 5 mg/kg diluted formaldehyde through IP route and groups IV, V and VI received oral administration of 10, 20 and 40 mg/kg extract as treatment groups also 5 mg/kg formaldehyde. The trial was continued for 40 days.

**Results:** The result showed that formaldehyde could lead to significant adverse effects on weight, volume, diameter of ovaries and number of different follicles and corpus luteum of ovaries compared to group I (P<0.001) as well as decreased serum level of estrogen and progesterone compared to group I (P<0.001). In groups IV, V and VI it were determined that the produced damages, caused by formaldehyde, were improved after extract administration compared to group III (P<0.01).

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

**Keywords:** Formaldehyde, Rose Damascene, Mice, Ovary, Sex Hormones

### **P-81: G-CSF Intrauterine and Thin Endometrium, and Pregnancy Outcome, Non Randomized Clinical Trial**

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**Background:** Is G-CSF has any effects on cancelled ART cycle due to thin endometrium. This study showed the beneficial effects of G-CSF on thin endometrium. In two previous studies, some beneficial effects were reported and in another no effects were seen.

**Materials and Methods:** In a nonrandomized clinical trial, fifteen cases were evaluated from June 2011 till September 2012. All cases had history of cycle cancellation due to thin endometrium which are referred to our center. They had used estradiol, and sildenafil citrate unsuccessfully in their previous cycle. Intrauterine infusion of G-CSF was done on the day of oocyte pick-up or 5 days before embryo transfer in recipient cycles. Primary outcome was endometrial thickness at least till 6.5 mm and secondary outcome as clinical pregnancy rate and then take home baby. All previous cycle was considered as control for each patient, the mean of age, gravidity, parity, FSH, were 35.13 ± 9.531, 3(20%), 1(6.6%), 32.78 ± 31.10 mIU/ml, respectively. (46.6%) had diminished ovarian reserve according to the FSH levels and averagely they had 1.2 ± 0.532 cycle cancellation due to thin endometrium.

**Results:** The G-CSF infused at the day of oocyte puncture or 5 days before embryo transfer, the endometrial thickness reached from 3.593 mm ± to 7.120 mm ± 0.84, the difference was 3.53 mm ± 0.88, and clinical pregnancy rate was 20%, one missed abortion, a mother death at 34 weeks, and a pre-term labor at 30 weeks due to PROM.

**Conclusion:** Remarkable results for a small group of patients who had no choice except cycle cancellation or surrogacy, but respond to G-CSF and acceptable outcome.

**Keywords:** ART Cycle, Thin Endometrium, G-CSF

### **P-82: Antagonist/Letrozole Protocol in Poor Ovarian Responder Patients Undergoing Intracytoplasmic Sperm Injection-Embryo transfer Cycles**

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**Background:** The optimal stimulation protocol for poor responder patients is a therapeutic challenge. GnRH antagonist protocol has been proposed as a potentially proper option for poor responders. Nevertheless, there is no significant difference in terms of clinical pregnancy and cancellation rates be-

tween the GnRH antagonist and agonist cycles. Therefore, this subset of patients might be the best to benefit from new treatment strategies that make better outcomes. The use of aromatase inhibitors such as letrozole were advocated by some studies. The aim of this study was the evaluation of aromatase inhibition by letrozole on outcome of antagonist protocol in poor responder patients undergoing ICSI cycles.

**Materials and Methods:** It is a Double-blinded randomized control trial in the University-affiliated IVF unit. Based on Bologna criteria, 83 infertile women were labeled as poor ovarian responder. They were enrolled in two groups; letrozole/GnRH-antagonist (LA) group (n=35) involved at letrozole 2.5 mg over 5 days and recombinant human follicle stimulating hormone (rhFSH) with starting daily dose of 225IU and placebo/GnRH-antagonist (PA) group (N=35) received placebo over 5 days and (rhFSH) at the same dose and starting day as group (PA). In both groups, the central blockage was achieved by the use of the GnRH antagonist (cetrotide) in a flexible fashion. The ICSI outcomes were compared between the groups.

**Results:** There were no significant differences in demographic characteristics between the groups. There were no significant differences between the groups regarding the number of oocytes retrieved ( $2.8 \pm$  vs.  $2.6 \pm P=0.81$ ), number of embryos transferred ( $1.02 \pm 0.75$  vs.  $1.23 \pm 0.84, P=0.82$ ), implantation rate (11.9% vs. 9.5%,  $P=0.72$ ), total cycle cancellation rate (20% vs. 22.9%,  $P=0.08$ ), clinical pregnancy rate (17.85% vs. 14.81%  $P=0.76$ ).

**Conclusion:** There is no benefit for adding of letrozole in GnRH-antagonist protocol for poor responder patients undergoing ICSI.

**Keywords:** Letrozole, GnRH-Antagonist, Intracytoplasmic Sperm Injection, Poor Ovarian Responder

### **P-83: Development of Mouse Preantral Follicles in Fibrin-Alginate Matrix during In Vitro Culture**

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**Background:** This research was conducted to assess preantral follicle development in fibrin alginate matrix following ovarian tissue vitrification

**Materials and Methods:** Ovaries of 13-day-old NMRI female mice were removed and placed in control and vitrification groups. Vitrification group ovaries were transferred in media containing ethylene glycol, dimethyl sulphoxide, and sucrose then were plunged in LN2 by acupuncture needle. Medium sized preantral follicles of fresh control and vitrified-warmed ovaries were mechanically isolated and cultured in fibrin-alginate matrix for 12 days. Finally, survival and growth rate and also quantitative oocyte maturation genes (Gdf9 and Bmp15) expression of follicles were evaluated on 1st and 12th culture days

**Results:** Although higher follicle survival rate was shown in

control group rather than vitrification one, follicle diameter was being increased until the last culture day in both groups. Quantitative oocyte maturation genes expression evaluation did not reveal any significant difference between control and vitrification groups on first and last days of culture, but they were significantly decreased on 12<sup>th</sup> culture day compared to the first in both groups

**Conclusion:** According to the observed similarities in both groups, it seems that fibrin-alginate could be an appropriate matrix for mouse preantral follicle development and in spite of decreased follicle survival rate in vitrification group, applied vitrification method did not affect developmental competence of ovarian follicles

**Keywords:** Vitrification, Preantral Follicle, Fibrin-Alginate Matrix, Oocyte Maturation Genes

### **P-84: Evidence for Differential Expression of The Pluripotency Factors c-MYC, KLF4 and LIN28 in Normal Endometrium and in Endometriosis**

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**Background:** Endometriosis is a common gynecological disease characterized by the presence of endometrial tissue outside the uterine cavity. This disease affects approximately 10% of women in reproductive age and is associated with pelvic pain, dysmenorrhea and infertility. The theory of involvement of stem cells is a considered new hypothesis in etiology of endometriosis. The aim of this study was to quantify the expression of stemness genes *c-MYC*, *KLF4* and *LIN28* in the endometrium of women with and without endometriosis.

**Materials and Methods:** For this respect, 10 ectopic and 10 eutopic endometrial samples and 23 normal endometrium as control group were tested in this study. The expression of *c-MYC*, *KLF4* and *LIN28* genes were analyzed with quantitative real-time PCR technique.

**Results:** Differential expression of *c-MYC*, *KLF4* and *LIN28* genes were observed in normal, ectopic and eutopic endometrium; in the way that *c-MYC* and *KLF4* showed significantly higher expressions in ectopic tissues, compared with normal and eutopic endometrium, but the *LIN28* mRNA expression was not significant between normal, ectopic and eutopic endometrium. Also, there were differences in expression of these genes in normal endometrium during the menstrual cycle.

**Conclusion:** Higher expression of *c-MYC* and *KLF4* mRNA in ectopic endometrium can be considered as a molecular biomarker for endometriosis development and pathophysiology.

**Keywords:** Endometriosis, Pluripotency, *C-MYC*, *KLF4*, *LIN28*

**P-85: Effect of Metformin on Anti-Mullerian Hormone (AMH) Level in Women with Infertility and Polycystic Ovary Syndrome**

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**Background:** Women with PCOS have increased AMH levels due to accumulated preantral and small antral in follicles. Ovulation improvement following decrease in levels of AMH could occur in these patients. The purpose of this study was to evaluate the effect of metformin on anti-Mullerian hormone levels in women with polycystic ovary syndrome and infertility.

**Materials and Methods:** This randomized clinical trial study was done on 30 infertile patients with polycystic ovary syndrome (using the Rotterdam criteria) in 1393, in infertility center of Shahid Beheshti hospital in Kashan. Demographic data included age, BMI, history of abortion and contraception, and hirsutism were recorded. Metformin 1, 500 mg for 8 weeks were prescribed, then AMH level was checked before and after treatment. Results were analyzed by using X<sup>2</sup>, t test, Mann-Whitney and logistic regression.

**Results:** Mean age of patients was 25.2 ± 4.2 years and BMI was 26.2 ± 3.8. Patients had 60% hirsutism, 93% oligomenorrhea and 80% infertility. AMH levels before treatment with metformin was equal to 10 ± 3.75 and after treatment was 7.8 ± 3.7 which decreased significantly (P=0.008).

**Conclusion:** The results of this study showed that metformin for 2 months could decrease AMH significantly.

**Keywords:** Metformin, Anti-Mullerian hormone, Polycystic Ovary Syndrome, Infertility

**P-86: Evaluation of Telomere Length, Telomerase and Telomeric Repeat Containing RNA (TERRA) Expression Levels in Cumulus Cells of PCOS Patients**

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**Background:** Polycystic ovary syndrome (PCOS) is one of the reasons of infertility in women with chronic anovulation. Ovulation process is tightly regulated by molecular mechanisms controlling proliferation/differentiation of cells. Telomeres, TTAGGG tandem repeats, are transcribed into a non coding RNA, named TERRA. Recent studies suggest that TERRA sustain several important functions at chromosome end such as regulation of telomere length through modulation of telomerase. Telomerase is an enzyme that binds to the telomere and maintains telomere length and integrity. In the reproductive system, telomerase is present in germ cells, proliferative granulosa cells and germline stem cells but it is absent in differentiated cells. Each oocyte is covered by cumulus cells (CCs) which are in bi-directional communication. The role of these cells in the maturation, ovulation and fertilization of oocytes has been studied extensively. It seems that the length of telomere is related to proliferation and differentiation events during follicular development including the mechanisms which regulate successful reproduction.

**Materials and Methods:** Cumulus cells and blood samples were collected from 20 PCOS patients and 20 healthy women with male factor infertility as control group. Informed consents were obtained from the participants. Expression of telomerase, TERRA genes were evaluated in CCs and telomere length in blood samples were quantified by using q-PCR.

**Results:** Obtained data from CCs showed increase in mRNA levels of telomerase and also significant increase in TERRA transcription level in PCOS patients vs. control group. However, there were no significant alterations in telomere length of blood cells among studied groups.

**Conclusion:** According to increased telomerase expression and different roles of TERRA, we speculate that TERRA mRNA increasing may have a role in inhibiting telomerase activity of cumulus cells which at least affect on their telomere length. Overall, these findings suggest a correlation of telomerase and TERRA levels with infertility anovulation in PCOS patients.

**Keywords:** Cumulus Cells, TERRA, Telomere, Telomerase, PCOS

**P-87: Dietary Patterns in Relation to Ovarian Infertility: A Case-Control Study**

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**Background:** Previous studies have focused on a single or few dietary nutrients, and scarce data is available on dietary

patterns related to infertility. Therefore, we aimed to investigate the relation between female ovulatory infertility and major dietary patterns among women attending fertility clinics.

**Materials and Methods:** This case-control study was conducted on 167 infertile women with PCOS and 251 controls. PCOS was determined by using 2003 Rotterdam criteria. Usual dietary intake was assessed using a validated 168-item semi-quantitative food frequency questionnaire. Major dietary patterns were identified using factor analysis.

**Results:** Two main dietary patterns, healthy dietary pattern and western dietary pattern, were identified. Healthy dietary pattern was high in green leafy vegetables, fruits, low fat dairy products, nuts, fish, poultry, vegetable oils and whole grains and the Western dietary pattern was high in red meat, processed meats, refined grain, French fries, high fat dairy products, snacks, starchy sweets, soft drinks and hydrogenated fats. Cases were statistically more overweight and abdominally fat than controls ( $P=0.00$ ). No statistical significant difference was seen in total energy intake, nutrient intakes and dietary fiber between the two groups. Lower adherence to western dietary pattern was associated with decreased chance of infertility (OR=0.61; 95% CI: 0.41-0.91,  $P=0.01$ ). The association remained significant even after taking other confounders into account (OR=0.62, 95%CI: 0.41-0.96,  $P=0.03$ ). However, after adjusting for energy and macronutrient intakes, the association altered to marginally significant relation ( $P=0.07$ ). Associations between having healthy dietary pattern and infertility regarding PCOS was not statistically significant ( $P=0.45$ ).

**Conclusion:** Lower adherence to western dietary pattern may protect women in reproductive age against infertility. Further studies are needed to confirm the role of different dietary patterns on fertility outcomes.

**Keywords:** Dietary Pattern, Nutrition, Infertility, Polycystic Ovarian Syndrome

### **P-88: The Effect of Micronutrient Supplements on Female Fertility**

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**Background:** Several recent reviews, based mainly on observational studies, have however identified that micronutrient concentrations in the peri-conception period influence female fertility and embryogenesis, and may prevent adverse pregnancy outcomes. The possible effects on subfertility of two micronutrients (Zinc and Selenium), Components of antioxidant enzymes which are commonly included in oral supplements, are discussed here.

**Materials and Methods:** This is review article.

**Results:** Serum Zn concentrations are almost twice as high as follicular concentrations, although the high expression of Zn transport genes in the oocyte suggests active Zn transport during the first stages of pre-implantation development. Simi-

lar to studies on males, studies report conflicting findings as to whether differences exist in serum Zn concentrations between infertile and fertile women. Lower follicular fluid and serum Zn and selenium levels were found in IVF patients than in fertile women, with normalization to those of fertile women following multivitamin supplementation, although the effect on pregnancy rate was not investigated.

**Conclusion:** Apart from lowering the malformation risk by periconceptual supplementation of folic acid, substitution with different micronutrients, particularly folic acid, vitamin B6, vitamin C, vitamin D, vitamin E, iodine, selenium, iron, and DHA might have a positive impact on infertility treatment. The multivitamin formulation should take the pathophysiology, clinical studies, and upper limits into account.

**Keywords:** Zinc, Selenium, Fertility

### **P-89: Evaluation of Anxiety and Depression in Women with Infertility**

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**Background:** The aim of this study was to evaluate the anxiety and depression in women with infertility.

**Materials and Methods:** A case control study was carried out on 150 subjects with infertility and on 150 subjects with tubal ligation. The two groups were matched in demographic and personal characteristics. Data collection tool was a questionnaire including questions regarding demographic and obstetrical characteristics. Anxiety and depressive symptoms were evaluated by the Hospital Anxiety and Depression Scale (HADS). Each subscale's scores can range from 0 to 21. Sum scores < 8 indicate normal range; scores from 8 to 10 reflect mild alterations, and scores  $\geq 11$  represent clinical relevance of symptoms.

**Results:** The overall prevalence of anxiety and depression in the infertile couples and non-infertile couples was 88% and 70.7% respectively ( $P<0.0001$ ). The mean scores of anxiety and depression were found to be higher in infertile couples as compared to the control group, and the differences between the two groups were statistically significant in anxiety scale ( $11.41 \pm 3.54$  vs.  $8.61 \pm 3.06$ ;  $P<0.0001$ ), depression scale ( $7.36 \pm 3.32$  vs.  $5.91 \pm 2.64$ ;  $P<0.0001$ ), and total HADS scores ( $18.78 \pm 6.00$  vs.  $14.16 \pm 4.99$ ;  $P<0.0001$ ).

**Conclusion:** Infertility may be a risk factor for anxiety and depression. We found significant differences in anxiety and depression between women with and without infertility.

**Keywords:** Case-control Studies, Tubal Ligation, Infertility, Anxiety, Depression



### **P-90: Comparison of The Effects of Two Types Fixative in Preparation of Mouse Ovarian Tissue**

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**Background:** Sample preparation is the first step in the evaluation of tissue samples. In this research tried to using two types of formalin and Bouin fixative ovarian tissue morphogenesis be evaluated practical.

**Materials and Methods:** Ovaries of 6-8 week-old Naval Medical Research Institute female mice were randomly divided into 3 groups: I. Ovaries that immediately fixed in Bouin's fixative; II. Ovaries that immediately fixed in Formalin's fixative; and III. Ovaries that in the first for 24 hours at Bouin's fixative and were subsequently fixed in Formalin for 24 hours. The samples were serially sectioned and stained either with H&E or immunohistochemistry kit of pro-caspase-3.

**Results:** Morphologically healthy primary, preantral and antral follicles showed significant reductions in II group compared to I and III groups. The difference in the number of primordial, primary, preantral and antral healthy and dead follicles between the I and III groups was not significant. In comparison with healthy follicles, there were significantly more dead follicles in the II group than the other groups. The apoptotic follicles increased significantly in III group compared to I group. No results were obtained from formalin-fixed specimens immunohistochemistry study.

**Conclusion:** The results showed that formalin solution is not appropriate fixative for immunohistochemistry studies. According to the results of this research, it can be stated that, for fixing ovarian tissue Bouin's fixative is a more appropriate solution.

**Keywords:** Ovary, Bouin's Fixative, Formalin's Fixative

### **P-91: Vitamin E and Selenium Supplementation Affects Aldehyde Oxidase, Xanthine Dehydrogenase/Oxidase Activities In Diabetic Rat Ovaries**

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**Background:** Any factor like diabetes that changes the oxidant/antioxidant balance in favor of oxidants could possibly disrupt the physiologic function of ovaries. So, we have investigated the effect of vitamin E and Se supplements on antioxidant defense in ovaries of diabetic rats with focus on Xanthine dehydrogenase/oxidase (XDH/XO) and Aldehyde oxidase (AO) activities.

**Materials and Methods:** This study was conducted on three groups: control C; STZ-induced diabetic, DM; and diabetic rats with orally administered vitamin E and selenium, DM+S. After 4 weeks treatment, plasma and ovarian levels of Malon-dialdehyde (MDA), total antioxidant status (TAS) and also, ovarian activities of Superoxide dismutase (SOD), Glutathione peroxidase (GPX), AO and XO/XDH were evaluated.

**Results:** Ovarian GPX activity and TAS level in DM group were lower than DM+S and control (P<0.001). Although the supplementation significantly decreased MDA level and activities of XO, XDH and AO in ovaries (P<0.05) but they were significantly higher than those in C group (P<0.05). In group C, there were positive correlations between XO activity with ovarian TAS levels and also between AO with GPX activities (r=0.83, p=0.01; r=0.77, P=0.02 respectively). Furthermore, a significant positive correlation was seen between XO and GPX activities in DM+S group (r=0.85, P=0.007). There was a statistically positive correlation between plasma and ovary levels of MDA in control group (r=0.787, P=0.02).

**Conclusion:** Our study showed that in ovarian tissue of diabetic rat the activity of GPX and TAS levels were decreased and the level of MDA and AO, XDH and XO activities were increased. It also became clear that vitamin E and Se supplementation in diabetic rats could increase the activity of antioxidant enzymes and decrease activity of oxidant enzymes to improve the situation.

**Keywords:** Xanthine Dehydrogenase/Oxidase, Aldehyde Oxidase, Antioxidant, Ovary, Diabetes.

### **P-92: Compare The Efficacy of Two Protocols of Ovarian Stimulation Using Recombinant FSH Plus Recombinant LH versus Recombinant LH Alone in Hypogonadotropic Hypogonadal Women Undergoing In Vitro Fertilization/ Intracytoplasmic Sperm Injection (IVF/ICSI)**

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**Background:** To compare the efficacy of recombinant follicle-stimulating hormone (rFSH) plus recombinant luteinizing hormone (rLH) versus LH alone during the late follicular phase for ovarian stimulation in hypogonadotropic hypogonadal (HH) women undergoing *in vitro* fertilization/ intracytoplasmic sperm injection (IVF/ICSI).

**Materials and Methods:** In this prospective, single-blind, randomized clinical trial, 51 HH infertile women undergoing IVF/ICSI were enrolled and received 150 IU/d rFSH (with the possibility of dose adjustment) and 75 IU/d rLH for ovarian stimulation. When at least one follicle reached 14 mm in diameter, treatment with rLH alone or rFSH plus rLH were randomly assigned. The main outcome measures were the number of mature follicles, the endometrial thickness on the day of human chorionic gonadotropin (hCG) administration, the number of oocytes retrieved, implantation rate and clinical pregnancy rate.

**Results:** The number of mature follicles, the endometrial thickness on the day of hCG administration and the number of oocytes retrieved were similar in the two groups. The implantation and clinical pregnancy rates were higher in group rLH as compared with group rFSH/ rLH, but the differences were not significant (implantation rate: 34.7% vs. 20.0% with  $P=0.112$ ; clinical pregnancy rate: 50.0% vs. 38.9% with  $P=0.492$ ).

**Conclusion:** The ovarian stimulation by rLH alone versus rFSH/rLH during the late follicular phase showed better effect on final outcomes of IVF/ICSI in HH women. Therefore, the use of rLH alone during the late follicular phase can be effective in treatment of HH women undergoing IVF/ICSI cycles.

**Keywords:** Hypogonadotropic Hypogonadal Women, Recombinant FSH, Recombinant LH, Ovarian Stimulation, *In Vitro* Fertilization / Intracyto- Plasmic Sperm Injection Outcome

### **P-93: The Accuracy of Hysteroscopy in Diagnosis of Polyps and Myomas in Patients with Abnormal Vaginal Bleeding**

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**Background:** Abnormal vaginal bleeding (AUB) is among the most prevalent gynecologic complaints, which is responsible for nearly 33% of all gynecology clinic referrals. Intrauterine anatomic lesions are one of the etiologies leading to AUB and hysteroscopy is the gold standard method for evaluating these problems. The accuracy of hysteroscopy vary in different intra-cavitary problems. Gold standard method for diagnosis of myoma or polyp is pathologic examination. The aim of this study was to compare the accuracy of hysteroscopy in diagnosis of myomas and polyps compared to pathologic examination.

