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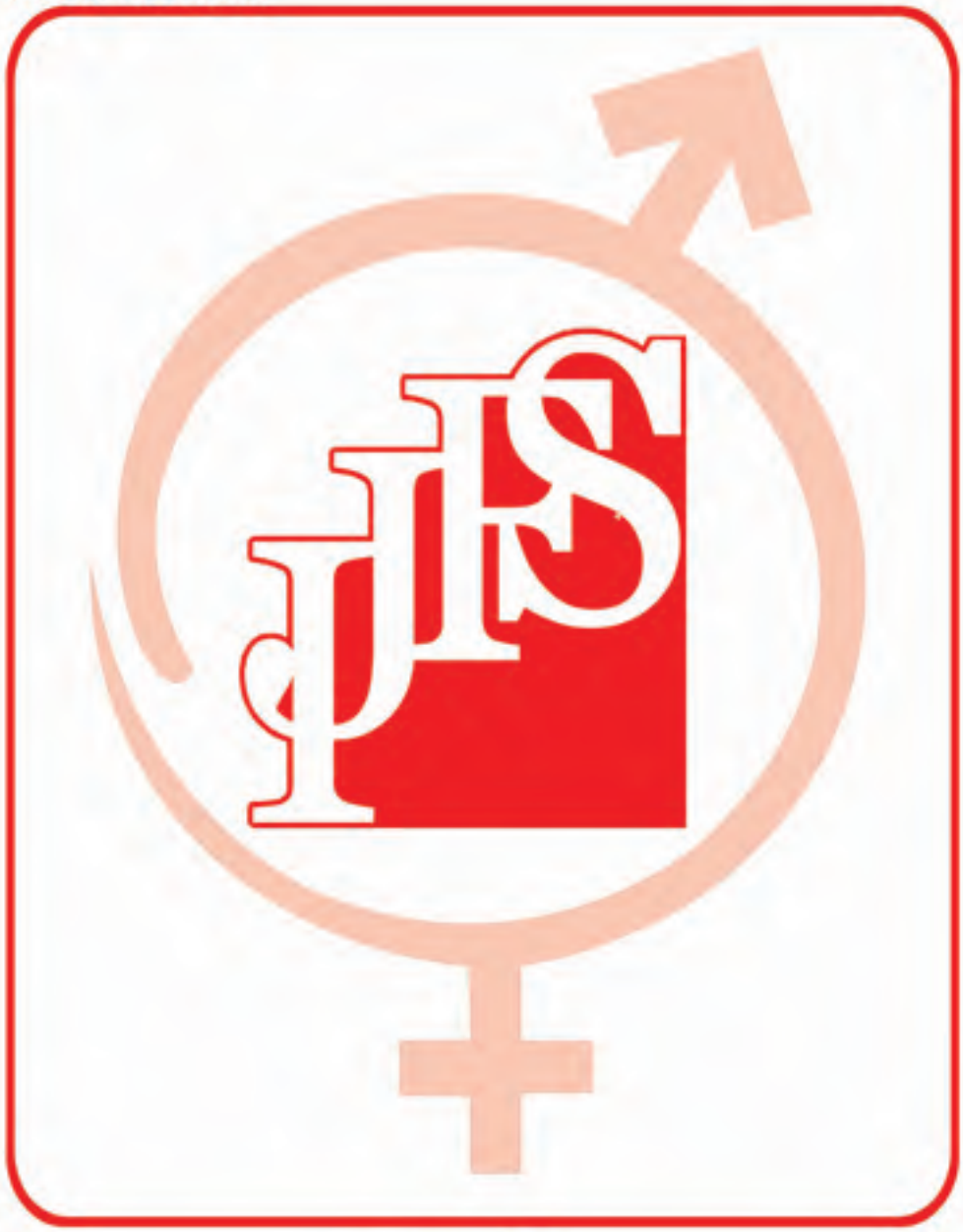