**Materials and Methods:** Medical records of patients who

were undertaken hysteroscopy for AUB were reviewed for the period of 5 years, concerning their sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in diagnosis of intra-cavitary myoma and polyps.

**Results:** One hundred and seven women with the mean age of  $40.63 \pm 10.15$  years (ranging from 18 to 70), were assessed. The sensitivity, specificity, PPV, and NPV of hysteroscopic diagnosis for polyps were 94.2, 85.4, 85.9, and 94%, respectively. The sensitivity, specificity, PPV, and NPV of hysteroscopic diagnosis for myomas were 97.7, 88.7, 86.2, and 98.2%, respectively.

**Conclusion:** Hysteroscopy is a safe and accurate diagnostic modality. Its sensitivity, specificity, PPV, and NPV of hysteroscopy is higher in diagnosis of uterine myomas compared to polyps.

**Keywords:** Myoma, Polyp, Abnormal Vaginal Bleeding

### **P-94: Acupuncture as A New Infertility Treatment**

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**Background:** Infertility has long been a common problem in our human history and still continues to be a problem in the modern era. Fortunately Western Medicine has developed various treatment methodologies, especially the new assisted reproductive technology, which does increase the success rate. Despite this, nearly half of the couples among these who get treated by WM still fail conceive. The main objective of the present study was to investigate the acupuncture strategies for female infertility in world.

**Materials and Methods:** 166 cases of infertility, the ages of patient 27-47 with an average being 37. Traditional Chinese Medicine periodic therapy, which included both acupuncture and Chinese herbal medicine from 1 day to 28. the average time of infertility was three and half years. All the patients had been evaluated and diagnosed by their WM doctor and most of them had received regular treatment. The main causes of infertility was :62 with ovulatory factor (37%); 31 with tubal fac-

tor (18%); 36 with endometriosis (21%); 29 with unexplained factor (17%); 8 with uterine immune factor (5%).

**Results:** Among the 166 cases, 68 patient became pregnant after application of the above therapies. The success rate was 41%.

**Conclusion:** Because of various limitation, researcher cannot use the standard method for these clinical observation but acupuncture with TCM show cure infertility at a significant rate, and that the treatment provides new hope for those couple

**Keywords:** Infertility, Acupuncture, Herbal Medicine, Western Medicine

### **P-95: Seasonal Changes in Hypothalamic Gonadotropin-Inhibitory Hormone Expression in Alectoris Chukar**

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**Background:** Gonadotropin-inhibitory hormone (GnIH), initially discovered in birds as a hypothalamic neuropeptide, inhibits the synthesis and release of gonadotropins via affecting GnRH neurons and gonadotropes. Therefore, it could be a key neuropeptide in regulating seasonal breeding in birds. The aim of the present study was to investigate the expression of GnIH in the hypothalamus of male and female chukar partridges during the breeding and non-breeding seasons.

**Materials and Methods:** In breeding (May) and non-breeding (January) seasons, the brains of male (n=10) and female (n=10) sexually mature chukar partridges were removed following fixation. Sections (30 µm) were prepared from the entire hypothalamus and stained immunohistochemically (GnIH antibody was provided by Prof. K. Tsutsui).

**Results:** GnIH-immunoreactive (-IR) neurons were primarily found in PVN nucleus and few positive neurons detected in DMN nucleus. The number of GnIH-ir neurons were significantly lower in the breeding season than non-breeding season in both male and female partridges (P<0.05).

**Conclusion:** The results showed that GnIH neurons may play part in regulating the seasonal breeding in the chukar partridge.

**Keywords:** GnIH, Partridge, Hypothalamus

### **P-96: Extensive Fundal Uterine Rupture in Post-resection Bicornuate Uterus in a Term Pregnancy: A Case Report**

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**Background:** Uterine rupture in a term pregnancy is an adverse and rare event with serious maternal and fetal consequences. The history of uterine septum resection is considered as a risk factor for uterine rupture. Women with such circumstances are thus recommended to be considered as having a high-risk pregnancy. Accordingly, their prenatal care should be implemented in shorter intervals during which more attention should be paid to auscultation of fetal heart rate and warning signs (e.g. hemorrhage and severe and sudden pain). We report a case of asymptomatic fundal uterine rupture in a woman with term pregnancy.

**Materials and Methods:** Uterine rupture can be caused by various reasons. However, according to previous studies, the most important reason is the history of a previous cesarean scar in a pregnant uterus. In the case we presented at Akbarabadi Hospital, the history of uterine septum resection was the cause of uterus rupture. Incidence of uterus rupture is possible during the whole stage of pregnancy, especially during the labor and due to induction with oxytocin. In our case, asymptomatic uterine rupture was observed in a female with term pregnancy who was not induced with oxytocin. Such a case has never been reported. It should be noted that despite a previous cesarean scar in our case, the uterine fundus was ruptured due to a scar caused by uterine septum resection. Possible causes of such ruptures in previous reported cases included deep resection of myometrium and weakening of the fundus and/or an undiagnosed rupture.

**Results:** Maternal health care providers should pay more attention to the outcomes of the septum resection itself. They are required to prevent uterine rupture with more accurate control of patients and faster diagnosis of a uterine susceptible to rupture.

**Conclusion:** Maternal health care providers should pay more attention to the outcomes of the septum resection itself. They are required to prevent uterine rupture with more accurate control of patients and faster diagnosis of a uterine susceptible to rupture.

**Keywords:** Post-Resection Bicornuate Uterus, Term Pregnancy, Uterine Rupture

### **P-97: The Effects of Catalase Addition to The Cryopreservation Medium on Follicles Apoptosis and Oxidative Stress in Human**

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**Background:** Today, cryopreservation of ovarian tissue has been the effective procedure to restore fertility in can-

cer patients. It is well known that oxidative stress (OS) is a widespread phenomenon that occurs in ovary cryopreservation. Numerous studies show that follicular atresia in mammalian species due to the accumulation of toxic metabolites often results from oxidative stress. Therefore, currently there is a great interest to the use of antioxidants to prevent ROS generation and ROS-induced apoptosis during the ovary cryopreservation processes. It has been revealed that including catalase as an antioxidant in sperm freezing medium reduced lipid peroxidation. In this regards, the aim of this study was to investigate the effects of catalase addition to the cryopreservation medium on cell morphology, viability, apoptosis, ROS generation and lipid peroxidation in human ovary.

**Materials and Methods:** Biopsies of ovarian cortex from cancer patient (n=14) were divided into 4 groups: without catalase, with catalase in freezing medium, with catalase in thawing medium and with catalase in both medium. After 2 weeks the morphology, viability and incidence of apoptosis were evaluated using Hematoxylin and eosin (H & E), Calcein-AM and Ethidium homodimer-1 staining and TUNEL. H<sub>2</sub>O<sub>2</sub> generation and LPO were assessed by means of DCFH-DA fluorescence and MDA assay kit.

**Results:** Compared with the control group, cryopreservation with catalase resulted in significant decrease in the percentage of DCFH-DA fluorescence, apoptosis, and increase in viability of the follicles. There was no difference between groups in follicular morphology.

**Conclusion:** Catalase addition to cryopreservation medium can be a potential tool against oxidative damage and apoptosis in human ovarian tissues.

**Keywords:** Catalase, Oxidative Stress, Ovarian Cryopreservation, Apoptosis

### **P-98: The Effect of Primary pH Values of Medium Culture on Expression of Human Follicle Stimulating Hormone in Recombinant Chinese Hamster Ovary Cells.**

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**Background:** One of the common gonadotropin hormones used for infertility treatment is recombinant Follicle stimulating hormone (rFSH). pH is an environmental factor that can affect production of recombinant proteins with no imposing cost. Culture pH is known as a parameter that significantly affects cell growth and protein production. In this study, tried to investigate the effect of different pH values on cells growth and rFSH production.

**Materials and Methods:** The primary pH changes were

carried out by adding NaOH and HCL 1 N to medium culture. The range of considered pH values was (6.7-7.6). Trypan blue staining method was applied for cells counting and measuring viability of the cells. Total protein concentration was determined by Bradford assay and Western blotting used for detection of rFSH. qRT-PCR and ImageJ software were used for quantification assays.

**Results:** According to obtained data, at the first stationary phase of cells growth, by evaluating the pH values to 7/6, the cells viability, mRNA ratio of rFSH and rFSH production significantly was decreased. The highest cells viability and mRNA ratio of FSH and FSH production were seen in medium with pH=7.0. The achieved results from relative gene expression at pH=6.7 and pH=7.0 didn't show a significant difference.

**Conclusion:** Considering obtained data, by lowering pH value to 7.0 for cells, without any cost, the highest proliferation, will be achieved.

**Keywords:** Follicle Stimulating Hormone, pH Optimization, Infertility

### **P-99: Relationships between Serum Luteinizing Hormone Level, Endometrial Thickness and Body Mass Index in Polycystic Ovary Syndrome Patients with and without Endometrial Hyperplasia.**

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**Background:** It is well documented that ultrasonographic endometrial hyperplasia in polycystic ovary syndrome (PCOS) women is strongly related to pathologic endometrial thickness, but there is no consensus on the relation between serum luteinizing hormone (LH) and either of these factors: pathologic endometrial hyperplasia and body mass index (BMI).

**Materials and Methods:** An observational cross-sectional study was designed. Three hundred fifty infertile PCOS women were involved. An endometrial biopsy was taken by using a pipelle instrument, regardless of menstrual cycle's day and all samples were reported by the same pathologist. Basal serum LH was compared in two subgroups (hyperplasia and non-hyperplasia). The studied population was divided into three groups according to BMI and basal serum LH and the comparison was made in three groups. Chi-square test, Mann-Whitney U and one way analysis of variance (ANOVA) tests were used to compare variables among groups.

**Results:** The frequency of endometrial hyperplasia was 2.6%. Endometrial thickness in the patients with endometrial hyperplasia was significantly higher than that of those with

a normal endometrium ( $10.78 \pm 3.70$  vs.  $7.90 \pm 2.86$  respectively,  $P=0.020$ ). Also, there was no relation between endometrial hyperplasia and serum LH ( $P=0.600$ ). The ANOVA test showed serum LH levels were not equal among three BMI groups ( $P=0.007$ ). Post hoc test revealed that the LH level in normal BMI group was higher than other groups significantly ( $P=0.005$  and  $P=0.004$ ), but there was no statistical difference between overweight and obese groups ( $P=0.8$ ). We found no relationship between BMI and endometrial thickness in PCOS patients ( $P=0.6$ ).

**Conclusion:** Sonographic endometrial stripe thickness is predictive for endometrial hyperplasia in PCOS women. We could not find any relationship between serum LH level and BMI with endometrial thickness in PCOS patients. However, our study confirmed a diverse relationship between serum LH level and BMI in PCOS patients.

**Keywords:** Polycystic Ovary Syndrome, Endometrial Hyperplasia, Luteinizing Hormone, Body Mass Index

### **P-100: Family Intervention Effect in The Private Lives of Infertile Couples**

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**Background:** The purpose of this study, the appraisalment of family intervention effect in the private lives of infertile couples.

**Materials and Methods:** 150 women were selected from Dr. Rasekh infertility clinic by census method in the fall of 2014. It was used from questionnaires to collect data. Data was analyzed with SPSS statistical software.

**Results:** The average age of women were  $28.17 \pm 5$  years and duration of infertility was 57.72 months. The most important reason for refer to infertility clinic; 71.5% women's desire to have a child, 14.6% their partners desire, 8.5% with the agreement of each other, 5.4% due to the insistence of family and relatives. Intervention of the husband's family in Married life and create resentment and emotional problem; 10.6% too much, 10.6% much, 12.8% moderate, 26.2% low, 39.7% very low. Up questions were also asked about intervention of women's family; 2.9% too much, 6.2% much, 12.2% moderate, 28.8% low, 49.6% very low. Intervention by neighbors and acquaintances; 6.4% too much, 8.5% much, 11.3% moderate, 29.1% low, 43.3% very low. They are Fortunate couples without children, If someone did not intervene in the lives of infertile couples; 69.3% agreement completely, 22.9% agreement, 5% Apathetic, 1.4% opposite, 1.4% opposite completely.

**Conclusion:** The patients should be consulted by psychologist frequently that will be a fundamental role in reducing stress and even accelerate the outcometreatment. Finally, the

couple must be said "Life is just not parenting but also has a sacred purpose that couple have spiritual tranquility together".

**Keywords:** Stress, Anxiety, Infertility, Intervention, Family

### **P-101: Advantages of Recombinant Follicle-Stimulating Hormone over Human Menopausal Gonadotropin for Ovarian Stimulation in Intrauterine Insemination: A Randomized Clinical Trial in Unexplained Infertility**

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**Background:** To compare two different gonadotropin preparations, human menopausal gonadotropin (hMG) and recombinant follicle-stimulating hormone (rFSH), combined with clomiphene citrate (CC) in women with unexplained infertility undergoing intrauterine insemination (IUI).

**Materials and Methods:** In this prospective clinical trial, couples prepared for IUI cycles were randomly allocated to two groups either to receive CC and hMG (group A, n=127) or CC and rFSH (group B, n=132) for ovarian stimulation. Outcomes including rates of clinical pregnancy, miscarriage, OHSS, multiple pregnancy, cancellation, and live birth were compared between groups.

**Results:** Duration of gonadotropin therapy was significantly shorter in group B ( $5.1 \pm 0.84$  vs.  $4.7 \pm 0.8$  days, CI=95%,  $P<0.001$ ). The total dose of administered gonadotropin was also significantly lower in group B ( $386.9 \pm 68.2$  vs.  $348.2 \pm 56.3$  IU, CI=95%,  $P<0.001$ ). Dominant follicle number ( $>17$ mm), mean follicular diameter, and endometrial thickness on the day of HCG injection were similar. Clinical pregnancy, multiple pregnancies, abortion, live birth, ovarian hyper stimulation syndrome (OHSS), and cancellation rates were not statistically different between the groups.

**Conclusion:** IUI cycles in which rFSH had been administered may require shorter duration and a lower total gonadotropin dose.

**Keywords:** Human Menopausal Gonadotropin, Intrauterine Insemination, Recombinant Follicle-Stimulating Hormone, Unexplained Infertility

### **P-102: Recurrent In Vitro Fertilization Failure and Hereditary Thrombophilia**

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**Background:** The largest percentage of failed *in vitro* fertilization (IVF) cycles are due to lack of implantation. As hereditary thrombophilia can cause in placentation failure, it may have a role in recurrent IVF failure. The aim of this case-control study was to determine whether or not hereditary thrombophilia is more prevalent in women with recurrent IVF failures.

**Materials and Methods:** Case group comprised of 96 infertile women, with a history of recurrent IVF failure. Control group was comprised of 95 healthy women with proven fertility who had conceived spontaneously. All participants were assessed for the presence of inherited thrombophilia's including: factor V Leiden, methylen tetrahydrofolate reductase (MTHFR) mutation, prothrombin mutation, homocysteine level, protein S and C deficiency, antithrombin III (AT-III) deficiency and plasminogen activator inhibitor-1 (PAI-1) mutation. Presence of thrombophilia was compared between groups.

**Results:** Having at least one thrombophilia known as a risk factor for recurrent IVF failure (95% CI=1.74-5.70, OR=3.15, p=0.00); Mutation of factor V Leiden (95% CI=1.26-10.27, OR=3.06, P=0.01) and homozygote form of MTHFR mutation (95% CI=1.55-97.86, OR=12.33, p=0.05) were also risk factors for recurrent IVF failure. However, we could not find significant difference in other inherited thrombophilia's.

**Conclusion:** Inherited thrombophilia is more prevalent in women with recurrent IVF failure compared with healthy women. Having at least one thrombophilia, mutation of factor V Leiden and homozygote form of MTHFR mutation were risk factors for recurrent IVF failure.

**Keywords:** Hereditary Thrombophilia, *In Vitro* Fertilization, Recurrent Implantation Failure

### **P-103: Polscope Analysis of Meiotic Spindle and Zona Pellucida Birefringence of Metaphase II Oocytes in Polycystic Ovarian Syndrome Patients**

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**Background:** Polycystic ovarian syndrome (PCOs) is one of the most common causes of infertility in women of reproductive age. Currently, one of the best therapeutic options for PCOs patients is ICSI. Moreover, IVM can be a useful technique for women with PCOs who are at the risk of ovarian hyperstimulation syndrome (OHSS). On the other hand, the oocyte quality can be a determining factor for outcome of ICSI cycles. The goal was to compare both zona pellucida (ZP) birefringence and meiotic spindle (MS) of the in-vivo with in vitro-matured oocytes from PCOs patients using non-invasive PolScope system.

**Materials and Methods:** This prospective study included immature oocytes (30 GV and 5 MI) undergoing IVM, and MII oocytes obtained from PCOs patients (mean age  $\pm$  SD, 29.64  $\pm$  5.31 years) in ICSI program. Using a PolScope, the presence of MS and ZP birefringences were assessed in both in vivo-matured (n=32) and matured oocytes after IVM (n=24). Oocytes were classified as high birefringent (HB) ZP and low birefringent (LB) ZP. Furthermore, the rates of fertilization and embryo development were evaluated.

**Results:** The oocyte maturation rate was 68.5% after IVM. Analysis revealed that the percentage of a HB ZP was significantly higher in the IVM oocytes than in vivo-matured ones (58.3 vs. 31.2%, P=0.04). There was insignificant relationship between MS detection and either in vivo-maturation or IVM oocytes (P=0.53). Likewise, there were similar outcomes for the rates of fertilization and embryo development after ICSI between two groups, respectively (P=0.80 and P=0.13,).

**Conclusion:** Clinical IVM is a safe technology for the maturation and maintenance of oocytes integrity in PCOs patients. Furthermore, the non-invasive PolScope could be used to select healthy oocytes for insemination in ICSI patients with immature oocytes after retrieval.

**Keywords:** PCOs, IVM, PolScope, ZP Birefringence, Meiotic Spindle.

### **P-104: The Effect of L-Carnitine on Mouse Transplanted Ovarian Tissue**

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**Background:** The aim of this study was to evaluate the effect of L-carnitine as an antioxidant on transplantation of ovarian tissue.

**Materials and Methods:** Ovaries from 6-week old mice were grafted into back muscle. Transplanted ovaries were divided into three groups. 150 mg/Kg LC (solved in normal saline) was injected intraperitoneally one hour before surgical operation and repeated one other day until 21 days. Follicular morphology and apoptosis were examined in all groups and compared to age-matched non grafted ovaries. Anti procas-

pase-3 antibody was used to evaluate apoptosis in all groups.

**Results:** Morphological analysis revealed that the number of follicles decreased in all transplanted groups in comparison with non grafted ones. The percentage of normal and degenerative follicles in all developmental stages had no significant differences. The rate of apoptosis in all groups had no significant differences

**Conclusion:** It seems that this concentration of LC have no effect on morphological and apoptosis of follicles in grafted ovaries. However, further studies are required for the fertilization rate of oocytes.

**Keywords:** L-Carnitine, Ovarian Transplantation, Mouse

### **P-105: Genetic Variation of Kinase Insert Domain-Containing Receptor Gene and Its Association with Recurrent Spontaneous Abortion**

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**Background:** Recurrent spontaneous abortion has been defined two or more consecutive miscarriages at 20 weeks' pregnancy and one of diseases that can lead to physical, psychological and economical for the individual problems. Recently number of polymorphisms in several genes was examined for association analyses in pregnant women which related to endanger the life of the fetus. In present study we investigated allele frequency of SNP in Kinase insert Domain Receptor (*KDR*) gene in women with recurrent miscarriage.

**Materials and Methods:** One hundred women with recurrent spontaneous abortion of the fetus with at least 2 or more miscarriages before 20 weeks and without anatomical problems of the uterus, cytogenetic, hormonal problems as patients and 100 women with no history of abortion and with having had successful birth were chosen as controls. For polymorphism analysis of functional SNP rs1870377, PCR-RFLP was performed by using the restriction enzymes *AhaI* and digested products were visualized on 12% acrylamide gel respectively. The differences between allele frequency in two grouped were calculated by chi square test with P value < 0.05. The results were analyzed in the both groups by using SPSS version 18, SNPalyze 7 and GenAlex Ver. 6.4.

**Results:** The estimated risk of subjects with one or two copies of risk alleles in different inheritance models showed no significant differences for crude odd ratio in 95% confidence interval (OR = 1.62, 1.05 for rs1870377 respectively. while, rs1870377 with  $X^2 = 3.249$ ,  $P = 0.08$  showed slightly significant difference ( $P < 0.1$ ). K means clustering showed  $k = 8$  as the best fit for the optimal number of genetic subgroups in our studied materials similar to NJ cluster analysis.

**Conclusion:** Inconsistent results in different ethnic groups with different allele frequencies among RSA patients and

controls may be involving two main factors including, ethnic variation and sample size in these studies.

**Keywords:** Genetic Structure, KDR SNP Sites, PCR-RFLP, RSA

### **P-106: Comparative Expression of The Stemness Gene Oct-4, Nanog, Sox-2 and Rex-1 in Normal Endometrium and in Endometriosis**

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**Background:** Endometriosis is a gynecological disease defined as the presence of endometrial tissue outside the uterine cavity, which caused by various factors. Recent evidences support the presence of endometrial stem cells and their possible involvement in endometriosis. Related studies mainly focus on stemness-related genes, and pluripotency markers may play a role in the etiology of endometriosis. The aim of this study was to analyze transcription levels of the pluripotency factors sex-determine region Y-box 2 (*SOX-2*), Nanog homeobox (*NANOG*), Octamer binding protein 4 (*OCT-4*) and *REX-1* (*Zfp-42*) in the endometrium of reproductive-age women with and without endometriosis.

**Materials and Methods:** Ten ectopic and 10 eutopic tissue samples, as well as 23 normal endometrium specimens were tested in this study. The expression of *OCT-4*, *NANOG*, *SOX-2* and *REX-1* genes were analyzed with quantitative real time polymerase chain reaction.

**Results:** The transcription levels of *OCT-4*, *NANOG* and *SOX-2* mRNA were significantly increased in ectopic lesions compared with eutopic and control group, but the *REX-1* mRNA increase was not significant between endometriosis and the control groups. Also there were difference in expression of these genes in normal endometrium during the menstrual cycle.

**Conclusion:** Based on our data we can conclude that differential transcription of pluripotency factors are involved in ectopic endometrium and the expression of *OCT-4*, *NANOG* and *SOX-2* may contribute to the pathophysiology of endometriosis by stimulating the migration and invasion activity of endometrial cells.

**Keywords:** Endometriosis, *SOX-2*, *NANOG*, *OCT-4*, *REX-1*

### **P-107: Can Combination of Hysterosalpingography and Ultrasound Replace Hysteroscopy in Diagnosis of Uterine Malformations in Infertile Women?**

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**Background:** Müllerian anomalies are associated with in-  
fertility. Hysteroscopy as the gold standard of evaluating Mül-  
lerian anomalies is an invasive, expensive and risky procedure  
which requires enough experience. Transvaginal sonography  
(TVS) and hysterosalpingography (HSG) are less invasive  
procedures, but their accuracy is unknown. The aim of this  
study was to compare the accuracy of combination of TVS  
and HSG with hysteroscopy.

**Materials and Methods:** Medical records of an infertile  
population who were undertaken all three diagnostic modal-  
ities were reviewed concerning their sensitivity, specificity,  
positive predictive value (PPV), and negative predictive value  
(NPV).

**Results:** Ninety nine infertile women with the mean age of  
 $29.1 \pm 6.47$  years, mean duration of marriage of  $8.93 \pm 10.28$   
years, and mean duration of infertility of  $5.59 \pm 4.16$  years  
were assessed. The sensitivity, specificity, PPV, and NPV of  
TVS were 39.6, 76.5, 61.3, and 57.3%, respectively. HSG had  
a sensitivity of 39.6%, specificity of 96%, PPV of 90%, and  
NPV of 62.8%. When both modalities were combined, the  
sensitivity, specificity, PPV, and NPV were 60, 72.5, 67.4, and  
66%, respectively. The diagnostic accuracy of single or com-  
bined techniques was similar.

**Conclusion:** 2D TVS, HSG, and combination of both diag-  
nostic modalities are not accurate enough for assessing uterine  
malformation in infertile women.

**Keywords:** Ultrasound, Hysterosalpingography, Hysteros-  
copy

### **P-108: Efficacy of Intrauterine Injection of Granulocyte Colony Stimulating Fac- tor on Treatment of Unexplained Recur- rent Miscarriage**

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**Background:** Effects of GCSF (Granulocyte Colony-stim-  
ulating Factor) injection in patients suffering from recurrent  
pregnancy loss has been studied in some studies and we now  
know that transvaginal perfusion of G-CSF might be helpful  
for improvement of implantation rate among patients with thin  
endometrium and patients under *in vitro* fertilization /embryo  
transfer (IVF/ET) or intracytoplasmic sperm injection (ICSI)/  
ET. The aim of present study was to evaluate the impact of  
intrauterine injection of G-CSF in patients that suffering from  
unexplained recurrent miscarriage.

**Materials and Methods:** In present randomized clinical  
trial a total of 67 patients were randomly allocated into two  
study groups including intrauterine G-CSF (300 µg) injection  
and control group (no G-CSF injection). All patients were in  
ovulation induction (OI) cycle. In G-CSF group, intrauterine  
injection of G-CSF were done twice in cycle. All enrolled pa-  
tients were under 40 years old and had at least two times unex-  
plained abortion. Pregnancy was evaluated by titer of βhCG in  
institute's lab, presence of gestational sac (implantation) was  
assessed by vaginal sonography and finally clinical pregnancy  
was confirmed by detection of fetal heart rate (FHR).

**Results:** A total of 17 patients were excluded from the fi-  
nal analysis due to different reasons. No significant difference  
were observed between two study groups when we compared  
the rate of pregnancy (P=1.000), implantation (P=0.491), clin-  
ical pregnancy (P=0.414) and abortion (P=0.414).

**Conclusion:** In contrast to possible effect of G-CSF on im-  
provement of implantation rate that revealed by some other  
studies, based on the result of present study we couldn't sug-  
gest intrauterine injection of G-CSF for improvement of clinical  
pregnancy rate and reduce of abortion among patients with  
unexplained recurrent miscarriage. Further molecular biology  
studies are needed to clarify the mechanism in which G-CSF  
affects the pregnancy process.

**Keywords:** Granulocyte Colony-Stimulating Factor, Recur-  
rent Miscarriage, Intrauterine Injection

### **P-109: Cultural Beliefs and Values in Relation to Women's Preferred Mode of Birth in The North of Iran**

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**Background:** Pregnant women rely heavily on informal  
information while making decision on the mode of delivery,  
either as normal vaginal delivery (NVD) or cesarean section  
(CS). Through recognition of social attitudes towards differ-  
ent modes of delivery, societies can be directed towards a po-  
sitive understanding of vaginal delivery, which can ultimately  
lead to maternal health promotion. Thus, this study aimed  
to explore common beliefs, values and traditions regarding  
women's preferred mode of birth in the North of Iran.

**Materials and Methods:** Using a focused ethnographic  
approach twelve pregnant women, 10 women with previous  
experience of childbirth, seven midwives, seven obstetricians,  
and nine non-pregnant women were included in this study  
through a purposeful sampling in health clinics of Toneka-  
bon in the North of Iran. Semi-structured interviews and  
participant observations were used for data collection. Study



rigor was confirmed through prolonged engagement, member check, expert debriefing, and thick description of the data. Data were analysed using Braun and Clarke thematic analysis (2006) and MAXqda software.

**Results:** Through analysis, three major themes and 10 sub-themes emerged. They included: 1. sociocultural childbirth beliefs with five subthemes of CS as protector of genital tract integrity, blind imitation in choosing mode of birth, NVD as a low cost type of delivery, CS as prestigious mode of birth and NVD as a symbol of woman's power and ability; 2. traditional health beliefs with two subthemes of NVD as a guarantee for woman's health and traditional childbirth facilitators; and 3. religious beliefs and values with three subthemes of NVD as a symbol of God's power, call for help from the Mighty God and NVD as a sacred phenomenon.

**Conclusion:** The results of this study indicated that cultural beliefs, values and traditions can significantly affect individuals' attitudes towards mode of delivery, their definitions of different modes, and the decisions they make in this regard. In order to develop a positive cultural and religious attitude towards vaginal delivery, awareness has to be raised through various ways, and the existing misconceptions should be corrected.

**Keywords:** Birth, Cultural Beliefs, Women, Qualitative Study, Ethnography

### **P-110: Evaluations of WBC Crossmatch Results After Lymphocyte Immunization in Women with Recurrent Spontaneous Abortion in Sarem Women's Hospital**

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**Background:** Recurrent spontaneous abortion (RSA) is a complication in pregnancy that results into fetus rejection by mother and several factors such as anatomical, genetic and immunological problems can be its etiologic causes. Paternal lymphocyte therapy as an immunotherapy for these patients has been more evaluated. The purpose of lymphocyte therapy is stimulation of the mother immune system that contributes in the maintenance of fetus. This treatment enhances the immunological regulatory response using prepared mononuclear cells from the husband or third party. In present study we tried to examine the efficacy of paternal lymphocyte immunotherapy to produce suitable immune response for RSA patients by evaluation of WBC-crossmatch results.

**Materials and Methods:** A retrospective survey was conducted in 704 volunteer subjects with RSA, at Sarem Women's Hospital, during the years 2009-2013. Peripheral blood mononuclear cells (PBMCs) were isolated from husband and

were injected into the patients, two or three times. Two weeks after the last immunization, the patient's serum was tested for anti-paternal cytotoxic antibodies (APCA) by WBC cross match test.

**Results:** Positive result of WBC cross match test was found in 319(45.31%) of the 704 patients with RSA that received paternal lymphocytes two times. Immunization was repeated for patients with negative test result and then, positive result were observed in 219 (31.11%) of the treated women (76.42% in total).

**Conclusion:** Results of this study indicated the efficacy of immunization with paternal mononuclear cells to enhance production of APCAs in women with recurrent spontaneous abortion. Also, if lymphocyte therapy performed three times, it can increase alloantibodies production and induction of positive cross match test in these patients.

**Keywords:** Lymphocyte Therapy, Recurrent Spontaneous Abortion, Crossmatch

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## Genetics

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### **P-111: EGFR, ERK, MEK Genes Expression Level in Cumulus Cells of PCOS Women Compared with Healthy Women**

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**Background:** Poly cystic ovarian syndrome (PCOS) is known as a common endocrine disorder in women at reproductive ages and may cause developmental abnormality in oocyte. ERK has found as a regulator protein of Gap junctions (GJ) function and the level of exchanges between two neighbors cells, for example oocyte and surrounding cumulus cells (CCs) in the mammalian ovary. Such exchange is essential for many important events including resumption of meiosis in oocyte. *EGFR* and *MEK* are two genes important in the growth and maturation of oocytes. In this study we evaluate the expression Level of *EGFR*, *ERK* and *MEK* in CCs affected by PCOS.

**Materials and Methods:** In this case-control study 40 women (PCOS= 20, control= 20) were evaluated. Total RNA content was extracted from CCs obtained from cumulus-oocyte complexes (COC) of patients after puncture. The quantitative expression level of *EGFR*, *ERK* and *MEK* were measured using real time PCR. Data analysis was performed using two-way ANOVA parametric tests in R statistical analyzing software.

**Results:** PCOS women CCs demonstrated the significant reduction in expression level of the *EGFR* compared with control (P=0.04). There were no significant difference in expression level of *ERK* (P=0.35) and *MEK* (P=0.11) in PCOS

patients compared with healthy women.

**Conclusion:** Considering the observed reduction in expression level of EGFR and due to its important role in regulation of GJ function and gating, It seems that the regulation control of GJ function may affect by PCOS. In other hands, there was no significant difference in expression level of ERK and MEK between two groups. We hypothesized that interaction between mitogen activated protein kinase (MAPK) signaling and several signaling pathways occurring synchronously in same cell, may moderate the changes induced by PCOS.

**Keywords:** Pathway MAPK, Gap Junctions, Polycystic Ovary Syndrome

### **P-112: PGS-Array-CGH Technique: New Technical Approach to Promotion ART Outcome**

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**Background:** Chromosomal abnormalities are common in embryos from assisted reproductive technology, ranging from 60% abnormal embryos in women <35 to 80% in women ≥41 years. The majority of numerical chromosome abnormalities detected in cleavage-stage embryos are not compatible with implantation or birth, and their high frequency is likely to have a substantial effect on the success of assisted reproductive treatments.

**Materials and Methods:** Preimplantation Genetic Screening (PGS) aims to provide a means of identifying potentially viable euploid embryos, which may have higher chances of producing a pregnancy, to be prioritized for transfer. Prior PGS strategies using Fluorescence In Situ Hybridization (FISH) have involved the analysis of 5, 9, or 12 chromosomes. Nowadays the new generation of PGS methods, 24-chromosome tests, such as CGH, array CGH or single nucleotide polymorphism arrays, are demonstrated to help increase successful outcome rate of ART especially in repeated implantation failure's cases. The combined use of array CGH and single blastocyst transfer can provide an efficient tool for improving IVF clinical outcomes without increasing the number of transferred embryos and the risk of unwished multiple pregnancies.

**Results:** In view of these, Array-CGH is proved to be highly robust and specific when applied to rapid (24-hour) analysis of single cells biopsied from cleavage-stage embryos. This tool does not require prior testing of parental DNA and thus advance planning and careful scheduling are unnecessary and with respect to the review of the literature, array-CGH will detect approximately 42% more abnormalities and 13% more abnormal embryos than the standard 12-probe FISH approach.

**Conclusion:** In a wider perspective, this technique can also be used in patients who, independent of a RIF's history, wish to limit the number of transferred embryos to a single one for different personal, social or economic reasons.

**Keywords:** PGS, Array-CGH, ART Outcome

### **P-113: The Effect of HSP70 Polymorphism in Infertile Male with Varicocele**

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**Background:** Varicocele is dilatation of the veins of the pampiniform plexus within the spermatic cord and is one of the amendable causes of male infertility. Many studies have revealed increase of oxidative stress in serum, semen and testicular tissues of patients with varicocele which may cause DNA damage. HSP70 are a family of proteins that can be expressed in response to a variety of stress, including reactive oxygen species (ROS) and play an important role in the maintenance of cellular integrity. The aim of this study was the evaluation of HSP70-hom polymorphism in infertile men with varicocele.

**Materials and Methods:** The total number of 86 men (37 patients with varicocele and 49 normal controls) was enrolled into the study. Genomic DNA was isolated from 1 ml peripheral blood. Genotyping was performed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Statistical analysis was performed using the MedCalc program (Version 12.1).

**Results:** The frequency of the TT, TC and CC genotype of HSP70-hom polymorphism in varicocele patients were 75.5, 20.41 and 4.1%, respectively, while in controls were 72.9, 8.1 and 18.9%, respectively.

**Conclusion:** It is suggested the HSP70-hom may affect the susceptibility to varicocele in the studied population. While the randomized multicenter studies with greater, sample size are still needed to clarify our results.

**Keywords:** Varicocele, Gene Polymorphism, HSPA1L, Infertility

### **P-114: Evaluation of GSTM1 Null Allele in Infertile Men with Varicocele**

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**Background:** Varicocele is the abnormal inflexion and distension of veins of the pampiniform plexus within spermatic cord and is the most common and correctable known risk factor for male infertility. Varicoceles are found in approximately 40% of infertile men, whereas the incidence in the general male population is approximately 15%. Some studies suggest that varicocele is associated with elevated levels of reactive oxygen species (ROS) in sperm and reduced seminal plasma oxidant capacity. It has been postulated that human semen contains a significant amount of glutathione S-transferase (GST)

and that the enzyme could attenuate the toxicity of ROS to sperm. The GST gene family produces isoenzyme that is important in protection against oxidative stress and an increase of ROS associated with reduced activity of GST may lead to sperm membrane damage. Our objective was to investigate the genetic polymorphism of the glutathione S-transferase M1 gene (GSTM1) and to assess the oxidative damage in Iranian infertile men with varicoceles. This study was performed on 35 patients with varicocele and 58 control subjects.

**Materials and Methods:** Genomic DNA was extracted from peripheral blood of both groups. Genotyping was performed via Multiplex PCR and Statistical Analyses were performed using MedCalc software.

**Results:** The frequency of GSTM1 null (-) genotype was observed to be 22.85% in infertile men with varicocele as against 5.17% in fertile men. Association (OR= 5.43, P=0.026, 95% CI=1.33-22.13) between GSTM1 null (-) genotype and varicocele-induced infertility was confirmed, overall.

**Conclusion:** GSTM1 null (-) genotype may be an important contributor factor in infertile men with varicocele. Larger population-based studies are needed to determine the relation between this deletion and varicocele-associated infertility.

**Keywords:** GSTM1, Varicocele, Oxidative Stress, Male Infertility.

### **P-115: The Role of G22A Adenosine Deaminase 1 Gene Polymorphism and The Activities of ADA Isoenzymes in Fertile and Infertile Men**

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**Background:** Some studies have revealed enzymatic and non-enzymatic roles of adenosine deaminase (ADA) in male reproductive system such as regulating adenylate cyclase activity by decreasing adenosine concentration, helping to interaction between prostasomes and spermatozoa.

**Materials and Methods:** In this study, we evaluate frequency distribution of ADA1 G22A alleles and genotypes in 200 fertile and 200 infertile men. The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique was used for determining of ADA1 G22A variants. In addition, ADA isoenzymes activities (ADA1 and ADA2) were measured using Giusti and Galanti colorimetric method.

**Results:** The frequency of GG genotype was significantly higher and GA genotype was lower in infertile males compared with fertile men (P=0.048 and P=0.045 respectively). However, there was not any noticeable difference in allele dis-

tribution between groups (P>0.05). Based on logistic regression analysis, the GA genotype has a protective role and can decrease the risk of male infertility 1.7 times (P=0.046). There were significantly higher activities of ADAT and its isoenzymes in infertile males compared with fertile men (P<0.05). Also, ADA1 activity with GG genotype was higher than GA carriers in all population (P=0.001).

**Conclusion:** Our results revealed that activity of ADA isoenzymes and distribution of ADA1 G22A genotypes were different among fertile and infertile men and more likely the GA genotype which had lower ADA1 activity and was higher in fertile men is a protective factor against infertility. Moreover, activity of ADA<sub>total</sub>, ADA1 and ADA2 enzymes were significantly higher in infertile men compared with fertile subjects which can indicate importance of this enzyme in male fertility.

**Keywords:** Adenosine Deaminase, Male Infertility, Genetic Polymorphism, Reproduction

### **P-116: Absence of JMJD1A, A Testis-Specific Histone Demethylase, in Tissue Samples of TESE Negative Men**

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**Background:** During mammalian spermatogenesis unique and dynamic epigenetic events occur leading to chromatin condensation. Through these events, histone demethylases such as JMJD1A play important roles in compaction of sperm chromatin, due to regulation of histone methylation dynamics and alteration of chromatin structure. As “histone methylation” is one of the best-characterized modifications in the study of germ cell development, evaluation of presence/absence of JMJD1A protein in impaired spermiogenesis was aimed in this study.

**Materials and Methods:** For this respect, based on spermogram and presence/absence of sperm in testicular sperm extraction (TESE) process, testis tissue samples of infertile men referred to Royan Institute were collected as two groups of TESE negative (n=9) and TESE positive (n=3), respectively. Immunohistochemical analysis of paraffin embedded tissue samples was performed qualitatively, using anti-JMJD1A antibody to elucidate presence/absence of JMJD1A in nucleus of germ cells.

**Results:** Immunohistochemical analysis data showed absence of JMJD1A protein in nucleus of germ cells in TESE negative group compared to TESE positive group.

**Conclusion:** It can be concluded that there is an obvious association between absence of histone demethylation as a chro-

matin condensing state with impairment of spermatogenesis and male infertility.

**Keywords:** Spermatogenesis, Infertility Men, Epigenetic, JMJD1A, Demethylation

### **P-117: Association of G16129A and T16172C in Mitochondrial D-Loop with Azoospermia**

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**Background:** Almost 15% of couples suffer from infertility and the men account for 50% of infertility factors. The most prevalent reason of male infertility is due to problems in sperm production that include low number of sperms or low mobility of sperm and production of sperm with improperly function. Sperm cell needs ATP to perform its functions which provided by mitochondria. Presence of point mutations, polymorphisms and eliminations in mitochondrial DNA especially in D-loop region influence the ATP production and subsequently impair the performance of sperm.

**Materials and Methods:** We collected 360 blood samples (24 azoospermia and 336 healthy controls) from men attending the infertility clinic at Research and Clinical Center for Infertility (Yazd, Iran). Genomic DNA was extracted from blood using salting-out procedure. To amplification of HV1 region, the universal primers of ONP98 F and ONP77 R and to amplification of HV2 region, universal primers of ONP38 F and ONP79 R were used.

**Results:** Our data revealed that there were total 85 variations in HV1 and HV2 regions. From these variations, 78 mutations were previously reported and 7 mutations has not been reported yet. However, the D-loop G16129A and T16172C polymorphisms were associated with azoospermia ( $P < 0.05$ ).

**Conclusion:** D-loop is contained two areas, namely, HV1 and HV2. The rate of mutation is higher in this area as a non-coding site. We found two polymorphisms (G16129A and T16172C) in HV1 region and then we compared the frequency of them between case and control groups. Our data revealed a significant differences between control and case groups ( $P < 0.05$ ). Since HV1 has an essential role in DNA replication therefore we suggest that G16129A and T16172C may change the DNA replication deficiency. The issue may change the copy number of mtDNA and mitochondrial function subsequently.

**Keywords:** Azoospermia, mtDNA, D-Loop, HV1 Region, HV2 Region

### **P-118: Triplet Nucleotide Repeats Expansion (CAG and GGN) of Androgen Receptor Gene in Infertile Patients with Abnormal Spermogram**

### **tor Gene in Infertile Patients with Abnormal Spermogram**

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**Background:** The infertility has recently been estimated to affect approximately 9% of couples worldwide. Androgens and a functional androgen receptor (AR) are essential for normal development of the male gender, and for maintenance of spermatogenesis throughout the life. Two polymorphic trinucleotide repeats, CAG and GGN, encoding for the amino acids glutamine and glycine, respectively are located on the longest exon of *AR* gene. The effect of these trinucleotide repeats expansions on *AR* actions and male infertility is still under investigation as an interesting question. This study determines to answer this question in Iranian population.

**Materials and Methods:** Therefore 80 infertile patients and 40 control fertile subjects were elected. The infertile group was divided into two subgroups according to the sperm count (40 asthenospermic men and 20 severoligospermic men). After DNA extraction from peripheral blood and amplifying the *AR* gene via polymerase chain reaction (PCR), number of CAG and (GGN)<sub>n</sub> in each group were determined by sequencing method.

**Results:** Median lengths CAG for the asthenospermic men and the control group respectively were 24.12 and 21.8. Statistical analysis of the CAG repeats shows significant difference ( $P=0.0$ ) between the asthenospermic and the control group. Nonsignificant difference was elicited between CAG repeat in the severoligospermic group and the control group (mean lengths 21.4 and 21.8,  $P=0.96$ ). AR-GGN repeats have demonstrated nonsignificant difference in two asthenospermic and severoligospermic in compared with the control group.