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Abstracts of

# Royan International Twin Congress
## 14th Congress on Reproductive Biomedicine
4-6 September 2013

## 8th Royan Nursing and Midwifery Seminar
4-6 September 2013

# Royan Institute

Tehran, Islamic Republic of Iran

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# 14th Congress on Reproductive Biomedicine

## 8th Royan Nursing and Midwifery Seminar



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# Scientific Board

# Collaborators in Award Jury and Scientific Board of14th Congress on Reproductive Biomedicine

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# Congress Chairperson

**Ensieh Shahrokh Tehraninejad, M.D.**

Dear Friends and Colleagues,

We are delighted to welcome you to Royan International Twin Congress, 14th Congress on Reproductive Biomedicine and 9th Congress on Stem Cell Biology and Technology in Tehran, September 4-6, 2013.

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

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

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

**Best Regards,**
**Ensieh Shahrokh Tehraninejad, M.D.**
**Congress Chairperson**
**Reproductive Biomedicine Congress**

Abstracts of

# 14th Royan International Congress on Reproductive Biomedicine

4-6 September 2013

# Royan Institute

## Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

# Andrology

## I-1: Surgical Treatment of Male Infertility

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Male factor is the sole reason or a component for infertility in 20 and 30% of cases respectively. The management of the disease may be via medical or surgical treatment. The surgical approach is classified as techniques which improves sperm production and delivery in order to achieve spontaneous pregnancy or sperm retrieval techniques prior to assisted reproductive techniques (ART).
Varicocelectomy is the most common surgery performed for the sperm production improvement. After the surgery the prominent improvements are observed in motility and morphology with a higher chance of success in men with >10 million sperm/ml. Testosterone increase, approximately 100 ng/dl, is also reported for patients with a baseline testosterone below 400 ng/dl. The spontaneous pregnancy odds ratio after varicoselectomy was reported as 2.63 in patients with palpable varicocele and abnormal semen parameters.
Surgery to allow sperm delivery are applied to patients with proximal and distal seminal duct pathologies. The obstructed passageway may be caused by a congenital or acquired anomaly or iatrogenic but the existence of sperm in ejaculate to allow spontaneous pregnancy is the main objective of this surgery. Microscopic vasavasostomy and epididymovasostomy are the elementary surgeries of this subject but technological developments such as robotics may be applied as developed. Microscopic vasovasostomy was reported to have patency and pregnancy rates of 92 and 53% with an interval of 3 years. For vasoepididymostomy patency and pregnancy rates of 48-63% and 21-45% had been reported with a mean interval of 16 months. Usage of robotic assisted VE has been suggested for increased precision and decreased operation times. TUR-ED is the endoscopic technique which is performed in distal duct pathologies. Sperm parameter improvement had been reported up to 94% in men with distal duct obstructions.
Sperm retrieval techniques are treatment modalities used to gather sperm from the testis and epididymis of azospermic infertile males prior to ART. These techniques are applied to obstructive azoospermic (OA) and non-obstructive azoospermic (NOA) males.
General sperm retrieval techniques are listed as:
Percutanoues epididymal sperm aspiration (PESA)
Microscopic epididymal sperm aspiration (MESA)
Testicular sperm aspiration (TESA)
Conventional testicular sperm extraction (TESE)
Micro-surgical testiküler sperm ekstraksiyonu (m-TESE)
PESA: Despite its minimal invasive, easy and prompt nature, inadequate material aspirates and greater risk of hematoma complication had made this technique obsolete.
MESA: This technique is used for obstructive azospermia not suited for reconstructive surgery. Number of gathered sperm is which is sufficient for both ART and cryopreservation. The SRR and pregnancy rates are 90% and 14-66% respectively. The ART success rates of epididymal sperms are similar to testicular ones.
TESA: It become reserved for obstructive azoospermia subsequent to the introduction of TESE. The general SRR for OA and NOA patients are 100 and 27% respectively. Although adequate number of sperms are gathered for ART, cryopreservation may not be possible all the time. This technique is not preferred anymore because of possible vascular injury and insufficient sperm numbers.
TESE: The SRR of this technique is 36% via multiple biopsies and drops to 23% for single biopsy. Conventional TESE is replaced by mTESE in routine practice because of low SR rates.
mTESE: Requirement of micro surgical skills, greater learning curve and longer operation time is balanced by much higher SRR for this technique. In addition preservation of testicular tissue and vascular structure are further advantages. The SRR is correlated with seminefer tubule diameters with a threshold value of 110 microns. This rate was demonstrated to be 84 and 36% for >300 micron and <300 micron diameters of seminifer tubule respectively. Up to 60% sperm retrieval rate was reported for mTESE which is superior to its conventional counterpart. The predictive factors for SRR are accepted as experience of the surgeon, duration of the operation and histopathology of testis.