**Conclusion:** This evidence suggests *AR* with long CAG stretches can display lower activity than the AR of median length. Reduced *AR* activity causes defect in sperm count and motility. In this study Association between GGN repeats and AR acts and sperm production was not conformed and needs more research.

**Keywords:** Polymorphic Trinucleotide, Infertility, Asthenospermic, Severoligospermia

### **P-119: Survey of Genetic Alterations in Exon1 of Androgen Receptor Gene in Azoospermic Patients**

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**Background:** Androgen receptor (AR) mediates androgen actions such as initiation and promotion of spermatogenesis and growth of accessory sex organs. There are two trinucleotide polymorphisms (CAG and GGN repeats) in exon1 of AR gene that are vary in length in population. The CAG and GGN repeats association with infertility is still unknown and this study is planned to assess the distribution of CAG and GGN repeat expansion and their roles in Iranian men's possible infertility.

**Materials and Methods:** To achieve this aim, eighty men were chosen (40 fertile men as a control and 40 azoospermic verified using spermogram test as an infertile group). The patients genomic DNA was extracted from their peripheral blood and the AR gene was amplified by polymerase chain reaction (PCR). For determining the number of repeats, sequencing method was used.

**Results:** Our result demonstrated the means of CAG and GGN repeat length in infertile group were 23.4 and 23.7 respectively, that were significantly higher than these trinucleotide repeats in fertile men (AR-CAG mean =21.7, P=0.014 and AR-GGN mean =22.3, P=0.001). In addition, the maximum CAG repeat in azoospermic men was 29 repeats and in control group it was 27 repeats. Longest GGN repeat in infertile men was measured 30 vs. 25 in control group.

**Conclusion:** This finding indicates that some men with deficiency in spermatogenesis have longer trinucleotide repeats in the AR gene. According to the results, long stretches of tandem repeats of AR gene may negatively affect the function of the gene and consequently lead to male infertility in Iranian men.

**Keywords:** Androgen Receptor, Trinucleotide, Polymorphism, Azoospermia

### **P-120: Evaluation of OGG1 Ser326Cys Polymorphism in Idiopathic Male Infertility**

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**Background:** Male infertility is a multifactorial syndrome encompassing a wide variety of disorders. In more than half of infertile men, the cause of their infertility is unknown (idiopathic) and could be congenital or acquired. OGG1 is a bifunctional glycosylase, in that it has both AP lyase and DNA

glycosylase activity and one of its functions is to excise and remove 8-oxo-dGuo from DNA. Among the common single nucleotide polymorphisms (SNPs) of the OGG1 gene, one located in exon 7, resulting in an amino acid substitution of serine (Ser) with cysteine (Cys) at codon 326 (Ser326Cys, rs1052133), has been demonstrated to affect OGG1 function. Functional studies have revealed that 326Cys allele of OGG1 was associated with the reduced repair capacity toward oxidized DNA lesions. The aim of this study was to elucidate the relationship between OGG1 Ser326Cys polymorphism and Idiopathic male infertility.

**Materials and Methods:** Frothy eight men with idiopathic infertility and 50 healthy men were included in this study. DNA was extracted from 1 ml of peripheral blood samples. Ser326Cys polymorphism was determined using PCR-RFLP method. Statistical analysis was performed using the MedCalc program.

**Results:** In the control group, the distribution of genotypes was as follows: 88% were Ser/Ser, 10% were Ser/Cys and 2% were Cys/Cys. For the infertile group, the distributions were 83% Ser/Ser, 13% Ser/Cys and 4% Cys/Cys.

**Conclusion:** The results of this study suggested that the OGG1 Ser326Cys polymorphism may not associated with male infertility. However, additional studies are needed to validate our findings.

**Keywords:** OGG1, DNA Damages, Polymorphism

### **P-121: Cloning and Expression of The Inosine Triphosphate Pyrophosphatase Gene Variant II in E.coli**

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**Background:** Environmental and cellular inappropriate conditions can cause damages to cell's nucleotide pool. Deamination and oxidation damages interfere with cell's vital reactions. Inosine triphosphate pyrophosphatase (ITPA), an evolutionary conserved enzyme, plays a critical role in elimination of non-canonical bases. In human genome, the ITPA gene is located on chromosome 20 short arm and transcribed into three different alternative transcripts in various tissues. The main alternative variant is variant I (coding isoform a), which is expressed in all human's cells. Although, ITPA variant II (coding isoform b) is expressed in large cell carcinoma, astrocytoma grade IV, brain and Embryonic Stem Cells (ESCs). Comparing with isoform a, isoform b is 17 amino acids shorter in length. It's not yet clear whether the variant II can code any protein or not.

**Materials and Methods:** RNA was extracted from cancerous cell line. cDNA was synthesized by oligo dt and random hexamer primers. Primers specific to the variant II amplified the relevant cDNA. The PCR fragment was cloned into TA vector. By applying restriction enzymes, cloned fragment was subcloned in the pET expression system. For its expres-

sion, pET32a vector was transformed into BL21 E.coli strain, which can code T7 RNA polymerase. Expression was induced by exertion IPTG into the liquid bacterial culture (LB).

**Results:** Sequence file was analyzed and the accuracy of cloned fragment was endorsed by sequencing. Expression of isoform b was validated on SDS-PAGE.

**Conclusion:** The size of the cloned fragment was confirmed by protein ladder. The size difference between two isoforms was observed on SDS-PAGE, approving the precision of cloned fragment. This cloned variant, can be used for further investigations such as, activation analysis, protein-protein interaction and sub-cellular localization.

**Keywords:** ITPA, SDS-PAGE, pET Expression System

### **P-122: The Effect of Beta Globin Intron on Human LH Hormone Expression in CHO Cells**

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**Background:** Luteinizing hormone (LH) is a heterodimeric glycoprotein composed of alpha and beta subunits. This hormone is secreted from the pituitary gland. LH, in women triggers Menstrual cycle and ovulation. In men, LH stimulates production of testosterone, which plays a specialized role in sperm production. Up to day, LH hormone have produced in different ways such as codon optimization and also in various cell lines. Between mammalian expression systems, Chinese hamster ovary (CHO) cells, due to rapid proliferation and post-translational modifications, are more common than other hosts. One way to increase gene expression is use of introns. Beta-globin intron is composed of 3 exons and 2 introns. Human beta globin gene is located on chromosome subband 11p15.5. In this study, the beta-globin gene intron-I was used to enhance the expression of LH hormone. The purpose of this study, was to investigate the effects of intron I beta globin gene on LH expression.

**Materials and Methods:** The gene construct was made by PCR and Soeing-PCR techniques. Then, this structure was cloned into pVito2-neo-mcs expression vector.

**Results:** In continuous, recombinant colonies were approved by colony PCR, digestion and sequencing.

**Conclusion:** In the future, gene construct will be transferred into CHO cells by electroporation technique and examined the expression level of recombinant protein by SDS-page, Western blotting and ELISA methods.

**Keywords:** Luteinizing Hormone, Beta Globin Intron, Chi-

nese Hamster Ovary

### **P-123: Genetic Variation of AKAP3 Gene in Infertile Men with Abnormal Spermogram Referred to Royan Institute**

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**Background:** One kind of sperm abnormality that leads to men infertility is short flagella sperms. In this defect, fibrous sheath and axoneme are disorganized, the sperms tail is short, the numbers of sperms in the semen fluid reduce and the sperms are immotile. There are also other kinds of oligoasthenoteratozoospermia (OAT) disorder that causes infertility in men. A kinas anchoring protein 3 (*AKAP3*) gene encodes a protein that is involved in the fibrous sheath structure, regulation of sperm motility and head-associated functions such as capacitation and the acrosome reaction. In the present case-control study, 30 patients with short tail sperms and 30 patients with OAT disorder and 40 males with normal spermogram referred to Royan institute were enrolled as case and control groups, respectively.

**Materials and Methods:** The genetic variation in exon 5 of *AKAP3* gene which encodes the functional domain of this protein was studied using PCR-sequencing technique on extracted DNA from blood samples of control and patient groups.

**Results:** According to the results, four haplotype polymorphisms; 1378 T>C, 1573 G>A, 1391C>G, 1437T>C were observed in all samples of both patient groups and the control group. However a polymorphism 1982T>C was just observed in 2 short tail sperm samples and one OAT defect sample as heterozygotes (T/C) (P value=0.268). Another polymorphism was 1499T>C, which was only seen in 5 short tail sperm samples and 6 OAT samples. A significant difference was seen between the studied groups after analyzing the data by SPSS (P Value=0.004). This alternation changes the hydrophobic (I) amino acid with medium size changes to a medium size and polar (T) amino acid.

**Conclusion:** It can be concluded that 1499T>C polymorphism may be associated with these defects in Iranian men.

**Keywords:** *AKAP3* Gene, Abnormalities Spermogram, Polymorphism

### **P-124: Comparative Study of The Effect of Fetal Bovine Serum Concentrations on Expressing of Human Follicle Stimulating Hormone.**

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**Background:** Follicle stimulating hormone (FSH) produced in recombinant Chinese hamster ovary (CHO) cells used for infertility treatment. Fetal Bovine Serum (FBS) is known as a factor in medium culture that provides the tolerance towards physiochemical variations and protects cells against apoptosis; on the other hand, FBS is the most expensive component in medium culture and collection that cause unnecessary suffering for the unborn calf. This study tried to investigate the effect of FBS levels on growth of recombinant cells producing FSH and FSH titer for determination the optimum concentration of FBS in medium culture.

**Materials and Methods:** Trypan blue staining method was applied for cell counting and measuring Cells Viability. SDS-PAGE and Western blotting used for detection of rFSH. In continuous, qRT-PCR and ImageJ software were used for quantification assays.

**Results:** The obtained results in this study showed that the most productivity of the cell at 10% FBS. Also, Increase in cell viability and longevity was indicated at 3% FBS. Quantification assays 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:** The maximum expression was seen at medium culture with 10% FBS. Cells viability and longevity increased in medium containing 3% FBS, also the need for FBS reduced to less than one-third in medium with 3% FBS. Considering these, medium containing 3% FBS suggested in this study.

**Keywords:** Follicle Stimulating Hormone, Fetal Bovine Serum, Infertility

### **P-125: Identification of Novel Missense Mutations of The TGFBR3 Gene in Chinese Women with Premature Ovarian Failure**

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**Background:** The aim of this study was to assess the association between human transforming growth factor b receptor,

type III (TGFBR3) and idiopathic premature ovarian failure (POF) in a Chinese population.

**Materials and Methods:** A total of 112 Chinese women with idiopathic POF and 110 normal controls were examined. DNA samples prepared from blood leukocytes were used as templates for polymerase-chain reaction amplification of DNA fragments from TGFBR3. The gene fragments were sequenced. Web-based programs, including PolyPhen, Sorting Intolerant from Tolerant (SIFT), Prediction of Pathological Mutations (PMUT), ScanProsite and ClustalW2, were used to predict the potential functional and structural impacts of the missense variants of TGFBR3.

**Results:** A total of 11 novel variants were identified. Among them, six were found only in the POF patients. Two missense variants, p.E459G and p.P825L, which are conserved in primates, were predicted to have functional and structural impacts on the TGFBR3 protein.

**Conclusion:** The other four variants (c.381+12A>C, c.24317A>G, p.S172S and p.C220C) were considered benign. However, further functional studies are necessary to confirm these findings.

**Keywords:** Infertility, Mutation, Premature Ovarian Failure, TGFBR3

### **P-126: VEGFA Gene Polymorphisms and Its Association with Recurrent Spontaneous Abortion**

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**Background:** Spontaneous abortion has been defined as two or more consecutive miscarriages at 20 weeks pregnancy. Vascular endothelial growth factor (VEGF) plays a main role in fetal and placental angiogenesis which secretes from different cells like endometrium and placenta. The objective of this study was to investigate of association of VEGFA gene polymorphisms and recurrent spontaneous abortion (RSA).

**Materials and Methods:** In this project, a case-control study of 100 women with RSA and 100 fertile controls was performed to evaluate four VEGF gene polymorphisms including +936C/T (rs3025039) and -154G>A (rs1570360). The polymorphisms of VEGF gene were studied by using PCR-RFLP technique. Restriction enzymes consist of NlaIII and MnlI were used for digestion. Digestion products were visualized by polyacrylamide gel (12%PAGE). The results were analyzed in the two studied groups by using SPSS version 18, SNPalyze 7 and GenAlex Ver. 6.4.

**Results:** We found association between -154G/A (rs1570360, 5'-UTR) [OR (95% CI)=0.01 (0.03-0.29); P<0.0001] heterozygous genotype (GA) and RSA. The VEGFA single nucleotide polymorphism (SNP) +936C/T (rs3025039, 3' UTR region) [OR (95% CI)= 0.73 (0.33-1.60); P<0.43] did not show association with RSA pathogenesis.

The genetic variation of studied population was estimated by Nei's genetic diversity and Shannon index. Cluster analysis including Neighbor Joining and K-means clustering as well as analysis of molecular variance (AMOVA) supported genetic differentiation of women with RSA and controls.

**Conclusion:** Allelic polymorphisms in common VEGF SNPs was associated with RSA samples and haplotypes with at least one minor allele showed association with RSA pathogenesis.

**Keywords:** Genetic Diversity, K Means, VEGF Gene, RSA

### **P-127: The Effect of Beta Globin Intron on Human FSH Hormone Expression in CHO Cells**

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**Background:** Follicle stimulating hormone (FSH)- a heterodimeric glycoprotein- is secreted by pituitary gland. This hormone stimulates growth and maturation of the follicles in females and sperms in male. Up to now, glycoprotein hormones such as FSH have produced in different cell lines. Among of the mammalian expression systems, the Chinese hamster ovary cells (CHO) have taken into consideration owing to high level of expression and post-translational modifications on proteins. Nowadays, recombinant FSH is produced in CHO cells. One of the pathways of gene expression optimization is use of introns. Introns are non-coding sequences that is located between coding sequences in the majority of eukaryotic genes. Human beta globin gene is located on chromosome subband 11p15.5. This gene is composed of 3 exons and 2 introns. The length of intron is 130 and 850 base pairs, respectively. The aim of this study was to investigate the effects of intron I beta globin gene on FSH expression.

**Materials and Methods:** At first, the gene construct was made by PCR and SoeingPCR. Then, this structure was cloned into the pTZ57R/T vector and pViro2-neo-mcs expression vector.

**Results:** In continuous, recombinant clones were confirmed by colony PCR and sequencing techniques.

**Conclusion:** In the future, this gene construct will be transformed into CHO cells and examined the expression level of recombinant protein by SDS-page and Western blotting methods.

**Keywords:** Follicle Stimulating Hormone, Beta globin Intron, Chinese Hamster Ovary

### **P-128: Optimization of Human LH Gene**

### **Expression by Codon Usage Adaptation in CHO Cell Line**

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**Background:** Human luteinizing hormone (hLH) belongs to glycoprotein hormones which is composed of two non-covalently linked subunit,  $\alpha$  and  $\beta$ . The  $\alpha$ -subunit is similar in all glycoprotein hormones, whereas the  $\beta$ -subunit is conferring the hormonal specificity.

This hormone has important roles in the growth and maturity of sexual organs and secondary sexual characteristics and steroid biosynthesis in the ovary. LH currently is produced by two methods: isolated from the urine of menopausal women and recombinant technology, but it is not sufficient for medical demands. The purpose of this study is to increase the expression and production of LH. Since each amino acid codon frequencies are not the same in different organisms, replacing codons for each amino acid in the desired gene with the same amino acid codon which is more abundant in the host cells, can increase expression level.

**Materials and Methods:** In this study, open reading frame (ORF) of  $\beta$  subunit was changed based on codon usage in Chinese hamster ovary (CHO). The synthesized gene was purchased in the vector PGEM-B1 and cloned by PCR and inserted in the expression vector pVITRO2-neo-mcs. The  $\alpha$ -subunit was already present in the vector. All processes were confirmed by PCR, digestion reaction and sequencing. Recombinant pVITRO2-neo-mcs expression vector was linearized and transfected into the CHO cells. rLH Protein expression was analysed by Bradford's technique, SDS page, Western blotting and ELISA.

**Results:** Our results showed that According to the standard curve, the levels of intracellular rLH protein was increased but secreted protein was decreased (1.17 and 0.87 folds respectively).

**Conclusion:** Our results showed that the codon optimizing of LH gene for expression in CHO cell does not change the expression level comparing to the normal gene considerably. It may be because of the LH gene that is a GC rich sequence.

**Keywords:** Luteinizing Hormone, Codon Usage, Chinese Hamster Ovary, Optimal Gene Expression

### **P-129: Significant Association of TNF- $\alpha$ and IL-6 Gene with Male Infertility: An**



## Explorative Study in Indian Populations of Uttar Pradesh

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**Background:** In this study, we aimed to identify the association of SNPs candidate genes of TNF- $\alpha$  and IL-6 with hormones levels and sperm cells death in infertile subjects of Uttar Pradesh population in North India

**Materials and Methods:** The study population comprised fertile donor (control group) and infertile group patients i.e. normozoospermic (idiopathic unexplained), oligozoospermic and asthenozoospermic groups, with 260 subjects in each group. Subjects were selected from the Departments of Urology, K.G's Medical University and Urology, SGP GIMS, Lucknow, India. The allele-specific polymerase chain reaction (PCR) and PCR-RFLP were used to investigate the substitution of the guanine (G)-to-adenosine (A) at position-308 and guanine (G)-to-cytosine (C) at position-174 in the promoter regions of the TNF- $\alpha$  and IL-6 genes, respectively. Further their relation to male fertility and sperm function were also investigated.

**Results:** It was found that the substitution levels from G to A and from G to C in the TNF- $\alpha$  and IL-6 genes, respectively, were significantly higher in the infertile subjects as compared to that of control group. The apoptosis and necrosis levels were also higher in oligozoospermic and asthenozoospermic infertile subjects. Further it was found to be associated with increased level of reactive oxygen species as observed in oligozoospermic and asthenozoospermic subjects. However, a significant decrease in testosterone and luteinizing hormone with increased prolactin and follicle stimulating hormones was observed in infertile subjects.

**Conclusion:** The study of populations indicated a strong association between TNF- $\alpha$  G-308A and IL-6 G-174C substitution with infertile men which is further supported by allele and genotype meta-analysis and thus established it as a risk factor.

**Keywords:** Apoptosis, Cytokines, Gene, TNF- $\alpha$ , IL-6

## P-130: Piwil2 Reprograms Human Fibroblasts to Germ Cell Lineage

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**Background:** The piwi family genes are highly conserved during evolution and play a crucial role in stem cell self-renewal, gametogenesis, and RNA interference in diverse organisms ranging from Arabidopsis to humans. Piwil2, also known as Hili, is one of the four human homologues of piwi. Piwil2 was found in germ cells of adult testis, suggesting that this gene functions in spermatogonial stem cell (SSCs) self-renewal and formation. Therefore, as a bid for infertility treatment, we examined the piwil2 potential to trans-differentiate human fibroblasts, as terminally differentiated cells, to germ cell lineage.

**Materials and Methods:** Primary Human Neonatal Foreskin Fibroblasts (Nff) were transfected by plasmid overexpressing hpiwil2 under the control of CMV promoter. After establishing stable cell line, semi-quantitative RT-PCR analysis was then applied to assess the expression level of our target genes including those related to pre-meiotic and late meiotic germ cell lineage.

**Results:** Our semi-quantitative gene expression analysis on the human fibroblasts ectopically expressing hpiwil2 demonstrated an increase in the expression level of many premeiotic germ cell markers including HSP90, CD49f, C-kit, Stella, Fragilis, Spoc1, RBM. However, the expression of late meiotic markers such as Scp3, Pgk2 or Prm1 were not detectable under the standard cell growth condition which were applied in our study. notably, in line with the pluripotent characteristics of premeiotic spermatogonial stem cells (SSCs), the hpiwil2 expressing human fibroblasts demonstrated an elevated level of pluripotency markers including Oct4, C-myc, Klf4 and Nanog as well.

**Conclusion:** Our results clearly indicated the capability of hpiwil2 gene in reprogramming of human fibroblasts to premeiotic germ cell lineage like SSCs, providing novel approach for treatment of male infertility

**Keywords:** Piwil2, Spermatogonial Stem Cell, Male Infertility, Pluripotency

## P-131: The Study of Nitric Oxide Synthase 3 (NOS3) 4a4b Gene Polymorphism in Iranian Infertile Men with Varicocele

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**Background:** Varicocele is an abnormal dilation and tortuosity of veins of pampiniform plexus that drains the testis and causes an important change in semen. This abnormality is often one of the most common risk factors for male infertility. The aim of this study was to investigate the relationship between nitric oxide synthase 3 (NOS3) 4a4b (rs61722009) gene polymorphism, as a common genetic factor, with the risk of varicocele in Iranian infertile men. Three types of eNOS gene polymorphisms have been described. G894T, T-786C

and 4a4b, a variable number of tandem repeats (VNTR), within intron 4 of the eNOS gene, accounts for >25% of basal plasma NO generation, suggesting that this gene might have an important role in NO-mediated physiology.

**Materials and Methods:** Sixty infertile men with varicocele (mean age  $31.58 \pm 6.16$  years) and forty-five healthy control (mean age  $31.26 \pm 5.3$  years) with documented fertility were recruited in this study. Both group were analyzed for the VNTR polymorphism in intron 4 of the nitric oxide synthase 3 by the conventional polymerase chain reaction (PCR).

**Results:** As a result, in patient group genotype frequency of AB is 20% and AA is 80%. Significant differences were not found between infertile cases and healthy controls with regard to 4a/b polymorphism. Therefore, no genetic relationship was observed between NOS3 gene 4a4b polymorphism and varicocele.

**Conclusion:** The findings demonstrate that, in Iranian population studied, genetic variation (4a/b) of the NOS3 gene, are not associated with Iranian infertile men with varicocele. Furthermore, studies with larger samples need to be done to confirm these finding.

**Keywords:** NOS3, Varicocele, 4a4b Polymorphism, Conventional PCR

### **P-132: Analysis of Genetic Variation of Interleukin 1- $\alpha$ in Idiopathic Male Infertility**

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**Background:** Despite enormous progress in the understanding of human reproductive physiology, the underlying cause of male infertility remains undefined in about 50.0% of cases, which are referred to as idiopathic infertility and already affects about 5.0-7.0% of the general male population. Interleukin-1 is a regulator that plays an important role in maintaining a safe environment for testes and differentiation during spermatogenesis. IL-1 $\alpha$  is expressed in all stages of the epithelial cycle of the seminiferous epithelium and applies its biological effects through pairing with its receptor on both sertoli and germ cells. It also affects spermatogenesis and cellular integrity of germ cells. The primary cellular goal of IL-1 $\alpha$  is to maintain the binding of sertoli cell actin cytoskeleton. The gene encoding interleukin-1 $\alpha$  has several polymorphism sites and our aim in this study is to assess the IL -889C/T polymorphic loci in men with idiopathic infertility.

**Materials and Methods:** Blood samples were collected from 80 patients diagnosed with idiopathic male infertility and 90 control subjects and genotyped by Multiplex-PCR. To estimate the association between genotype and allele frequencies in cases and controls, P values were assessed by chi-square ( $X^2$ ) analysis.

**Results:** Among cases, the distribution of genotypes was as follows: 73 % was CT , 20 % was CC , and 7 % was TT . Among controls, 76 % was CT , 15 % was CC , and 9 % was TT . There was no significant differences in the IL-1 $\alpha$  -889C/T genotypes and allele frequencies between infertile cases and controls.

**Conclusion:** IL-1 $\alpha$  -889C/T polymorphism appeared to be unrelated to the risk of idiopathic male infertility in our population. While the results which have been done will be change if the gene pool of populations be varied. Further studies are needed to confirm the results.

**Keywords:** Polymorphism, Idiopathic Male Infertility, IL-1 $\alpha$

### **P-133: Investigation of Genetic Variations in Exon 6 of AKAP4 Gene in Infertile Men with Immotile Short Tail Sperm**

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**Background:** The immotile short tail sperm (ISTS) defect is one of the disorders that cause male infertility. Men with this condition have immotile short-tail sperm with structural defects in the fibrous sheath(FS). A Kinase Anchoring Protein 4 (*AKAP4*) is one of the most abundant proteins in the fibrous sheath of sperm flagella and provides scaffold for the correct assembly of FS. Since exon 6 of *AKAP4* gene, codes more than 80% of the protein, in this study its genetic variations in ISTS men was evaluated.

**Materials and Methods:** In this study, 32 infertile men with ISTS defect and 50 normozoospermic men as control were selected. To study the genetic variations, DNA was extracted from peripheral blood cells and then PCR-Sequencing was done.

**Results:** Sequence analysis results did not show any single nucleotide polymorphisms (SNP) or mutation in target region of *AKAP4* gene in the case or control groups.

**Conclusion:** Although our data did not reveal any mutation or SNP in exon 6, but according to the key role of *AKAP4* in sperm tail formation, study in a larger population can bring more accurate results.

**Keywords:** *AKAP4*, ISTS, Male Infertility

### **P-134: Effect of Tribulus Terrestris Extract on Sperm Parameters in Male Mouse**

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**Background:** Spermatogenesis is a process in which the gametes are produced. Dysfunction in any of part of the process could result in infertility. Tribulus terrestris is a flowering plant which traditionally is used to sexual instinct. Reports indicate that this plant could elevate blood levels testosterone and enhance libido.

**Materials and Methods:** In this experimental study, twenty six mature inbred male balb/c mice with average weight of 20g and age of 6 weeks were used. Animals were treated by Alcoholic extract of Tribulus terrestris flowers once daily for 8 weeks. After the last treatment, the mice were sacrificed. Semen samples were extracted from testis and epididymis tissue and were assessed by CASA.

**Results:** Clinical data indicated that median of weight gain in treated mice was statistically higher than non-treated ones. Besides, a significant difference in the number and motility of sperms from treated and non-treated mice was observed.

**Conclusion:** Results revealed that treatment with Tribulus terrestris extract can improve sperm parameters in mice. According to our previous knowledge, using Tribulus terrestris could result in sexual wellbeing as well as inducement more testosterone, and also erection in laboratory animals. This presumably means that this herb could affect human sperm parameters as well.

**Keywords:** Tribulus Terrestris, Sperm Parameters, Spermatogenesis, Male Mouse, Testosterone

### **P-135: Protective Activity of Tacrolimus Contralateral Epididymal Sperm Fertilizing Capacity Following Unilateral Vas Deferens Obstruction: A Murine Model**

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**Background:** Auto-immunization was proposed as the mechanism of contralateral testicular damage following unilateral vas deferens injuries. The objective of this experimen-

tal in vitro study was to elucidate the effect of tacrolimus (TS) as a powerful immunosuppressant on contralateral epididymal sperm fertilizing capacity following unilateral vas deferens obstruction (UVO) in mice.

**Materials and Methods:** Adult male mice were randomly assigned to four experimental groups. Two groups of mice were undergone to UVO under anesthesia with an intraperitoneal administration of ketamine (45 mg/kg) and xylazine (10 mg/kg). Following caudal abdominal incision, UVO was induced via left vas deferens ligation by a 4/0 silk suture 2 cm from the epididymis. One of these groups received TS (10 mg/kg per day, orally) for 5 days starting from the day of induction of experimental UVO. Corresponding control groups were also included. Contralateral epididymal sperm fertilizing capacity of all animals was evaluated following in vitro fertilization at 5 weeks postoperatively.

**Results:** UVO resulted in significant decreases in fertilization rate and embryonic development along with increased rates of embryo arrest. TS administration noticeably attenuated all UVO-induced negative changes in the above-noted parameters.

**Conclusion:** These findings suggest that TS therapy may have beneficial effects in UVO-induced fertility problems. However, further studies have to be contemplated to anticipate the effects of TS in human cases.

**Keywords:** Tacrolimus, Vas Deferens, In Vitro Fertilization, Mouse

### **P-136: Association of Matrix Metalloproteinase-2,-9 Overexpression and Imbalance PR-A/PR-B Ratio in Endometriosis**

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**Background:** Matrix Metalloproteinases (MMPs) degrade extracellular matrix components to provide normal remodeling and contribute to pathological tissue destruction and cell migration in endometriosis. It is accepted that MMPs are resistant to suppression by progesterone in endometriotic tissues. The physiological effects of progesterone are mediated by its two progesterone receptor (PR) isoforms, namely PR-A and PR-B. The capacity of progesterone affect to gene expression is dependent on the PR-A/PR-B ratio. The imbalance ratio in endometriotic tissue may be an important mechanism to be resulted in progesterone resistance and modify progesterone action via differential regulation of specific progesterone response genes and improve endometriosis disease.

**Materials and Methods:** RNA was extracted from twenty ectopic (endometriotic) and eutopic (endometrial) tissue sam-

ples of women undergoing laparoscopy for endometriosis and 20 healthy fertile women at Royan Institute, Tehran, Iran. Analysis of PR-A, PR-B, MMP-2 and MMP-9 mRNA expression was performed using Real-time PCR in ectopic and eutopic tissues.

**Results:** Quantitative RT-PCR analyses of PR-A and PR-B mRNA revealed that there was significance different in mRNA expression level of PR-A/PR-B ratio between ectopic, eutopic and control tissues. We were able to demonstrate low expression levels of PR-B isoforms in ectopic tissues. Although, PR-A expression was higher in the same ectopic samples compared to control groups. This method permitted us to demonstrate overexpression of MMP-2 and significant high level of MMP-9 expression in ectopic tissues compared to control groups, as well.

**Conclusion:** Our data suggest that low expression levels of PR-B and overexpression of PR-A can alter PR-A/PR-B ratio in endometriotic ectopic tissues. Imbalance ratio of PRs in endometriotic tissue may be able to consequence MMP-2 and MMP-9 overexpression which can be important in pathogenesis and treatment of disease.

**Keywords:** Endometriosis, Matrix Metalloproteinases, Progesterone Receptor- A and -B, PR-A/PR-B Ratio

**Abstracts of  
Royan International Twin Congress**

**10<sup>th</sup> Royan Nursing and Midwifery Seminar  
2-4 September 2015**



**Royan Institute**

**Reproductive Biomedicine Research Center**

**Tehran, Islamic Republic of Iran**

## Invited Speakers

### **I<sub>nm</sub>-1: Development of Quality Indicators for Infertility Care**

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As assisted reproductive technology is advancing, it should arrange all principles and indexes to improve its quality of functions to provide optimal services to infertile patients. There are few infertility care-specific quality indicators. The indicators were developed by the existing quality dimensions. Safety, effectiveness, and patient centeredness are the most important quality dimensions. One study covered six dimensions, including efficacy, timeliness, and equity in addition to three important dimensions mentioned. They assessed six dimensions by both professionals and infertile patients using a three-round iterative Delphi survey. Participants developed 24 indicators for all six dimensions. Another study examined only patient centeredness using the Delphi method and evaluated and added indicators from patients' perspectives rather than professionals, who did not adequately evaluate the indicators. In all studies, the existing dimensions and indicators are only applicable in infertility clinics. There is no guideline based indicators for monitoring and evaluating all departments of infertility care centers, such as laboratory, etc. There is also no enough effort to provide adequate criteria in Iran. Hence, we used all dimensions and indicators existed during recent years to assemble a primary questionnaire. We send the questionnaire to all professionals in this field to make a comprehensive standard questionnaire using three round Delphi infertility centers. It seems there are a lot of different opinions in whole content required deep analyses with nominal group of specialists to prepare for the final widespread implementation. It could help all quality managers to monitor their performance as well as accurate possible complications to evaluate effects.

### **I<sub>nm</sub>-2: Assessment of Relaxation Effect on Anxiety in Infertile Women Undergoing Assisted Reproduction Techniques during Ovulation Induction**

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**Background:** Infertility and diagnostic and therapeutic procedures of which during assisted reproduction technologies (ART) make discomfort and anxiety for infertile couples. Several studies have demonstrated that infertility treatment procedures, particularly during ovulation induction period, lead to high levels of distress and anxiety in infertile women. The aim of this study was to evaluate the effect of relaxation on anxiety levels of infertile women undergoing IVF/ICSI treatment during ovulation induction period.

**Materials and methods:** This was a randomized controlled trial. One hundred infertile women undergoing IVF/ICSI were recruited in this study and were randomly assigned into an intervention (N=50) or a control (N=50) group according to simple sampling method. Data collection tools contained a demographic (designed by researcher) and "Spielberger State Anxiety Inventory" standard valid and reliable questionnaire. All samples were evaluated at the beginning of the study (first day of ovulation induction) and again at the oocyte retrieval day at the operating room just before the anesthesia. Relaxation training was done for intervention group and they were asked to do exercises 10-20 minutes daily until operation. Data was analyzed via SPSS18 software.

**Results:** There was no significant difference between two groups in demographic features. Although the average anxiety level of the intervention group decreased from 43.73 at the first day of ovulation induction to 42.69 at the oocyte retrieval day, but there was no significant statistical difference. Furthermore, significant difference was not seen in the control group and between two groups ( $P>0/05$ ).

**Conclusion:** Relaxation leads to reduction of anxiety levels among infertile women undergoing IVF/ICSI treatment during ovulation induction period. Despite of the insignificant difference caused in anxiety levels, relaxation could be clinically important due to patient's satisfaction of doing relaxation exercises.

**Keywords:** Anxiety, Infertility, Assisted Reproduction Technologies, Relaxation

### **I<sub>nm</sub>-3: Patient Management in Ultrasonography Department**

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**Background:** Ultrasound has a pivotal role in imaging modality in the study of the female pelvis, and provides fundamental information in detecting uterine, ovarian, or adnexal origin, but to reach to the best results we need to use suitable procedure.

The aim of this article was to describe management of infertile patient in sonography department during infertility treatment cycles.

**Materials and Methods:** A narrative review was performed within articles published at "PubMed", "Elsevier", "SID" and original text books to reach the aim.

**Results:** Every patient in the process of infertility treatment needs to use sonography to roll out disorders for deciding about the best treatment method and to manage the correct scan for the right patient.

**Usage of Sonography in Treatment Procedure:**

**Base sonography** is used for examination of the:

- Overall evaluation of the pelvis and determining any pathologic malformations in uterus such as polyps, fibromas.
- Ovarians for inspection of ovarian cysts and polycystic ovary syndrome (PCOS).

**Pre-treatment sonography** is applied for checking the result of operative surgeries - like Hysteroscopy, Laparoscopy and Myomectomy.

**Monitoring sonography** is used in every stages of treatment cycle:

- Ovulation stimulation period, in order to investigate ovarians
- In picking up the eggs
- Measurement of endometrial thickness in response to drugs.

**Post-Cycles assisted reproductive techniques (ART)** is done for:

- Confirmation of pregnancy (gestational sac, fetal heart)
- Pregnancy complications such as ectopic pregnancy (EP) and mole
- Side effects of treatment cycles such as ovarian hyper stimulation syndrome (OHSS)

**Conclusion:** Ultrasound has a key role in diagnosis and treatment protocols. Primary aim of the managers is to achieve a discipline that defines how and when and for whom to use this device in best way..

**Keywords:** Patient, Management , Sonography, Infertility

**I<sub>nm</sub>-4: A Decade of Cord Blood Banking in Iran**

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The research made on stem cells through the last decade and following that the great achievements made applying stem cells in treating vast number of diseases have turned the issue of Cord Blood collecting and saving (cryopreserving) to

a critical one. A prompt demand and access to stem cells and applying them in treating a wide range of terminal diseases have led to an increasing interest and attention toward establishing Cord Blood Stem Cells Banks. Holding up such banks will facilitate access to these cells and saving more lives as its consequence.

Since the first cord blood transplantation, performed by Prof. Elian Gluckman in 1988, more than 30,000 stem cells transplantations have been performed all around the world which were mainly concerned with blood diseases.

Cord Blood Banks, basically, may appear in two forms: public, and private.

1. Public Banks collect donated cord blood for research or for use by anyone who may need it. There is usually no charge associated with this service. After birth, blood is collected, anonymously marked, and sent to a public bank to potentially save the life of another child one day.

If you choose this option and your child or a family member later develops a disease that requires a stem cell transplant for treatment, you won't be able to obtain the donation you made to the bank.

2. Private Banks store cord blood for personal use by the family. There is a fee associated with this service. People who have a family history of disease that can be treated with stem cell transplants sometimes consider this option.

In our country, Cord Blood Banks, both public and private, came into existence from 1380s (2000s). There have been about 10,000 samples cryopreserved in public banks including Shariati Hospital, Iran Blood Transfusion Organization and Royan Research Institute. While the number of samples cryopreserved in Royan private cord blood bank is about 54,000.

The cord blood samples cryopreserved in public banks include mostly the ones collected from Tehran, whereas the cord blood samples cryopreserved in private bank come from more than 23 provinces. So far, more than 40 cord blood samples have been released from both public and private banks to be applied in hematopoietic stem cells transplantations.

## Oral Presentations

### **O<sub>nm</sub>-1: Maternal Outcomes after *In Vitro* Fertilization**

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**Background:** This study aimed to investigate maternal outcomes of pregnancy after *in vitro* fertilization (IVF) in primigravida women admitted to 3 different hospitals in Tehran.

**Materials and Methods:** This study is a retrospective cross sectional analytical study to evaluate and compare the maternal outcomes (preeclampsia, multiple pregnancy, diabetes, pregnancy, delivery mode, preterm delivery) among 620 pregnant women in three hospitals: Shahid Akbar Abadi (310 samples), Arash (155 samples) and Baharloo (155 samples). Chi-square, Fisher's exact test, t student and Mann – Whitney was used.

**Results:** The mean and standard deviation of age was  $25.3 \pm 4.56$  and  $29.8 \pm 5.31$  in spontaneous pregnancy and IVF groups respectively. There was significant differences between two groups regarding to preeclampsia ( $P=0.003$ ), gestational diabetes ( $P=0.012$ ), multiple pregnancies ( $P<0.0001$ ), Cesarean delivery ( $P<0.0001$ ) and preterm delivery ( $P<0.0001$ ). After adjustment of confounding variable (age) preeclampsia and gestational diabetes showed significant difference between two groups.

**Conclusion:** Since pregnancies after IVF showed some negative maternal outcomes, special attention is necessary for these women in their pregnancy.

**Keywords:** Maternal Outcomes, Pregnancy, Infertility, *In vitro* Fertilization

### **O<sub>nm</sub>-2: Evaluation of The Quality of Life in Iranian Women with Infertility: A Case-Control Study**

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**Background:** The aim of this study was to evaluate the hypothesis that infertility may result in a decrease in quality of

life (QOL).

**Materials and Methods:** In this case control study that carried out on 150 women with infertility and 150 women undergone TL as the control group. The participants were asked to fill out the Short Form Health Survey (SF-12) questionnaire, a generic instrument to measure health-related QOL (HRQOL). SF-12 consists of 12 items in the physical and mental domains. The analysis was performed by using SPSS 20.

**Results:** The mean total score of SF-12 was significantly lower in infertile couples compared to non-infertile couples ( $65.59 \pm 9.60$  vs.  $69.98 \pm 10.80$ ;  $P<0.0001$ ). The physical and mental components (PCS-12 and MCS-12) mean scores were significantly lower in the infertile couples as compared to the non- infertile couples (PCS-12:  $61.14 \pm 11.57$  vs.  $72.97 \pm 13.30$ ; MCS-12:  $66.84 \pm 12.82$  vs.  $63.00 \pm 12.95$ , respectively), ( $P<0.0001$ ).

**Conclusion:** Our findings revealed the adverse effects of infertility on the QOL of women. It is recommended that the awareness and knowledge of healthcare professionals regarding the QOL in women with infertility should be increased.

**Keywords:** Case - Control Studies, Tubal Ligation, Infertility, Quality of Life

### **O<sub>nm</sub>-3: Ginseng and Male Reproductive Function**

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**Background:** Ginseng is often referred to as the King of all herbs, and is found to be a promising agent to improve general well-being. Ginseng has also been reputed as an aphrodisiac, a disused to treat sexual dysfunction as well as to enhance sexual behavior.

**Materials and Methods:** In this trial, thirty three adult male rats were divided into three groups. Experimental groups received ginseng orally for 60 days in two different sub lethal doses; 100 mg/kg as high dose and 50 mg/kg as low dose, whereas the control group received distilled water. The rats were sacrificed on the 60th day, Blood was centrifuged and serum was stored for determined testosterone. The testes and seminal vesicles were collected and weighed. The testes excised, and processed for microscopic examination. The study was approved by the Experimental Ethics Committee on Animals Use of Shahrekord University of Medical Sciences.

**Results:** The administration of the ginseng result did not show any significant difference in the weight of the seminal vesicle, liver and kidney of the treated groups relative to the control ( $P \geq 0.05$ ). On the other hand, the results showed a significant decrease in the body weight of both the low and high dose-receiving groups in comparison to the control group. The extract of this plant caused a decrease of the following in



the two experimental groups, compared to the control group: sperm count, motility and normal morphology, pregnancy rate and diameter of seminiferous tubules. Also, distortion of morphology of the seminiferous tubules and arrest in spermatogenesis were observed in the experimental groups.

**Conclusion:** From the present study, we can conclude that ginseng acts as an anti-fertility agent.

**Keywords:** Ginseng, Seminiferous Tubule, Sertoli Cells, Testosterone

#### **O<sub>nm</sub>-4: Body Mass Index and Sexual Function and Infertility in Women: A Cross-Sectional Survey**

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**Background:** Infertility as the bitterest life experience can affect sexual function. Also, infertility is a reproductive health problem. Many studies showed that agitation, stress, depression, marital dissatisfaction and sexual dysfunction are psychological outcomes resulted from infertility. There are many factors influencing the female sexual function, one of them is body mass index.

The present study is focusing on body mass index and sexual function. The objective of this study was to assess prevalence female sexual dysfunction and relationship between sexual function with body mass index among Iranian infertile women who had attended the infertility clinic.

**Materials and Methods:** A cross-sectional study was conducted between April 2012 and December 2012 using 502 infertile women who had attended the infertility clinic, Iran. Infertile cases were grouped into three according to body mass index: 20-24.9 (Group I), 25-29.9 (Group II), and >29.9 and longer (Group III). The Female Sexual Function Index (FSFI) questionnaire was used for sexual function assessment of sexual problems on six different domains, including sexual desire, arousal, lubrication, orgasm, satisfaction and pain. The data were analyzed by Descriptive Statistic, ANOVA test and Student's T test used SPSS11.5 software program.

**Results:** A total of 502 infertile women were surveyed. The mean age of women  $30.95 \pm 6.80$  years were. Result showed that 430(87.1%) of women had sexual dysfunction. The sexual dysfunction among infertile women was rated as 23.30%, 31.47%, and 45.23% in the Group I, Group II, and Group III, respectively. The mean FSFI scores of the BMI groups showed normal weight women were at  $21.65 \pm 1.70$ , overweight at  $18.08 \pm 1.52$ , and obese women  $12.21 \pm 3.62$ . The assessment of body mass index showed all scores and total FSFI scores were different significantly between all three groups ( $P < 0.05$ )

**Conclusion:** Sexual dysfunction in infertile women was very high, which might be due to the lack of knowledge about marital issues, lack of training in the society. If body mass index is too high, it can have a great affect on fertility. Overweight and

obesity based on body mass index among infertile women had a negative effect on woman's sexual function.