## I-2: Medical Treatment of Male Infertility

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Male factor is the sole reason or a component for infertility in 20 and 30% of cases respectively. Medical treatment is preferred for patients with testicular failure due to endocrine or exocrine diseases. Although the infertility may be due to a pathology in the hypothalamic- pituitary axis, the majority of the cases occurs as results of testicular failure.
In general medical treatment modalities may be listed as:
1. Hormonal treatment (Gonadotropins, Dopamine receptor agonists, Anti-ostrogens and Aromatase inhibitors)
2. Anti-oxidant therapy
Gonadotropins:
This is the mainstream treatment for hypogonadotropic hypogonadism presented with low FSH, LH and testosterone levels. Although the pulsatile GnRH treatment may be administered for these patients, it not preferred for difficulty of continuous infusion pumps. The treatment protocol is initiated by human chorionic gonadotropin (hCG) administration (1500-2000 IU 2-3 times/wk) to achieve normal range of testosterone in 48 hours. If patient continue to be azoospermic at 6. month, recombinant human FSH (100-150 IU 2-3 times/wk) may be added to hCG. Improvement for both testicular volume (4 to 12 cc)and total sperm concentration (5 million) had been achieved by using hCG alone. In patients who were refractory to hCG treatment but reached normal T levels, 84% of spermatogenesis was achieved after the

administration of rhFSH. Testicular volume greater than 8 cc and post pubertal onset hypodonadism had been identified as positive prognostic factor for this treatment, while requirement of orchiopexy, small testis volume (<4 cc) count as poor prognostic factor.
Although there is no evidence based study, the usage of rhFSH has been suggested in a study for select sub group of non HH oligo-asthenospermic patients with normal FSH values and maturation arrest histology. This treatment is still experimental and not approved by guidelines.
Anti-estrogens (AE):
Prior to introduction of ICSI, anti-estrogens such as tamoxifen and clomiphene were preferred for idiopathic male infertility in which these anti-estrogens improve testicular testosterone and spermatogenesis by blocking negative feedback of estrogen. In a meta-analysis, the pregnancy rate was reported as 15% in comparison with 12% of control group for oligoasthenospermic patients. In another placebo controlled study in which AEs were combined with vitamin E, the pregnancy rate was reported as 37 versus 13%. Clomiphene treatment was administered to NOA patients to achieve testosterone levels of 600-800 ng/dl and in 64% of patients who had a pathology of either maturation arrest or hypospermatogenesis, sperm was observed at the ejaculate (average 3.8 million sperm/ml).
Aromatase inhibitors:
These drugs improve androgen levels by blocking the conversion of them into estrogens. Although its usage has been suggested in trials without placebo, further scientific evidence is required. Aromatase inhibitors (anastrazole 1 mg 1x1, testalactone 100-200 mg 1x1, letrozole 2.5 mg 1x1) seems to restore T/E2 balance in patients with low testosterone and high estrodiole levels. Patients with T/E2 ratio lower than 10 and klinefelter may benefit from inhibitor treatment.
Antioxidant therapy:
Reactive oxygen species which present in higher levels of infertile males are suggested to effect the sperm function, DNA structure. Despite the numerous studies about the effects of anti-oxidant therapy on male infertility, most of them lack the necessary scientific design and mechanism of success had not been explained still. Current literature suggests carnitines, vitamin E and C for the improvement of semen parameters. A recent cochrane review had stated a 4-5 fold increase in pregnancy and live birth rates for men using anti-oxidants prior to ART. In another systemic review, an improvement in semen parameter or pregnancy rates had been reported in 82% of trials. Out of the 10 trials investigating pregnancy rates, 6 of them showed significant improvement. Despite all those trials, many more studies with better design and scientific approach should be performed before the routine administration of anti-oxidants.