**Keywords:** Infertility, Female Sexual Function Index, Body Mass Index, Obesity

#### **O<sub>nm</sub>-5: Domestic Violence in Iranian Infertile Women**

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**Background:** Millions of men and women suffer from infertility worldwide. In many cultures, infertile women are at risk of social and emotional problems. Infertility may affect the public health in many countries. Domestic violence is the intentional use of physical force, power or threat against oneself, another person or another group or community which leads to injury, death, mental harm, lack of development or deprivation. This study aimed to assess the prevalence of domestic violence against infertile women who referred to the infertility centers of Tehran, Iran in 2011.

**Materials and Methods:** This was a cross-sectional descriptive study conducted on 400 infertile women who were selected through convenient sampling method. The questionnaire used in this study included two sections: a demographic section with questions about demographic characteristics of the infertile women and their husbands; and the domestic violence questionnaire with questions about physical, emotional and sexual violence. Data were analyzed by SPSS 16; and descriptive statistics, Spearman's test, t test, one-way analysis of variance (ANOVA) and logistic regression were used for data analysis.

**Results:** Four hundred women with the average age of  $30.50 \pm 6.16$  years participated in the study; of whom, 34.7% experienced domestic violence physical violence (5.3%), emotional violence (74.3%) and sexual violence (47.3%). Domestic violence was significantly associated with unwanted marriage, number of IVFs, drug abuse, emotional status of the women, smoking and addiction or drug abuse of the spouse, mental and physical diseases of the husband ( $P < 0.05$ ).

**Conclusion:** Many of the current problems in this society, particularly in families are due to the transition of the society from a traditional model to a modern one. The majority of the infertile women experience violence in Iran. Domestic violence against infertile women is a problem that should not be ignored. Clinicians should identify abused women. Providing counseling services to women in infertility treatment centers is suggested to prevent domestic violence against infertile women.

**Keywords:** Infertile Women, Domestic Violence, Iran, Women

#### **O<sub>nm</sub>-6: Acceptance and Commitment Therapy with Hypnotic Effect in Positive Adaptation And Reduce Anxiety in Infertile Women**

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**Background:** Many researchers believe that it is one of the most stressful events in patients' lives. A considerable number of studies have consistently reported that infertility associates with psycho- logical distress and losses of hope, esteem and social roles by repeatedly attempting to have a baby but failing to achieve it. The measure of adaptation as one of the most important variables of personal and interpersonal adaptation and strong predictors indicates emotional maladjustment in infertile people. Therefore, the purpose of this article is to address the strategic treatment for solving conflicts and maintenance of peace of couple's life under this treatment in infertile people.

**Materials and Methods:** Among the 200 referred to a psychiatric and Gynecological clinic in 2014 in Babol with the cognitive problem and anxiety disorder toward pregnancy and delivery, 45 patients that were compatible with research conditions (Age 23-35, At least Associate Degree, without experience pregnancy, without special physical and mental disorders), 14 patients were divided in two equal groups (n=7) and pre-test and post-test were performed by the Millon Inventory (to check the level of anxiety, depression and quality of thinking), infertile women inventory (Social, parental, inclusive, sexual and relationship anxiety) three individual sessions, 10 sessions of group therapy with the content of acceptance and commitment, learning and Self-hypnosis (with bringing up the stories for each individual), while the control group only received individual counseling.

**Results:** The use of Self-hypnosis by designing the stories based on childhood with the acceptance and commitment therapy (in group therapy) resulted in reduction (41%) of anxiety and also has been effective on resilience compared to the control group. In addition, individual clinical interview after three and six months of treatment was performed, The experimental group had more adaptation to solve conflicts in life.

**Conclusion:** According to the results of this study, having a better understanding of content of each individual story with acceptance and commitment therapy treatment leads to more consciously and durable acceptance in the treatment of certain disorders, such as anxiety towards pregnancy and delivery. Although this research needs to more expansion, but it can help to integration of specific hypnotic texts for each person in the scene of acceptance and commitment therapy and ultimately for more cognition and readiness of individuals to confront to deal with personal and social stress in solving the crisis situations and problems to be more adaptive and thus help to sexual and psychological health.

**Keywords:** Acceptance and Commitment Therapy (ACT), Individual Hypnotic Texts, Positive Adaptation, Anxiety Infertility

## Poster Presentations

### **P<sub>nm</sub>-1: Infertility in Obese Men**

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**Background:** The obesity pandemic has grown to concerning proportions in recent years, not only in the Western World, but in developing countries as well. The corresponding decrease in male fertility and fecundity may be explained in parallel to obesity. Obesity in men is associated with infertility in numerous studies.

**Materials and Methods:** In this review article, electronic searches were undertaken in PubMed and Google Scholar, up to date since 2014.

**Results:** The prime hormonal defect in obese men is hypotestosteronemia, which results in impaired spermatogenesis leading to poor fecundability. In massively obese individuals, reduced spermatogenesis associated with severe hypotestosteronemia may affect infertility. In addition to impaired semen quality, fertility among obese men may be affected by sexual dysfunction, endocrinopathy, aromatization activity, psychological and thermal effects, sleep apnea, leptin and minor toxins and possibly the inflammatory and obstructive elements of epididymitis pathology. The frequency of erectile dysfunction increases with increasing body mass index. Recently, genetic factors and markers for an obesity-related infertility have been discovered and may explain the difference between fertile obese and infertile obese men.

**Conclusion:** Studies have shown that most mechanisms accounting for reduced fertility potentials in overweight men are reversible. Treatment of obesity may improve androgen imbalance and erectile dysfunction, the major causes of infertility in obese men. Treatments are available for not only infertility related to obesity, but also as a treatment for the other comorbidities arising from obesity. Natural weight loss, as well as bariatric surgery are options for obese patients and have shown promising results in restoring fertility and normal hormonal profiles. Therapeutic interventions including aromatase inhibitors, exogenous testosterone replacement therapy and maintenance and regulation of adipose-derived hormones, particularly leptin, may also be able to restore fertility in obese male.

**Keywords:** Male Infertility, Obesity, Oligospermia

### **P<sub>nm</sub>-2: Sexual Function in Women from Infertile Couples and Women with Tubal Ligation**

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**Background:** The aim of this study was to compare sexual function between women of infertile couples and women who have undergone TL.

**Materials and Methods:** In this case control study in Royan Institute and in a health center in Gilan province (Iran), between 2013 and 2014. There were 150 infertile women (cases) and 150 women undergoing TL (controls). A questionnaire was completed for each patient. The participants' sexual function was evaluated and compared between two groups by using the Female Sexual Function Index (FSFI) questionnaire. Scored data were collected on six different domains: desire, arousal, lubrication, orgasm, satisfaction, and pain.

**Results:** The greatest positive correlation in the TL group was between orgasm and arousal (0.754), and in infertile group between desire and sexual satisfaction (0.773). Infertile women and fertile women who want to undergo surgical sterilization have low sexual function scores in all mean values.

**Conclusion:** Our findings indicated that adverse effects for the sexual life in women undergoing TL and infertile couples. Awareness and knowledge of healthcare professionals regarding the sexual dysfunction in women should be increased.

**Keywords:** Case-Control Studies, Tubal Ligation, Infertility, Sexual Dysfunction

### **P<sub>nm</sub>-3: The Impact of Life Quality in The Incidence of Endometriosis**

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Endometriosis is a gynecological benign tumor that is estrogen-dependent, in this kind of disease uterus tissue grows into the pelvic cavity of ectopic, and 20-30% of these patients are infertile. Clinical symptoms such as dysmenorrhea, dyspareunia and depression and in some cases arise in asymptomatic. Because the lack of ovulation and menstruation, the pain relief during the pregnancy. Several studies suggest that endometriosis reduce the quality of life, loss of ability to work, social relationships and sexual dysfunction in women. The most important sections that have been studied in endometriosis are psychological and social dysfunction, declining employment, frustration and depression, negative impact on the attitude of individual about himself. It effects on the mental health and personality of individual. The quality of Life, the impact of environmental pollutants, toxins, lifestyle, age and environmental factors such as stress, poor nutrition, inadequate sleeping are the most important indicators in disease management. Endometriosis is a multi-factorial disease that is caused by both genetic and environmental factors. It will occur more with the urban life style, and the risk of environmental pollution in large cities and incidence of mother's disease in girls

or one of the twins and also in close relatives. The proper nutritional supplements such as vitamin B-1 and B-9 and B-6, and fresh fruits and vegetables, exercise, use of Omega 3 fatty acids, vitamins E and selenium, emotional support of couple, learning the ways of reducing the anxiety and stress, as well as acceptance methods of assisted reproduction could have significant impacts on control disease.

**Keywords:** Endometriosis, Quality of life, Incidence

#### **P<sub>nm</sub>-4: Risk Factors Associated with Infertility**

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**Background:** Infertility is the inability to conceive children after one year of unprotected intercourse. The estimated prevalence of infertility in Canada is 11.5-15.7%. Fertility problems affect approximately one in seven couples in the United Kingdom (UK). Infertility occurs in conditions in which the pregnancies end with recurrent miscarriages (ASR) or birth of a child with multiple congenital anomalies.

**Materials and Methods:** This article is review article in which, articles about risk factors associated with infertility have been reviewed. Searching performed in some databases like PubMed, Scopus, Springer and Science Direct. Fifteen full text articles in English from 2010 until 2015 were found which their topic was similar to our topic. Keywords that have been used included: infertility, infertility risk factors.

**Results:** One of the causes of damage to both female and male reproductive systems was a delayed or untreated sexually transmitted infection (STI). The most common STIs that cause female and male infertility were Chlamydia and gonorrhea. Articles showed that The risk of infertility was increased with advanced age of the female partner. Reviewing articles revealed that secondary infertility was a common, preventable but neglected reproductive health problem in poor countries. Risk factors for secondary infertility were a history of no prenatal care during the last pregnancy, early age of first pregnancy, unwanted pregnancies and stillbirths. According to articles, infertility was often associated with unbalanced chromosome abnormality. According to researches, women with obesity, especially those with central adiposity, had difficulty in getting pregnant and had decreased success in infertility treatment. The association of obesity with polycystic ovary syndrome (PCOS) was a contributor to infertility rates in women with obesity. Articles showed that about %10 of infertile/subfertile women are diagnosed with diminished ovarian reserve (DOR). By searching we found that the weight loss resulting from bariatric surgery can be very beneficial to the overall health of the woman, but the reproductive process can be impaired.

**Conclusion:** According to researches, female infertility may present as anovulation, obstructed fallopian tubes, endometriosis or uterine abnormalities. Male factor infertility is characterized by diminished production of morphologically normal, motile sperm. Genetic abnormalities, hormonal imbalances

and congenital/infectious malformations of the reproductive tract are some of the common causes of male and female infertility. Lifestyle factors such as obesity, diet, smoking and alcohol use along with environmental chemical exposures have been increasingly examined as additional modifiers of fertility.

**Keywords:** Infertility, Infertility Risk Factors, Risk Factors

#### **P<sub>nm</sub>-5: Anti-Mullerian Hormone in The Assessment and follow up of Cancer in Young Females**

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**Background:** The impact of cancer therapy on the reproductive potential of patients is increasingly recognized because survival rates of patients have clearly improved in recent years. The study stressed the use of AMH may allow better prediction of chemotherapy-related risk to further fertility.

**Materials and Methods:** A narrative review was performed within articles published at PubMed, Elsevier, SID and original text books to reach the aim.

**Results:** AMH is a dimeric glycoprotein, a member of the transforming growth factor (TGF)- $\beta$  super-family AMH is produced by the granulosa cells in the preantral and antral follicles. AMH is the most sensitive hormonal parameter in changes in ovarian reserve when compared with FSH of inhibin B. Anderson et al. explored forty-two women received chemotherapy for breast cancer, continuing menses 4–5 years after diagnosis closely reflected ovarian activity as assessed by a range of serum markers, including estradiol, inhibin B, FSH, and AMH. Pretreatment serum AMH, FSH, AFC, and age predicated late ovarian activity by univariate analysis. However, only AMH was predictive in a multivariate logistic regression; 0.71 ng/ml gave a peak likelihood ratio of 7.0 with 54% sensitivity and 92% specificity. In conclusion, measurement of AMH at cancer diagnosis predicts long-term ovarian function after chemotherapy. Lie Fong et al. Assessment of ovarian reserve in adult childhood cancer survivors using anti-mullerian hormone was studied in a total cohort of 185 survivors compared with 42 control subjects. The median follow-up time was 18.1 years. However, AMH levels were lower than the 10th percentile of normal values in 27% of our survivors. The relationship between serum AMH and the number of small growing and indeed primordial follicles has made it a prime potential tool for the investigation of gonadotoxicity of cancer therapy and loss of the ovarian reserve from ovarian surgery.

**Conclusion:** AMH can be used to identify subgroups of childhood cancer survivors at risk for decreased fertility or premature ovarian failure.

**Keywords:** AMH, Cancer, Fertility

### **P<sub>nm</sub>-6: The Primary Dysmenorrhea and Complementary Medicine in Iran: A Systematic Review**

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**Background:** Many women experience primary dysmenorrhea, and to get away from it, they resort to natural remedies and alternative medicine. Health care providers should help women to make a decision based on the evidence in relation with IRAN alternative treatments available and equipped to high quality information to reduce severity of pain during the menstrual cycle.

**Materials and Methods:** Electronic searching through Medline, Sid, Irandoc, Magiran, Journals of herbal drugs and sports sciences was performed. Moreover, through internet search engines (e.g. Google and Yahoo), randomized clinical trials of herbal and complementary medicine for dysmenorrhea in Iran were identified. The bibliographies of articles and students' theses were searched and evaluated. 24 clinical trial article alternatives that are associated with primary dysmenorrhea were studied.

**Results:** The data effectively means that exercise, acupressure, acupuncture, relaxation, vibration and heat, thyme, chamomile tea, Hypericum perforatum, platyloba, fennel, ginger, dill, celery, saffron, anise, lemon balm flower Maryam, valerian, mint extract, pure honey on pain of primary dysmenorrhea protection.

**Conclusion:** The data effectively means that exercise, acupressure, acupuncture, relaxation, vibration and heat, thyme, chamomile tea, Hypericum perforatum, platyloba, fennel, ginger, dill, celery, saffron, anise, lemon balm flower Maryam, valerian, mint extract, pure honey on pain of primary dysmenorrhea protection.

**Keywords:** Alternative Medicine, Complementary Medicine, Medicinal Plants, Primary Dysmenorrhea, Systematic Review

### **P<sub>nm</sub>-7: Sexual Function in Women with Cervical Cancer and A History of Pelvic Radiation Therapy**

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**Background:** Cervical cancer is the second most common cancer in women and is regarded as one of the leading causes

of cancer death in developing countries. In cervical cancer patients, both surgery and radiation therapy may bring about sexual dysfunction. Therefore, this study aimed to survey sexual dysfunction in women with cervical cancer and a history study of pelvic radiation therapy in Ghaem and Omid hospitals in Mashhad.

**Materials and Methods:** This study was carried out on 176 women with cervical cancer and pelvic radiation therapy referred to Ghaem and Omid hospitals in Mashhad who were selected using convenient sampling from 2010 to 2014. Research tools consisted of valid and reliable demographic and ROSEN Female Sexual Function Index (FSFI) questionnaires. Data analysis was carried out by SPSS software using descriptive statistics and correlation test.

**Results:** Women with cervical cancer and a history of pelvic radiation therapy had 30% sexual desire, 49% sexual arousal, 42% orgasm disorder and 35% problems in the lubrication. 40% of women complained of dyspareunia and 24% of women were dissatisfied from sexual function. There was a significant correlation between the overall sexual function and the Stage of cervical cancer (P= 0.001, r= - 0.25). There was a significant correlation between sexual desire (P= 0.04), Sexual arousal (P= 0.008), lubrication (P= 0.001), orgasm (P= 0.004), sexual satisfaction (P= 0.009) and dyspareunia (P= 0.000) with the stage of cervical cancer.

**Conclusion:** Sexual disorders in women with cervical cancer and a history of pelvic radiation therapy and correlation between sexual function with the Staging of cervical cancer reflects the fact that treatment, education and counseling programs would be helpful for these women.

**Keywords:** Cervical Cancer, Sexual Function, Pelvic Radiation

### **P<sub>nm</sub>-8: Effect of Nutrition in Women Fertility in Iranian Traditional Medicine**

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**Background:** Infertility, typically defined as the inability of couples to become pregnant after 12 months of regular unprotected sexual intercourse. Female infertility accounts for 45% of all infertilities among couples. In Iranian Traditional Medicine (ITM) there are variable recommendation for the prevention and treatment of female infertility and one of the most important is nutrition.

The purpose of this study is to review the nutritional effect on female infertility in view of Iranian Traditional Medicine.

**Materials and Methods:** In this study, specific data related to the subject among all referral ITM texts such as, Al-Qanun Fi Al-Tibb (The Canon of Medicine) by Avicenna, Kholaseh Al-Hekmat, Zakhireh Kharazmshahi, Exir Azam

were evaluated.

**Results:** In ITM, the etiology of infertility in addition to the reproductive system disorders can be due to failure of other organs such as the brain, liver, stomach and heart and also obesity and thinness. The present review showed that, ITM has two major aspects in female infertility regimen: recommended foods and foods to be avoided. These regimens may be modify distemperaments or strengthen reproductive system and other related organs. For example honey, figs, peas and deal are suitable for treatment of cold uterus distemperaments and these patients should be avoided some foods like yogurt, watermelon and cold water. On the other hand, fresh shrimp can be used for improving uterine strengthen.

**Conclusion:** Nutrition in ITM could be used for prevention and treatment of female patients who suffer from infertility in medical centers.

**Keywords:** Infertility, Iranian Traditional Medicine, Nutrition

### **P<sub>nm</sub>-9: Association between Air Pollution and Reproductive System Functions**

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**Background:** The highly sensitive period of preimplantation development represents that embryo is very vulnerable to exogenous agents. Many of the developmental abnormalities may arise from the inevitable maternal periconceptional exposures to toxic substances in the environment which may threaten reproductive capability and pregnancy outcomes. In this article, an overview of the association between air pollution and reproductive, fetal, and neonatal health outcomes is given.

**Materials and Methods:** A literature search was conducted through PubMed to identify air pollution and fertility related studies.

**Results:** Regardless of the consideration of different types of study design, the statistical methods used and residual uncontrolled confounding variables, critical appraisal of reported studies reveals a causal relationship between environmental air pollution exposure and reproductive functions. Among the components of air pollution, particulate matter (PM) appears to be the most harmful to human health. PM include a range of tiny particles, including acids, organic chemicals, metals, and soil or dust particles. Particles come in a wide range of sizes in which smaller size fractions (PM<10) have the highest toxicity.

A series of studies showed that exposure to ambient levels of air pollutants, specifically PM were associated with fetal development. Prematurity, low birth weight, neonatal and post-neonatal mortality were also reported to be associated with air pollutant exposure in some studies. It has been suggested that

third-trimester exposure is most associated with an increased risk of neonatal abnormality. Air pollution may affect fetal development and birth outcomes through multiple pathways including systemic alterations in systematic oxidative stress, pulmonary and placental inflammation, maternal blood coagulation factors, hematocrit, blood viscosity and endothelial dysfunction. Altered pulmonary and cardiovascular functions may also play a fundamental role in morbidity and mortality during the postnatal period. In addition, air pollution can negatively affect male fertility by decrements in semen measures including proportionately fewer motile sperm, less sperm with normal morphology and more sperm with abnormal chromatin. Alternatively, the increased risk of early pregnancy loss has been observed in women exposed to air pollution which could be related to maternal changes in the uterine vascular environment prior to pregnancy.

**Conclusion:** Exposure to air pollution could be associated with lower fertility rates in humans and adverse birth outcomes including fetal growth, prematurity, neonatal mortality and miscarriage.

**Keywords:** Air Pollution, Fertility, Birth Outcomes

### **P<sub>nm</sub>-10: Towards Integrating The Traditional Medicine with Modern Medical Approaches in Infertility**

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**Background:** ART is costly for governments and couples seeking treatment. In addition to any other costs that may arise during treatment, such as medication, consultations and extra procedures, women undergoing IVF experience physical and emotional stress. Furthermore, it is not always possible to get a favorable outcome. Despite all the remarkable scientific advances in modern medicine, traditional medicine (TM) has always been used alongside official medical practices. TM refers to a broad set of health care practices, products and therapies incorporate acupuncture, herbal medicines, special diets, meditative exercises, etc. The main concept of integrative medicine (IM) is that by the co-utilization of TM with mainstream medicine synergistic therapeutic effects can be achieved. This article provides an overview of the prospects of integrating the alternative medical services with modern services in infertility.

**Materials and Methods:** A literature search was conducted through PubMed.

**Results:** Complementary and alternative medicine plays important roles in women's health issue. A wide range of TM is used by subfertile couples. Their use depends upon the cause of infertility and the area studied. The reasons for the inclusion of TM in infertility are economic considerations, traditional beliefs, unsuccessful treatment, benefit of using less invasive therapy, etc. There is growing evidence that acupuncture as adjunct treatment with IVF can increase the success.

Traditional Chinese Medicine (TCM) addresses some support needs in infertility. In one study on TCM, women evaluated it positively and suggested a possibly broader future role for TCM in improving fertility. This group of women was satisfied with the personalized approach and continuity of care compared to their experience of fragmented care and looked for a better and earlier fertility education about TCM to enable well informed decision making about their treatment.

**Conclusion:** Since, there are considerable evidences about the effectiveness of some TM therapies among infertile patients, it seems that we need to extend IM as a possible approach for infertility management. In this regard, further clinical studies need to be conducted.

**Keywords:** Traditional Medicine, Complementary Therapies, Infertility

### **P<sub>nm</sub>-11: Male Infertility and Its Effect on Male Sexuality**

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**Background:** Male infertility may occur as a consequence of psychological or physical problems or a combination thereof. On learning of their infertility, patients usually go through an initial emotional crisis. The male response thereafter varies depending on the etiology of the infertility. Male factor infertility threatens the traditional male/father role and results in a feeling of personal and sexual inadequacy. This may resolve itself in extramarital affairs or by the sufferer throwing himself into his work.