## I-3: Hypogonadotropic Hypogonadism

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Hypogonadotropic hypogonadism (HH) is an uncommon cause of male infertility and a congenital or secondary disorder characterized by delayed or absent sexual maturation. Congenital abnormalities leading to HH are usually the consequence of deficient GnRH secretion occurring either in isolation (idiopathic hypogonadotropic hypogonadism (IHH)), or in association with anosmia (Kallmann syndrome; KS). Acquired causes of HH can be due to any disorder that affects the hypothalamic-pituitary axis. The induction of spermatogenesis in men with HH can be successfully achieved using gonadotropin therapy. In spite of low sperm count, motility and normal morphology, some of gonadotropin treated patients with HH have fertile sperm and can fertilize ovum naturally or by IUI and ART.
We are studied sperm DNA fragmentation index (DFI), testicular volume, semen parameters and hormone profile in hCG and hMG treated hypogonadotropic hypogonadism (HH) patients with and without a successful pregnancy. The study initially included 81 patients with HH and azoospermia at the Infertility Unit of Royan Institute between 2010 and 2012. 58 of 81 (71.6%) patients achieved > $1 \times 10^6$ sperm per ml during hCG and hMG therapy. These 58 patients were divided into two groups: 20 HH patients who achieved pregnancy in response to hCG/hMG (responders, 16 naturally and 4 by IUI) and 38 gonadotropin treated HH patients with failed pregnancy (non-responders, 29 naturally, 5 by IUI, 1 by IVF and 3 by ICSI).
It was shown that DFI in responders is significantly lower than DFI in non-responders and duration of hCG and hMG therapy in responders is significantly higher than those of non-responders. DFI could be predictive of conception.
It can be concluded that despite of low sperm quality, especially sperm concentration in these patients, decreasing sperm DNA damage may be resulted in successful fertilization.

## I-4: Sperm Retrieval: 2013 Updates

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Men with congenital absence or bilateral partial aplasia of vas deferens, or those with failed or surgically unreconstructable obstructions or azospermic men with testicular failure or genetic cause can now be treated by using sperm retrieval techniques in conjunction with *in vitro* fertilization/intra cytoplasmic sperm injection (ICSI/IVF). These techniques are:
Microsurgical epididymal sperm aspiration (MESA) procedure allows lower complication rate ,require anesthesia and microsurgical skills, has better motility than TESE and large number of sperm can be harvested for cryopreservation. It is indicated for Nonobstructive Azospermia.
Percutaneous puncture of the epididymis with a fine needle has been successfully employed to obtain sperm and achieve pregnancies.
Percutaneous epididymal sperm aspiration (PESA) results in lower retrieval rates than microsurgical testicular sperm extraction (TESE) and does not allow histologi-