**Materials and Methods:** Feelings about fertility and sexual adequacy are interconnected for many men especially through male factor infertility. Couples with long term infertility, who have faced much treatment failure, report higher levels of depression, low satisfaction with their sex lives, and low levels of well being. The stigma of male factor infertility described earlier, has huge effects on the man. The problems infertile men have with sexual inadequacy stem from social ridicule and resulting low self-esteem. It is estimated that 40% of infertile individuals experience significant emotional distress with possible long-term implications. The diagnosis of infertility causes many males to question their masculinity. Male factor infertility is frequently associated with high levels of stigma. Many people assume that infertile men cannot perform sexually. This stigma adds to the heightened insecurities in infertile men.

**Results:** The results of this research showed that infertility treatments can be stressful, intrusive, and emotionally demanding. As part of your infertility treatments, men and their partner may need to have sex at certain times, even if they do not feel like it. Some of the medications that they need to take as part of your treatment may make it harder to have sex.

**Conclusion:** All of these things can be bad for a couple's sexual relationship and may lead to sexual dysfunction. Be-

cause of the stress caused by the need for a male to "ejaculate on demand," some men may have erectile dysfunction. Both partners may have less sexual desire.

**Keywords:** Male, Infertility, Sexuality

### **P<sub>nm</sub>-12: A Survey on The Relation between Patient's Age, Baseline of FSH Serum, Ovarian Volume and Number of Antral Follicles in Anticipating The Response of Ovary to Ovulation Stimulation**

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**Background:** Ovulation is one of the most important stages in treatment of fertility. Considering the importance of this stage and the incidence of treatment failures, much effort has been made to anticipate in some way the responses of ovary to different protocols of ovulation stimulation. Some of the authentic procedure in determination of ovary response to ovulation stimulation methods includes the age of patient, baseline of FSH, ovarian volume and number of antral follicles at the beginning of ovulation stimulation cycle. This paper is a review with the goal of determining the relation between these four parameters in anticipating the response of ovary to ovulation stimulation.

**Materials and Methods:** Searching was conducted in electronic information resources including PubMed, Google Scoler, Science Direct, SID, using english keywords including patient age, FSH, Antral follicels, ovarian volume, in 1996 to 2014 and 8 papers were related.

**Results:** All studies reported a directed and significant relation between the patient's age and baseline of FSH serum. Three studies reported a direct relation in the stimulation of ovulation by HMG in IVF cycles between ovarian volume and number of antral follicels at the beginning of the cycle. This relation was not present in other treatment protocols. Contradictory result had been reported from antral relation.

**Conclusion:** The age of patient and baseline of FSH serum in third menstruation day in all protocols of ovulation stimulation, vaginal ultrasound in the evaluation of ovarian volume and number of antral follicels before and after gonadotropins in HMG protocols can anticipate the response of patient to treatment.

**Keywords:** Patient Age, FSH, Ovarian Volume

### **P<sub>nm</sub>-13: The Effect of Relaxation on The Compatibility of Infertile Women** Kheirkhah M<sup>1</sup>, Jenani P<sup>2\*</sup>

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**Background:** The infertility prevalence has increased about 5% in the last decade and has considered an epidemic in the community. Coping is defined as the ability of the individual to accept and deal with the grief and losses associated with their infertility. The goal of psychological interventions is to help the individual to cope with the situation. This study has investigated the relaxation effects on infertility adjustment.

**Materials and Methods:** This study was a randomized control trial. 92 infertile women with inclusion criteria who were referred to infertility clinic of Tabriz Al-Zahra Hospital were simple random sampling randomly divided into two intervention and control groups. Before intervention, two groups were similar in adjustment with infertility. The "infertility psychological adjustment scale" was used and relaxation group was divided into groups of 5 people that trained progressive muscle relaxation in a 2-hour session. At the end, the training CD was given to the participants and they were asked to perform the technique about 15 minutes every day for 4 weeks and record them. The control group received the routine care of the center. The infertility psychological adjustment questionnaire was given to participants after 4 weeks again. For data analyzing statistical tests such as Paired T, Independent T, Fisher Exact and Chi-Square were used in SPSS software, version 16.

**Results:** Comparison of mean scores of infertility adjustment showed significant reduction in relaxation group in comparison with control group ( $24.28 \pm 0.49$ ) ( $P=0.000$ ). The mean scores of infertility adjustment scale before and after intervention had a statistically significant reduction in relaxation group ( $12.87 \pm 2.74$ ) ( $P=0.000$ ). The mean scores of infertility adjustment scale before and after intervention had a statistically significant increase in control group ( $5.65 \pm 2.33$ ) ( $P=0.000$ ).

**Conclusion:** According to the results of the study, relaxation affects infertility adjustment and it is suggested to consider relaxation as a cost-effective solution beside physical treatments for infertile women.

**Keywords:** Relaxation, Infertility, Adaptation

### **P<sub>nm</sub>-14: The Role of Education and Counseling in Infertility**

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**Background:** The demand for infertility services are increasing all over the world. In the United States, approximately 1.3 million couples receive medical advice or treatment for infertility yearly. It is estimated that approximately one in ten couples have either primary or secondary infertility. Infertility is a noticeable medical problem in Iran, and about a quarter of Iranian couples experience primary infertility at some point in their lives. Since having children is a basic social value in Iran, infertility has an adverse effect on the health of the couple and affects their well-being that can reduce these problems

by education and teaching information that the couples need. The demand for infertility services are increasing all over the world. In the United States, approximately 1.3 million couples receive medical advice or treatment for infertility yearly. It is estimated that approximately one in ten couples have either primary or secondary infertility. Infertility is a noticeable medical problem in Iran, and about a quarter of Iranian couples experience primary infertility at some point in their lives. Since having children is a basic social value in Iran, infertility has an adverse effect on the health of the couple and affects their well-being that can reduce these problems by education and teaching information that the couples need.

**Materials and Methods:** This review article has been extracted from 22 articles that have been indexed in most valid scientific sites that has published from year 2009 to 2015.

**Results:** Inability to conceive children is experienced as a stressful situation by individuals and couples all around the world. When conception does not occur easily, couples can become confused and angry. The consequences of infertility are manifold and can include societal repercussions and personal suffering. The emotional responses that couples experience, which include distress, loss of control, stigmatization, and a disruption in the developmental trajectory of adulthood. It has long been recognized that there is a relationship between emotional stress and some forms of infertility. The biochemical finding of higher mean prolactin levels in the female patients. The costs of infertility treatments can also contribute to the stress of couples. The feeling caused by infertility, in most of the cases leads to disintegration of marital relationship. One of the issues that is highly influenced by infertility is sexual relationship. In over 40% of infertile women, sexual dysfunction is observed. Sexual relationship is less used among infertile couples.

Several psychological interventions appear to increase pregnancy rates. Communication skills can have a significant impact on the sexual function of infertile couples, and communication skills training can improve the quality of marital life skills through the counseling sessions conducted for infertile couples.

**Conclusion:** The communication improvement educational program increases the quality of marital life. Communication skills training teaches the appropriate form of relationship to the couples and improves their interactions. By teaching the techniques of efficient listening, speaking and giving non-verbal efficient responses and emotional skills to the couples, their perception, recognition, and empathy, as well as their quality of relationship are improved.

Teaching the steps of problem-solving process to the couples, in addition to helping them solve their own problems not only results in improvement of their natural relationship, their cooperation and empathy, and better recognition of their needs and desires, but also increases.

**Keywords:** Education, Counseling, Infertility

### **P<sub>nm</sub>-15: Men Role in Reproductive Health**

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**Background:** Male involvement in reproductive issues cannot be denied. However, throughout history, it is paid little attention to the role of men in reproductive health programs. At the International Conference on Population and Development in 1994, the importance of men in reproductive health programs was recognized as one of the most important aspects. Since then, the involvement of men in reproductive health in order to achieve the key objectives of the Millennium Development including the reduction of maternal mortality was considered an important issue. Male involvement in family planning and women's empowerment is very important and leads to better results in reproductive health, including acceptance and continuation of contraception and safe sexual practice. However, although benefits of men involvement are recognized, to create a situation for the participation of men in maternal health, will continue to be a challenge. This review study aimed to investigate the involvement of men in reproductive health, including factors affecting the outcomes and impact of the partnership was designed.

**Materials and Methods:** In the present case study, articles published in the last five years in the databases Magiran, Scopus, PubMed, SID; in English and Persian, were searched using these keywords: reproductive health, male involvement. A total of 20 papers were analyzed.

**Results:** The findings of this study showed that including the factors that limit the involvement of men in reproductive health issues are: traditional gender roles, lack of information, the desire to have more children, refusal of spouse or partner, fear of side effects, non-religious, lack of knowledge about contraception, belief and attitude that this is only an issue for women, limited selection available for male contraceptive, fear of vasectomy, concern that usage of contraceptives by women will lead to extramarital sexual relations, lack of time and limited awareness of the unique role of men in reproductive health. Among the reasons for the participation of women and men expectations were the love and support. Facilitating factors for the participation of men were: the second pregnancy, the willingness and enthusiasm of men, teaching men and increased age in men and their experience. Consequences that will follow the involvement of men, to better understand the events of pregnancy and childbirth and reducing labor pain

**Conclusion:** To promote the participation of men in care and prenatal care review into "family-friendly" services and educate fathers on the emotional support of her mother, with specific content and warning signs nutrition during this period and the method of face to face counseling with mothers to help their media education and counseling the doctor is required. Increased participation by parents to basic education and adolescence specifically courses before marriage and in detail, and proportionate to pregnancy and childbirth during pregnancy in attracting and employing them should be promoted.

**Keywords:** Reproductive Health, Male Involvement, Men Roles

### **P<sub>nm</sub>-16: The Effect of Pregnancy above 40 Years on The Fetal Health**

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**Background:** Women's Health is trying to Priority health programs. A natural event of a woman's life is pregnancy. Highly regard to maternal health during pregnancy, one of the most basic ways maintains health of mother and fetus. Age factors are affecting the outcome of pregnancy. The aim of this study was evaluation of the complications of pregnant women above 40 years referred to Jahrom's Pymanyh hospital.

**Materials and Methods:** This was a descriptive study. Patient's information was obtained from their files. Variables assessed were included: age, parity, gestational age, abortion, disease during pregnancy, birth weight, neonatal problems and congenital anomaly. Data was analyzed by SPSS software and using descriptive statistics (frequency, mean, standard deviation).

**Results:** Medical file of 2049 pregnant women were studied. 9.56% were above 35 years. 69.4% rural, 30.1% were urban patients. The average age of women was  $42.07 \pm 2.01$ . 25.5% of samples were pregnant for the fifth times (gravid 5). Pregnancy complications include: 29.6% abortion, 3.1% bleeding in pregnancy, 15.3% post term delivery, 9.2% had preterm labor. The frequency of different types of diseases in the samples includes: anemia 14.8%, hypertension 7.7%, diabetes mellitus 6.7%, gastrointestinal problems 3.1%, pyelonephritis 1%, Previous history of disease 8.2%. Fetal effects include: 8.2% low- birth weight (under 2500), 7.7% (over 4000 g), 7.7% neonatal genital problems, 0/5 % skeletal disorders.

**Conclusion:** According to the findings of this study, it was found that pregnancy at inappropriate age and suffering from some diseases, especially hypertension, diabetes, anemia were the most important health problems of pregnant women In this city. Health status during pregnancy depends on before pregnancy of health care. Prior pregnancy care must be basic prenatal care as logically.

**Keywords:** High Risk Pregnancy, Maternal Health, Fetal Complications, Pregnancy

### **P<sub>nm</sub>-17: 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 cause of anxiety and depression in infertile women may be 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 on 74 infertile women 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 was 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, was 106.08 and two weeks after completion of the training program was significantly decreased to 87/81 (P value=000.0). 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. Educating infertile patients with emphasis on the cognitive, affective and behavioral attitudes towards infertility is suggested.

**Keywords:** Infertility, Anxiety, Training, Women, Education

### **P<sub>nm</sub>-18: Complementary and Integrative Medicine And Pregnancy**

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**Background:** Many couples have difficulty in getting pregnant, and turn to a variety of treatments to help them conceive. Allopathic, or conventional, methods to help with infertility are often expensive and risky. There are many things a woman can do to increase the chances of conceiving and having a healthy pregnancy with alternative forms of medicine.

Integrative medicine with complementary treatments, like nutrition, dietary supplements, and acupuncture.

**Materials and Methods:** In order to increase information and knowledge about the topic, we searched the databases: OVID , CINAHL , SCOPUS , PubMed , Magiran , Iran doc , SID , Science direct, using Keywords: adherence; complementary medicine gestation; pregnancy; integrative medicine. Library resources were used for this purpose, then selection of related content.

**Results:** Studies have shown that integrative medicine such as acupuncture, can be used to treat infertility in combination with traditional medicine. The acupuncture might increase blood flow and assistance with handling emotions and stress. Acupuncture electronic stimulation can help reproductive hormones. Although more researches are needed to confirm the findings, a study showed that acupuncture given as a complement to IVF which increases the odds of achieving pregnancy. Supplements in diet help a woman become pregnant, stay pregnant, and maintain a healthy pregnancy. Several clinical studies showed that chaste berry herbal tincture successfully improved fertility. L-arginine, an amino acid, helps improve ovarian response, endometrial receptivity, and pregnancy rate. Yoga and meditation provide a way to relax, improve your attitude, and help you manage stress. Some women undergoing IVF experience more success with these forms of integrative medicine, maintaining a regular yoga and meditation practice.

**Conclusion:** Integrative medicine brings together treatments

from both traditional medicine and alternative or complementary medicine. More and more often.

**Keywords:** Complementary, Gestation, Pregnancy, Integrative

### **P<sub>nm</sub>-19: How Are Prenatal Screening Results after ART Success?**

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**Background:** Down syndrome screening at first-trimester may cause a higher false positive rate in pregnant patients who have undergone assisted reproductive technologies (ART). Combining the second largest series of biophysical and biochemical tests in the first trimester of pregnancy after ART is mentioned in few studies.

**Materials and Methods:** Ghisoni et al explored that NT measurement between ART pregnancies and matched controls was not significantly different. PAPP-A was reduced but it was not significantly lower in ART pregnancies. Free beta-HCG and PAPP-A were measured on dried blood spots and converted to MoMs.

**Results:** Nuchal translucency (NT) was measured by certified operators. Free beta-HCG was the only analyte that resulted in significantly higher values in ART pregnancies versus controls. No significant differences were found for biochemical values observed between ICSI and IVF patients. NT measurements were not affected by ART pregnancies. In another study that was performed in Italy, results were consistent with other reported series

**Conclusion:** The algorithm used to calculate the relative risk after the combined tests should not be changed until the detection rate of trisomies in ART pregnancies is not fully disclosed by larger series.

**Keywords:** Prenatal Screening, ART

### **P<sub>nm</sub>-20: Applications of Hysterosalpingography in The Investigation of Female Infertility (Instruction for Midwives)**

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**Background:** To introduce the application of hysterosalpingography in the investigation of female infertility for midwives.

**Materials and Methods:** A review was performed within articles published at "PubMed", "Elsevier", "Google Scholar", "EBSCO", original text books and etc. to reach the aim. Lots of unique high-quality hysterosalpingograms were provided

in this article, using the archive of infertile patients referred to imaging department of Royan institute, Tehran, Iran.

**Results:** Hysterosalpingography (HSG) is a contrast enhanced fluoroscopic and flat plate investigation of the endometrial cavity and fallopian tubes which is widely used to evaluate infertile women. As a reliable simple out-patient and cost-effective method, HSG can reduce the indications for diagnostic hysteroscopy/laparoscopy and plays a significant role in infertility workup. Thus, every midwife working at infertility treatment centers needs to be aware of its application. In this article we discussed the instruction of HSG, advantages & disadvantages of which in infertility workup for midwives. Besides, we provided several hysterosalpingograms taken from infertile women to introduce abnormalities found by HSG.

**Conclusion:** Hysterosalpingography is a reliable approach that helps Midwives and Obstetricians to evaluate female infertility. So every midwife needs to learn about it.

**Keywords:** Hysterosalpingography, Infertility, Uterus, Fallopian Tubes

### **P<sub>nm</sub>-21: Human Parasitic Protozoans-Infection to Infertility**

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**Background:** Protozoan parasitic diseases are endemic in many countries worldwide especially in developing countries, where infertility is a major burden. These infections can cause diminished or absent ability to conceive (infertility) due to impairment in male and female reproductive systems.

**Materials and Methods:** Medline, PubMed, Scopus, Google scholar manuscripts were used to identify the most relevant studies on protozoan parasitic infections and their implications in human and model animal infertility.

**Results:** Literature described that some of the protozoan parasites such as *Trichomonas vaginalis* may causes deformities of genital tract, tubal inflammation and effects on semen quality. *Toxoplasma gondii*, *plasmodium* spp. and *Trypanosoma brucei* spp. cause damage in the pituitary gland, hormonal disorders and decrease semen quality. *Trypanosoma cruzi* inhibits cell division in embryos and impair normal implantation and development of placenta. *Entamoeba histolytica* infection can cause pelvic pain, salpingitis, Tubo-ovarian abscess and genital ulcers. Cutaneous and Visceral leishmaniasis causes genital lesion, testicular amyloidosis, inflammation of epididymis, prostatitis and sperm abnormality in human and animals.

**Conclusion:** Findings of this review indicate that protozoan parasitic infections may be an important cause of infertility. According to the widespread prevalence of parasitic protozoa

diseases in worldwide we recommend further studies to better understand the relationship between these infections and infertility as well as examination of both partners in treatment of infertility.

**Keywords:** Infertility, Protozoan Parasites, Infections

### **P<sub>nm</sub>-22: Obesity and Male Infertility: A Systematic Review**

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**Background:** In today's worlds of advanced technology, obesity phenomenon, more than whatever seen before, is a risk and health- threatening factor. Recent studies show that there is a relation between obesity and infertility in men.

**Materials and Methods:** This paper is written with an overview of related articles published in Scencedirect, Pubmed, SID and Iranmedex for articles from the inception of each database to March 2015.

**Results:** The studies showed that obesity with an impaired level of nitric oxide, reduce blood perfusion; with formation of free radicals of oxygen also impaired hormonal axis as a relative hypogonadotropic hypogonadism, and lead to spermatogenesis disorder and infertility. The results of some studies show that obesity in men reduced fertility with decreasing in levels of testosterone, Inhibin B, SHBG, gherlin and increase estrogen and leptin. Related erectile dysfunction and hyperthermia in testis are the most important physical factors in men's subfertility. Metabolic syndrome in relation to central obesity, with cardiovascular disorder, insulin hypersensitivity and dyslipidemia decreases the chance of male fertility. In vitro studies showed that high-fat diet also increase oxidative stress, and reduces nitric oxide and testosterone level. Some studies also showed a significant positive correlation between BMI and sperm morphology, levels of LH, FSH and leptin.

**Conclusion:** Obesity as a physical factor related to the life style, can cause reduced fertility in men. Therefore, according to these studies, any intervention to reduce weight, improve endocrine system and basal metabolism, leads to an increase in sperm quality.

**Keywords:** Men Obesity, Infertility, Nitric Oxide

### **P<sub>nm</sub>-23: A Comparative Study of Infertile Couples' Knowledge and Attitudes toward Surrogacy**

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**Background:** Surrogacy is one of the most popular ART methods which is used when a woman is not able to breed a fetus through pregnancy in her own uterus. Gestational surrogacy is approved by law and religion in Iran. Infertile couples' decisions between possible treatments are influenced by their knowledge and attitudes. The aim of this study is to compare knowledge and attitudes of infertile couples toward surrogacy.

**Materials and Methods:** This cross-sectional study was performed on 200 infertile couples (N=400) via simple sampling method. A self-made questionnaire with three sections such as "demographic and clinical information", "surrogacy knowledge" and "surrogacy attitude", after standardization, was used as a valid and reliable research tool ( $\alpha=0.91$ ). Data was analyzed using descriptive and Inferential statistics and a value of  $P \leq 0.05$  was considered significant.

**Results:** About half of the women had poor knowledge (46%), while most of men had an average knowledge about surrogacy (38.5%) and although this difference was statistically significant, there was no significant difference between their attitudes. In overall acceptance of surrogacy, more than half of the couples had positive attitudes but generally about all aspects of surrogacy, most of infertile couples had neutral attitudes. In infertile men statistically significant reverse association was found between knowledge and attitudes about surrogacy.

**Conclusion:** While infertile couples have similar attitudes toward surrogacy, lack of knowledge leads to formation of a neutral attitude and ambiguity about different aspects of surrogacy. Therefore, couples decisions will be faltered. Improving knowledge and attitudes about surrogacy relieves future potential negative consequences for infertile couples, surrogate mother and children born through surrogacy otherwise, surrogacy concept gets away from altruistic goals and trends to a stigma in society.

**Keywords:** Knowledge, Attitude, Infertile Couples, Surrogacy

### **P<sub>nm</sub>-24: Neonatal Outcomes in Assisted Reproductive Technology Pregnancies**

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**Background:** Preterm birth is the most important factor in neonatal morbidity and mortality. In the case of pregnancy with assisted reproductive technology (ART) we have more percentage of multiple pregnancies which is a strong reason for preterm birth and low birth weight so suggested that ART can affect neonatal outcomes. In this article we searched about neonatal outcomes in ART pregnancies.

**Materials and Methods:** We have done a systematic re-

view to find neonatal outcomes in ART pregnancies by related search engine such as Google Scholar, PubMed, Cochrane library and etc., with these key words: neonatal outcomes and ART pregnancies.

**Results:** The majority of studies illustrated that ART is associated with multiple pregnancies and multiple pregnancies are the most important reason in neonatal mortality and morbidity due to increased preterm birth rate, low birth weight also it leads to longer duration of hospitalization and more NICU admissions. While there is controversy between these studies and the others that emphasize that although ART lead to preterm birth the neonatal outcomes is not affected by the method of pregnancy was occurred.