cal examination to detect carcinoma in situ (CIS) and testicular malignancies. PESA may also result in more tubular and vascular damage than TESE .
Testicular biopsy can be part of ICSI treatment in patients with clinical evidence of NOA. TESE is the technique of choice and shows excellent repeatability.
Royan institute randomized control study, which was done with a larger sample size than similar published studies indicate that multifocal TESA is more effective and simply done than TESE for sperm retrieval and could be the preferred approach. Spermatogenesis may be focal, which means that in about 50% of men with NOA, spermatozoa can be found and used for ICSI. Most authors therefore recommend taking several testicular samples .There is a good correlation between the histology found upon diagnostic biopsy and the likelihood of finding mature sperm cells during testicular sperm retrieval and ICSI. However no threshold value has been found for FSH, inhibin B, or testicular volume and successful sperm harvesting.
When there are complete AZFa and AZFb microdeletions, the likelihood of sperm retrieval is almost zero. Although Choi (2013) showed in NOA and OATS patients, no significant difference in the sperm retrieval rate was shown between patients with Y chromosome microdeletion and those with no microdeletion. Patients with short Y chromosome microdeletion such as AZFc microdeletion have better prognoses for sperm retrieval and an increased chance of conception than do patients with larger microdeletions such as AZFb-c microdeletion.
The use of an operating microscope for standard open diagnostic testes biopsy allows identification of an area in the tunica albu¬ginea free of blood vessels, minimizing the risk of injury to testicular blood supply and allowing a relatively blood-free biopsy specimen .Employing the microscope for testis biopsy, discovered that in men with nonobstructive azoospermia, some of the tubules were larger than others. The larger tubules are more likely to yield sperm.
Microsurgical TESE may increase retrieval rates versus conventional TESE, even though comparative studies are not yet available .Positive retrievals are reported even in conditions such as Sertoli cell only syndrome type II .
The results of ICSI are worse when using sperm retrieved from men with NOA compared to sperm from ejaculated semen and from men with obstructive azoospermia (OA) .Birth rates are lower in NOA versus OA (19 vs. 28%).
In OA, there were no significant differences in ICSI results between testicular and epididymal sperm. Also, no significant differences have been reported in ICSI results between the use of fresh and frozen-thawed sperm.
Pregnancies and live births are eventually obtained in 30-50% of couples with NOA, when spermatozoa have been found in the testicular biopsy.
Men who are candidates for sperm retrieval must receive appropriate genetic counseling. Testicular biopsy is the best procedure to define the histological diagnosis and possibility of finding sperm. Spermatozoa should be cryopreserved for use in ICSI. For patients with NOA who have spermatozoa in their testicular biopsy; ICSI with fresh or cryopreserved spermatozoa is the only therapeutic option.
Men with NOA can be offered TESE with cryopreservation of the spermatozoa to be used for ICSI. To increase the chances of positive sperm retrieval in men with NOA, TESE (single, multiple or microsurgical) should be used rather than PESA.
Among sperm acquisition methods, micro-TESE has higher success rates at obtaining sperm compared with testicular sperm extraction and testicular sperm aspiration.

## I-5: Semen Analysis - Clinical Laboratories; Is Standardization Applied?

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Reduced semen quality is commonly claimed to be one of the main signs of male infertility and thus, analysis of semen is recommended as the cornerstone of a male fertility investigation. However, to control possible sources of physiological and methodological variation, and to generate reliable information on semen quality that can be accurately compared across different laboratories, semen analysis should be performed according to generally accepted, international recommendations and standardized procedures.
Quality assurance (QA) and quality control (QC) are essential aspects of any laboratory testing process. Both ensure that the data generated by the laboratory are consistent from one day to the next and that the results from one laboratory can be compared with those generated by others. However, whereas QA and QC approaches in many areas of laboratory medicine (e.g., clinical biochemistry and haematology) were relatively well developed in the 1970s and 1980s, their application to the andrology laboratories has been a relatively recent development.
Andrology laboratories need to produce reliable results for appropriate diagnostic and health care decisions. Since semen analysis is highly complex and procedurally difficult to standardize, QC is essential to detect and correct systematic errors and high variability of results. The large discrepancies between assessments of sperm concentration and morphology in different laboratories underline the need for improved QC and standardization, with the goal of improving the quality and standardization of semen analysis and enhancing the comparability of results from different laboratories. Until there are universally accepted standard methods and definitions of motility and morphology, it will not be possible to compare results from different laboratories.
Whatever its size, each laboratory should implement a QA programme, based on standardized methods and procedures, to ensure that results are both accurate and precise. In some countries, QA programmes are required by law, in others, by accreditation bodies or health insurance systems.
The World Health Organization (WHO) laboratory manual is recognized worldwide as the gold standard for human semen examination. It provides clear guidelines for this analysis and for the reduction of laboratory errors. The manual was first published in 1980. Since then, it

has been regularly revised and updated, and the most recent fifth edition appeared in 2010. Although the WHO manuals have been available for more than 30 years, there are still reports revealing a lack of standardization of the methods used in andrology laboratories and wide variation in the results obtained among laboratory technicians in many countries.
The principle of good practice in laboratories is the implementation of standardized, validated methods and equipment for the evaluation of the defined parameters. In consequence, it assures a proper basis for achieving an accurate, reliable and controlled service in the laboratory and its accreditation. For more than 30 years, the WHO has attempted to improve the quality of semen analysis by publishing manuals providing a detailed description of standardized methods and procedures for the evaluation of semen analysis. Unfortunately, adherence to these guidelines is sometimes selective and laboratories adopt not all the recommended methods for their seminological service, which may result in difficulties in interpreting and comparing the results of semen analysis.

# Embryology

## I-6: Role of Actin Cytoskeleton during Mouse Sperm Acrosomal Exocytosis

**Buffone MG[1]*, Gerton GL[2], Okabe M[2], Gerton GL[3]**

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**Background:** Mammalian sperm must undergo a process termed capacitation to become competent to fertilize an egg. Capacitation renders the sperm competent by priming the cells to undergo a rapid exocytotic event called acrosomal exocytosis that is stimulated by the zona pellucida (ZP) of the egg or progesterone. Over the years, several biochemical events have been associated with the capacitation process; however, the question that has remained unanswered in investigations of capacitation is: What is the underlying reaction or set of reactions that transform the sperm cell from a state unresponsive to ZP or progesterone-stimulated acrosomal exocytosis to the state primed to respond to these stimuli? Our preliminary results demonstrate that the actin cytoskeleton plays a role in this process. Our long-term goal of this research: to elucidate the molecular mechanism whereby the actin cytoskeleton controls acrosomal exocytosis in mammalian sperm. In this proposal, we evaluate the establishment and stabilization of the primed state of acrosomal exocytosis that develops during the course of sperm capacitation. Additionally, we will examine the roles of intracellular calcium and actin in the destabilization of the primed state of acrosomal exocytosis that results in the propagation of the fusion of the outer acrosomal and plasma membranes. There are several human health-related reasons these studies are significant. For example, an understanding this process may lead to a better understanding of certain cases of male infertility and to the development of pharmacological approaches to interfere with this process, leading to new contraceptive agents. Most importantly, since actin has been implicated in exocytosis occurring in many types of somatic cells, information gathered from studying the less complicated sperm system will likely impact our understanding of secretion in other organ systems such as endocrine or digestive tissues.
**Materials and Methods:** This application also proposes several novel methodological approaches. These include: a. the simultaneous imaging of acrosomal GFP and calcium to study exocytosis, a novel approach that has several advantages compared to studies previously performed to evaluate this process; b. we will be analyzing live cells in real time, without fixation or any other procedure that may introduce artifacts; c. we will use flow cytometry to monitor the formation of intermediate stages of exocytosis by analyzing the acrosomal GFP and the particulate acrosomal matrix protein sp56. The use of GFP sperm provides an objective and sensitive method to study exocytosis; d. we will use natural stimulants of acrosomal exocytosis such as zona pellucida and progesterone, not chemical agents such as calcium ionophores.
**Results:** We have investigated the pathways governing the establishment and stabilization of the primed state of acrosomal exocytosis that develops during the course of sperm capacitation through the formation of intermediate stages of exocytosis.
**Conclusion:** We have investigate the connection between capacitation and acrosomal exocytosis, because it is not known why sperm do not undergo acrosomal exocytosis if they are not fully capacitated. Moreover, we propose a testable model of how actin helps to establish a metastable state during capacitation to prepare sperm for acrosomal exocytosis and how calcium may cause the dissociation of the peri-acrosomal cytoskeleton, effecting the complete fusion of the outer acrosomal membrane and the plasma membrane.