**Conclusion:** In today medical world, ART are used widely for infertility treatments. Although it is an effective treatment it is associated with numerous risks for mother and fetus. Multiple pregnancies are the main problem of ART treatment in infertile couples. Recent studies show that multiple pregnancies are associated with four to ten times greater prenatal mortality compared to singleton gestations. It has been proposed that infertility itself increases the risk of preterm birth and low birth weight. Considering the above issues, infertility and treatment of which are the important and vulnerable situations for mothers and neonates. Midwives as a health care providers and the first level of primary care, have an integral roles in the prenatal cares. With the better and more effective systematic prenatal care we can improve the neonatal health in ART pregnancies.

**Keywords:** Assisted Reproductive Technology, Neonatal Outcomes, Pregnancies

### **P<sub>nm</sub>-25: The Importance of Reproductive Health**

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**Background:** Reproductive health is defined as a state of physical, mental, and social well-being in all matters relating to the reproductive system, at all stages of life. Good reproductive health implies that people are able to have a satisfying and safe sex life, the capability to reproduce and the freedom to decide if, when, and how often to do so. Men and women should be informed about and have access to safe, effective, affordable, and acceptable methods of family planning of their choice, and the right to appropriate health-care services that enable women to safely go through pregnancy and childbirth. Reproductive Health has a particular interest on the impact changes in reproductive health have globally, and therefore we decided to write a systematic review article in this issue.

**Materials and Methods:** We searched the related search engines such as PubMed, Google Scholar, Cochran library Science direct and WHO database with these keywords: reproductive health, reproductive rights and sexual health.

**Results:** We found 23 articles in this issue in our search. The majority of articles indicated that reproductive health is one

of the most important parts of human rights but in some cultures and countries was ignored by political and governmental policies especially in developing country where this need is critical.

**Conclusion:** Within the framework of WHO's definition of health as a state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity, reproductive health addresses the reproductive processes, functions and system at all stages of life. Reproductive health, therefore, implies that people are able to have a responsible, satisfying and safe sex life and that they have the capability to reproduce and the freedom to decide if, when and how often to do so. Implicit in this is the right of men and women to be informed of and to have access to safe, effective, affordable and acceptable methods of fertility regulation of their choice, and the right of access to appropriate health care services that will enable women to go safely through pregnancy and childbirth and provide couples with the best chance of having a healthy infant.

Our roles as a health care providers and first level of primary health care are so important. We can notice this issue and improve the quality of reproductive health in our society by prepare educational and counseling programs for our clients and inform them their rights and roles. So we can help the society to increase the level of reproductive health.

**Keywords:** Reproductive Health, Reproductive Rights, Sexual Health

### **P<sub>nm</sub>-26: Assessment Mother's Expectations and Satisfaction of Prenatal Training**

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**Background:** Nowadays, one of the most components of clinical governance is to held childbirth education classes for pregnant women. On the other hand, assessment of patient's satisfaction from the services is the most important indicators of quality of services and monitoring health services. So patient satisfaction has an important role in medical care. Therefore consideration of patient's expectation is necessary for improving quality of midwifery care.

To identify Mother's Satisfaction of training during pregnancy after delivery and Find out how much of the needs and expectations have been met.

**Materials and Methods:** This is a descriptive and cross-sectional study involving 51 women who attended prenatal classes to prepare for labor during pregnancy. Samples completed the Questionnaire within 3-8 weeks of delivery in urban health centers in Shahrekord city between March 2014 and

September 2014. Data collected prospectively using a form including demographic data and a 20 item self-designed questionnaire. Data processing and statistical analysis were performed using SPSS 16.0.

**Results:** The sample mean age was  $26.67 \pm 3.59$  years old. Type of delivery in 74 percent (n=37) was normal vaginal delivery and 26 percent (n=14) had cesarean section. According to the data analysis, 80.25% of clients had high satisfaction from the educational content and 12.45% of them had poor satisfaction, 85% of samples believed that class content met the needs in labor. Over 60% of samples said that their instruction in prenatal class not consistent with their observations in hospital. Our findings revealed that a majority of mothers believed that the patient's right was not respected in hospital while just 25.5% of mothers evaluated the hospital care relatively good. About 55.45% said that care providers did not respect their autonomy. There was a statistically significant relationship between type of delivery and satisfaction ( $P < 0.05$ ).

**Conclusion:** According to this study, the majority of mothers were satisfied from prenatal education but from client view point, there was a major discrepancy between expectations and experiences of the occurrence of interventions in labor. Also based on this research results, the patient's right was not completely observed in the view point of patients. Therefore, giving more information about patient's right to physicians and patients is recommended.

**Keywords:** Maternal Satisfaction, Expectations, Prenatal Training

### **P<sub>nm</sub>-27: The Effect of Wi-Fi Waves on Adolescent Fertility**

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**Background:** Due to the advancement of technology and communication, mobile and Wi-Fi have become as an integral part of human life, particularly young population. Thus, a review of EM wave's effects on the reproductive health has become one of the critical matters of researches in medical field. The accession of using Wi-Fi around the world now has made it possible that about 450 million family members to have access to it in the year 2014, which the most part of those people are adolescents.

**Materials and Methods:** This study was performed based on iterative searches, reviewing of texts and searching on various sources. According of examination that carried out in the year of 2010 in India on the male rats that exposed in the time intervals of 35 days and every day 2.1/2 hours on the vicinity of mobile waves it was determined that their sperm have been reduced considerably. And in another survey the Argentina scientists has examined the effect of Wi-Fi waves on the sperm during of one 4-hour time intervals, which in this probe 25% of sperms were inactive and 9% of them showed DNA rupture. As well in the other probe 28 rates of female along with Wister strain divided into 4 groups which the number of

the secondary follicles of each three groups exposed on the waves with different times indicated of significant reduction as compared with the control group.

**Results:** Radiation exposure of mobile phone and Wi-Fi waves can result in the reduction of movement speed and sperm number with various mechanisms. However, it is not only men that are on the exposure of infertility, but Wi-Fi waves can also effect on women's fertility.

**Conclusion:** There is a considerable correlation between radio frequency waves and fertility of people but as Wi-Fi has changed into one of the elements of social life to escape out of it is impossible. The only solution that can be done is that we ought to take away from the resources of Wi-Fi waves and for the duration of not using of apparatus as modem and other instrument connected to it, turned them off.

**Keywords:** Waves, Wi-Fi, Infertility, Sperm, Ovum

### **P<sub>nm</sub>-28: Dietary Glycemic Index, Glycemic Load, and The Risk of Endometrial Cancer**

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**Background:** Endometrial cancer is a hormone-dependent disease and has been consistently associated with obesity. Non genetic lifestyle factors may account for more than 75% of endometrial cancer cases and represent potential targets for prevention of this disease. Insulin resistance induced by diet may play a particularly pernicious role in the development of endometrial cancer, possibly by reducing levels of sex-hormone binding globulin and insulin like growth factor binding proteins. Increases in IGF-1 are known to stimulate cell proliferation and differentiation, inhibit apoptosis, and promote tumor angiogenesis. Increases in expression of proteins involved in glucose transport and breakdown may promote tumor cell survival. Therefore there was a potential role for the glycemic nature of a diet in the incidence of endometrial cancer. The aim of this review article is to study the role of dietary glycemic index (GI), glycemic load, and the risk of endometrial cancer.

**Materials and Methods:** This review article prepared by studying articles obtained from Google and PubMed sites with key words such as endometrial cancer; insulin resistance; diet and glycemic index.

**Results:** The relationship of dietary GI or GL and endometrial cancer risk yielded contradictory findings. In case-control studies, there was a positive association of GI with endometrial Cancer. But in cohort studies this association was much

weaker or absent. Almost all studies observed a null association between total carbohydrate intake and endometrial cancer. The pooled results from observational studies in Australia supported an increased risk for high GL, but not GI. The result of a recent study in China conducted among 30–69 year old residents recruited between 1997 and 2003, showed intake of high GL or GI foods, but not carbohydrates per se, may increase risk for endometrial cancer. The positive GL–cancer association was reported to be more pronounced among premenopausal women, obese women, and non diabetic women

**Conclusion:** This review suggested that maintaining lower intake of foods with a high GL value may reduce the risk of endometrial cancer.

**Keywords:** Cancer, Diet, Endometrium

### **P<sub>nm</sub>-29: Effects of Vitamin D Supplementation in Reproductive Function of Women with Polycystic Ovary Syndrome**

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**Background:** Vitamin D deficiency is common in women with PCOS. Vitamin D is thought to influence the development of PCOS through gene transcription, and hormonal modulation influences insulin metabolism and fertility regulation. Therefore, the aim of this review article is to study the effects of vitamin D supplementation in reproductive function of PCOS women.

**Materials and Methods:** This review article prepared by studying of articles obtained from Google and PubMed sites with key words such as polycystic ovary syndrome; vitamin D; reproductive function.

**Results:** An intervention study investigated whether vitamin D and calcium dysregulation contribute to the development of follicular arrest, resulting in reproductive and menstrual dysfunction. Thirteen vitamin D-deficient women with PCOS were supplemented with vitamin D combined with calcium which increased 25OHD levels. This resulted in normalized menstrual cycles within 2 months for seven of the nine women with menstrual dysfunction, two women became pregnant and the other four maintained their normal menstrual cycles. In a randomized clinical trial investigating the effects of calcium–vitamin D and metformin in regulating the menstrual cycle, 60 infertile women with PCOS were randomized to one of the three treatments consisting of 1000 mg calcium + 400 IU vitamin D per day; 1000 mg calcium + 400 IU vitamin D

+ 1500 mg/day metformin, or 1500 mg/day metformin. The number of dominant follicles (14 mm) during the 2-3 months of follow-up was higher in the calcium-vitamin D-metformin group than in either of the other two groups. In a case control study, 100 infertile PCOS women were randomly divided into two groups. Group I (n=50) were treated with metformin, and group II (n=50) treated with metformin plus Calcium and Vitamin D for 6 months. A better improvement was gained in regulating menstrual abnormalities, follicle maturation and infertility in group II compared with group I.

**Conclusion:** The results of this review article indicated a beneficial effect of vitamin D supplementation on menstrual dysfunction.

**Keywords:** Vitamin, Reproduction, PCOS

### **P<sub>nm</sub>-30: Assessment of The Relationship between Embryo Quality after ICSI and IVF at The Second and Third Days after Fertilization with Mothers' BMI**

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**Background:** Obesity has become a major health problem across the world. In women, it is known to cause anovulation, sub-fecundity, increased risk of fetal anomalies and miscarriage rates.

However, in women going for assisted reproduction the effects of obesity on egg quality, embryo quality, clinical pregnancy, live birth rates are controversial

To assess the effect of women's body mass index (BMI) on the reproductive outcome of non-donor *in vitro* fertilization (IVF) and Intracytoplasmic sperm injection (ICSI).

**Materials and Methods:** Retrospective study of medical records of 204 women undergoing non donor IVF and ICSI cycles in Laleh and Taleghani Hospitals. Body mass index was calculated according to the generally accepted formula Weight/Height<sup>2</sup>, where weight is in grams and height is in centimeters. The patients were divided into three classes: <25 normal body mass, 26 to 29.9 overweight, and >30 obese and we must attend to oocyte quality and PCO.

**Results:** This study shows, increase in BMI lead to decrease in fertilization rate (P value=0.055) and increase or decrease in BMI don't have any impression on embryo quality but in people haven't male factor, the number of embryo type B in group with BMI>25 is seventy percent more than women with BMI≤25. Furthermore, reverse relationship is seen between embryo quality and male factor.

**Conclusion:** Increase in body mass index in women does not appear to have an adverse effect on IVF outcome. However, pre-conceptual counseling for obese women is a must as weight reduction helps in reducing pregnancy-related compli-

cations.

**Keywords:** BMI, IVF, ICSI, Embryo Quality

### **P<sub>nm</sub>-31: The Role of Drinking Customs in Increasing The Success of *In Vitro* Fertilization**

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**Background:** Despite the progress and development of diagnostic procedures and treatment of infertility in recent years, the success of advanced therapies, like IVF, is around 30-40 percent, even in the best medical centers. The aim of this study is to review the role of drinking customs in the success of IVF from the standpoint of Iranian Traditional Medicine.

**Materials and Methods:** To achieve this aim, the available texts on Iranian traditional medicine and the related articles from accredited databases were investigated. The materials relating to successful pregnancy and infertility prevention were studied then, content analysis was carried out and the results were summed up.

**Results:** From the standpoint of physicians each organ in the body has its own temperament (position, status, state of health). The organ will do its duty well if the temperament is in a state of moderation, otherwise abnormal temperament (position, status, state of health) or adverse temperament will arise. Following the instructions about eating and drinking is one of the most important principles to maintain the normal temperament of organs, including reproductive organs. The uterus temperament is dry and cold in normal condition. Any adverse temperament in uterus can reduce successful fertility, increase abortion and reduce fertility treatment success including IVF. Intense cold causes narrowing and compression of the uterine artery. This often occurs following non-compliance with proper eating and drinking procedures like, drinking water and cold drinks in early morning, immediately after bath and copulation, with meals, immediately after meal, during exercise and all strenuous physical activities.

**Conclusion:** If the relationship between drinking cold drinks (which are very common today) and infertility and assisted reproduction treatments (including IVF) is proven, following these measures can be effective as an alternative and no cost procedure in increasing the success of IVF and prevention of infertility. It is recommended to teach such these instructions to couples in infertility treatment centers

**Keywords:** Drinking Customs, Infertility, *In Vitro* Fertilization

### **P<sub>nm</sub>-32: The Importance of Consultation in Embryo Donation**

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**Background:** Infertility and decisions about the use of assisted reproductive technologies such as embryo donation have legal, psychological and social issues in addition to medical aspect. So, expert advice should be considered as an integral element in providing the services. Accordingly, this study designed and implemented to investigate the consulting needs of embryo donation applicants to clarify the strengths and weaknesses of the rules in this area in addition to provide a perspective on the current state of the process of embryo donation at infertility treatment centers to address weaknesses by government policy makers in the field of infertility treatment.

**Materials and Methods:** This study is a descriptive study through interviews with 100 couples applying for Embryo Donation from September 2013 to July 2014 to assess the cover of their consulting needs in the process of embryo donation. The instrument is a questionnaire validated using content validity and the reliability has been verified by calculating Cronbach's alpha and test-retest method. Data analysis had also been performed using SPSS version 18.

**Results:** Embryo donation infertility treatment center service providers just provided brief information in the medical aspects of embryo donation process on the majority of applicants (74%). As well, the physicians had attempted to provide advice on the legal and juridical aspects of the process for most applicants (56%). Altogether, the most of samples showed the lack of emphasis by service providers on need for specialized legal and juridical advices. Also, effective method was not provided by the assisted reproduction service providers about supporting of embryo donation applicants' prospective needs for advice in cases such as necessity of exposing of embryo donation issue for resulting children, the disclosure and appropriate time for it and the applicants were placed in a state of confusion.

**Conclusion:** We are faced with a hierarchy of obvious and hidden needs of donor/recipient and the offspring of this method in which knowing them and providing the correct answer to them is necessary to ensure benefits for individuals. In this regard, survey of problems and concerns of patients after treatment and investigation of offspring's situation and also several ongoing communications and consultations would be essential steps to improve the situation of these people. Therefore, it is recommended that officials, experts and planners associated with infertility should pay particular attention to those issues.

**Keywords:** Embryo Donation, Consultation, Infertility

### **P<sub>nm</sub>-33: Role of Maternal Age in Outcomes of Pregnancy**

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**Background:** Today, many of women postpone their birth of the first baby until fourth or fifth decade. This study was to evaluate the pregnancy outcomes in women aged 40 years and older in pregnant women referred to Ayatollah Rohani Hospital in Babol.

**Materials and Methods:** This case-control study was conducted of 160 patients in two groups,  $\geq 40$  age group and age group  $<40$  in pregnant women referred to Ayatollah Rohani Hospital in Babol from of 2009 to 2013. Demographic data and maternal and neonatal outcomes were collected from recorded file of patients then analyzed by SPSS.

**Results:** Mean of patient's age in the group of women 40 years and older was  $(42.35 \pm 2.078)$  and in women less than 40 years was  $(26.68 \pm 4.677)$ . Pregnancy outcomes in pregnant women 40 years and older and under 40 years showed respectively that gestational hypertension 38 (23.8%) versus 22 (13.8%) ( $P=0.022$ ), GDM 47 (29.4%) versus 17 (10.6%) ( $P=0.000$ ) and polyhydramnios 9 (5.6%) versus 1 (0.6%) ( $P=0.01$ ), had significant statistical difference. We had 7 IUFD cases (4.375%) in women 40 years and older, while there was no IUFD in women under 40 years ( $P=0.01$ ). The APGAR of 1 minutes between two group had significant difference ( $P=0.043$ ) but in another neonatal outcomes in two groups did not have any difference. The most of cause in of caesarean section after repeat caesarean 66 (41.2%) ( $P=0.035$ ) in women 40 years and older was, high risk pregnancy 19(11.9%) ( $P=0.0001$ ), pre-eclampsia 6 (3.8%) ( $P=0.23$ ), oligohydramnios 5 (3.1%) ( $P=0.21$ ) and fetal distress ( $P=0.04$ ), twin pregnancy ( $P=0.03$ ) and no response to induction ( $P=0.001$ ) were most of cause of caesarean.

**Conclusion:** This study showed that pregnancy complications such as gestational diabetes and pregnancy induced hypertension and polyhydramnios are greater in women 40 years and older. Then old age of mother requires more prenatal care.

**Keywords:** Hypertension, Age, Pregnancy, Outcome, Diabetes

### **P<sub>nm</sub>-34: Effects of Supplementing Mouse Maternal Diet by Omega-3 Fatty Acids on Male Offspring Reproductive Organs after Weaning**

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**Background:** The objective was to determine how feeding fish oil (FO) with or without vitamin E (VITE) for mothers influences reproductive organs of male offspring at weaning.

**Materials and Methods:** Sixty mature female NMRI mice were divided into 10 groups (n=6). The experimental groups consist of mothers who consumed several diets: I. control (CTR; standard diet pre and postnatal period), II. PRE-VITE; consumed VITE two fold greater than standard recommendations (2×) during prenatal period, III. POST-VITE; consumed VITE 2× during postnatal period, IV. PRE-POST-VITE; consumed VITE 2× during pre and postnatal period, V. gavages 0.01 ml/d/ mother fish oil (FO) + CTR diet during prenatal period, VI. gavages 0.01 ml/d/ mother FO + CTR diet during postnatal period, VII. gavages 0.01 ml/d/ mother FO + CTR diet during pre and postnatal period; VIII) gavages 0.01 ml/d/ mother FO + VITE 2× diet during prenatal period, IX. gavages 0.01 ml/d/ mother FO + VITE 2× diet during postnatal period and X. gavages 0.01 ml/d/ mother FO + VITE 2× diet during pre and postnatal period. After weaning, the testes were collected and histological data were analyzed using SAS software.

**Results:** Sex cells as well as testes length, width and weight was lower ( $P<0.05$ ) in offsprings which their mothers fed FO + CTR diet during pre, postnatal -pre and post period than other treatments. Dietary supplementation by only vitamin E prenatal or only postnatal period improved these characteristics. Supplemented FO diet by VITE improved responses, especially during postnatal period when the offspring consumed milk.

**Conclusion:** The positive effects of supplementation maternal diet by FO with VITE or sole VITE was observed. Sole FO may destroy offspring sex cells. Thus, our data support the theory that antioxidant requirements increase by omega-3 fatty acid supplementation.

**Keywords:** Maternal Nutrition, Fatty Acids, Offspring Male Reproduction

## Precongress Courses and Workshops



**Royan Institute**

Tehran, Islamic Republic of Iran

### Culture of Chick Embryo in Surrogate Egg Cell

- **Chairpersons:** Imani H, Ph.D, Mozdziak P, Ph.D
  - **Executive Manager:** Golkar A, M.Sc
  - **Date:** Aug 29, 2015
- 

### Culture of Chick Embryo PGCS

- **Chairpersons:** Imani H, Ph.D, Mozdziak P, Ph.D
  - **Executive Manager:** Golkar A, M.Sc
  - **Date:** Aug 30, 2015
- 

### Isolation and Purification of Mesenchymal Stem Cell from Various References: Bone Marrow, Dental Pulp and Adipos Tissue

- **Chairpersons:** Baghaban Eslaminejad MR, Ph.D
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### Semen Analysis

- **Chairpersons:** Salman Yazdi R, Ph.D
  - **Executive Manager:** Lakpour M, M.Sc
  - **Date:** Sept 1, 2015
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### Fertility Preservation and Reproduction in Cancer Patients

- **Chairpersons:** Moini A, M.D
  - **Executive Manager:** Jahangiri N, M.Sc
  - **Date:** Sept 1, 2015
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### Color Doppler Study of Male Infertility

- **Chairpersons:** Saedi D, M.D, Vosough Taqi Dizaj A, M.D
  - **Executive Manager:** Mokhah S, M.Sc
  - **Date:** Sept 3, 2015
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### Prenatal Care in ART

- **Chairpersons:** Hajihasan F, M.Sc
- **Executive Manager:** Etminan Zh, M.Sc
- **Date:** Sept 3, 2015

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**International Journal of Fertility and Sterility (Int J Fertil Steril)**  
**Guide for Authors**

**Aims and Scope:** The "International Journal of Fertility & Sterility" is a quarterly English publication of Royan Institute of Iran. The aim of the journal is to disseminate information through publishing the most recent scientific research studies on Fertility and Sterility and other related topics. *Int J Fertil Steril* has been certified by Ministry of Culture and Islamic Guidance since 2007. It has also been accredited as a scientific and research journal by IBI (Health and Biomedical Information) Journal Accreditation Commission since 2008. **This open access journal holds the membership of the Committee on Publication Ethics (COPE).**

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