## I-7: Maximizing Pregnancy Rates with The Use of Vitrification and Comprehensive Chromosome Screening

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**Background:** Vitrification and warming of blastocysts is an established procedure for the preservation of embryos during IVF. Vitrification is so successful that it allows for elective single embryo transfer (eSET) to be performed routinely, since the frozen embryos will be available with high implantation potential if the fresh embryo fails. Comprehensive chromosome screening (CCS) is also a procedure that can allow wider use of eSET, since patients can transfer a single euploid embryo to the uterus and expect high implantation rates. In the current study, we have combined CCS with vitrification of blas-

tocysts at the time of biopsy, and examined outcomes for patients returning for a frozen embryo transfer with their chromosomally normal embryos.
**Materials and Methods:** Blastocysts were biopsied and then vitrified on day 5 or 6 of development. Three to 7 trophectoderm cells were removed and sent for analysis to a genetics laboratory (Natera, Redwood City, CA, USA), and the embryos were vitrified using a Vitkit and Cryotips (both from Irvine Scientific, Santa Ana, CA, USA) shortly after biopsy. When patients returned for a transfer of the vitrified embryos, they were strongly encouraged to transfer one embryo regardless of their age. The embryos were warmed using a Warming kit (Irvine Scientific) and transferred within 1 hour of warming to the uterus on day 6 of progesterone in a natural or controlled cycle as appropriate.
**Results:** From 95 warming cycles performed between Jan 2012 and March 2013, an overall clinical pregnancy rate of 67% and implantation rate of 57% was achieved, and most patients warmed and transferred 1 embryo.
No differences were seen in the proportion of embryos that were abnormal on day 5 or 6, and the number of retrievals with biopsy decreased with patient age as expected, as did the number of euploid embryos.
**Conclusions:** The combination of comprehensive chromosome screening and vitrification gave good pregnancy and implantation rates across all age groups. Older patients were less likely to have any embryos biopsied or to have normal embryos available after CCS, but they avoided unnecessary transfers. Overall, the combination of vitrification and CCS worked well and facilitated the widespread use of eSET, even in older patients.

## I-8: Oxygen Consumption As A Marker of Oocyte and Embryo Quality

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## I-9: Role of Sperm Transcripts in Oocyte Activation, Fertilization and Development

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It is well established that in addition to generating the diploid state in the zygote, the sperm also endows the oocyte with paternal centrioles, proteins and RNAs. Although the exact roles of mRNA introduced into the oocyte by the sperm are not completely understood. However, literature background, suggested that RNAs may have a vital role in embryo cleavage and further development. Therefore, the RNA content of the sperm has been divided into three categories: non-functional, functional and foreign mRNA. Moreover, comparative sperm transcriptoms of fertile and infertile has revealed substantial difference in expression pattern of some genes. Thereby, confirming the fact that there are huge mRNA content differences between fertile and infertile individuals. In this study we assessed the expression of PLCz and PAWP involved in oocyte activation in fertile, individual with zero and low fertilization rate, individuals with high fertilization rate and globozoospermia. The results revealed that expression level of these transcripts may be used to predict the potential of sperms in semen sample, to induce oocyte activation and thereby result in successful fertilization.

## I-10: Embryo Assessment and Selection

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Over the years it has been an important goal to optimize the treatment of subfertility in order to obtain a better pregnancy rate and baby-take-home rate, usually combined with the goal of decreasing the rate of multiplet pregnancies.
This implies optimizing the development of embryos through improved *in vitro* culture methods. And it also implies optimizing the methods of selection of embryos for fresh and frozen transfers, as well as the timing and location of the deposition of the embryos during transfer.
Non-invasive techniques for assessment of implantation potential of embryos can improve the success rate and hence be of importance not least when transferring single embryos.
Using time lapse investigations it is possible to follow the morphological development without interfering with the cell culture processes and the natural cell divisions. Important parameters are e.g. morphology, cleavage pattern and fragmentation. This gives the possibility of evaluating the embryo's chance for survival and implantation - and ultimately for developing into a child.
An alternative method of improving this evaluation is metabolomic profiling. The type and amount of metabolites used and secreted by the embryo during culture can give an indication of the possibilities for survival and further fetal development. This profiling is carried out in the culture medium after transfer of the embryos to another dish - with other words without interference with the culture processes.

## I-11: The Search for The Best Spermatozoon - A Multi-Regional Study

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Assessment of sperm has over the years by many been regarded as something secondary to assessment of ova, oocytes and embryos. Often the most inexperienced of the laboratory staff have been asked to evaluate the semen sample. It is our belief that the outcome of subfertil-

ity treatment could be improved considerably, if semen and sperm assessment would be taken more seriously. This may not be as simple as it may sound. This lecture will deal with subjective and objective semen analysis. The lack of accuracy and standardization using the older subjective method can make it difficult, if not impossible, for physicians to interpret semen analysis results or to compare results obtained from different laboratories. We will discuss the mistakes, which can inadvertently be made during a subjective analysis, and the advantages - and disadvantages - of an automated computer aided sperm assay (CASA) system.
The total concentration of sperm is fairly easy to determine, but makes only sense, if we know the motility and morphology, both of which can be very hard to determine. If the motility is very poor, we still may be able to use the sperm for ICSI, if we can show that an immotile spermatozoon is actually alive. The presence of antisperm antibodies may prevent fertilization by IUI or IVF, but ICSI may still work. Severe sperm DNA fragmentation can make IUI and even IVF impossible, but again ICSI can maybe lead to success and a healthy baby. DNA fragmentation assays are relatively expensive, but in cases where the sperm sample has a good concentration, good motility, good morphology and good vitality, but does not bring about fertilization, it may be worthwhile to do a DNA fragmentation assay.
Based on this background, an extremely important subject, particularly in patients on the border line of fertility is that it is (wrongly) assumed that a two day period of abstinence is required for both good semen analysis as well as for artificial fertilization/ART procedures. Recent research on humans as well as a very wide group of animals show that an ejaculate obtained relatively few hours after a first ejaculate is better than the first.
A study of the sperm parameters in 2 or 3 consecutive sperm samples obtained within two days from a sufficient number of patients in several populations provides a quantitative (objective) knowledge in terms of reference values for semen/sperm using these newer techniques.

## I-12: The Use of Oxygen Consumption for Oocyte and Embryo Selection

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Assisted human reproduction is characterized by a high variability in results regarding pregnancy and birth rates. In fact, in a cohort of fertilized oocytes from the same stimulation cycle, the development rate varies drastically, even though produced at the same time and under similar conditions. This variability results in large differences in implantation potential of human embryos developed *in vitro*, despite similarities in observable parameters such as embryo development rates and morphology. There is no doubt that embryo morphology (determined by the number, size, and shape of blastomeres, the proportion of fragments, and the presence of multinucleated blastomeres) has some predictive value on development and implantation potential. Some noninvasive methods such as metabolomic profiling are still at initial stages of development, awaiting their verification in daily laboratory practice, but it could be applied in the future with further improvements. One of these noninvasive methods ,which is consolidating at the moment as a good indicator of overall metabolic activity and a valuable parameter for evaluating embryo quality is the $O_2$ consumption (OC), which is directly related with the capacity of an embryo to produce ATP via oxidative phosphorylation, a process that utilizes 30% and 60-70% of the oxygen consumed by the embryo at the early cleavage and blastocyst stages respectively. The purpose of this presentation is to describe new and potentially useful aspects of oxygen consumption as a tentative method for embryo selection.
We evaluated the influence of different stimulation protocols on the oocyte $O_2$ consumption as well as the influence of different oocyte dimorphisms in respiration rates. The next step was to study how the fertilization process might be affected by the oxygen uptake. In the second part of the presentation we have focused our attention on embryos, measuring the OC during 3 days of culture until embryo transfer and evaluating its correlation with embryo development and reproductive outcome.

## I-13: Omega-3 Fatty Acids Accompanied with α-Tocopherol Improved Fresh and Post-Thaw Sperm Quality in Ruminants

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**Background:** The plasma membrane is a highly dynamic structure that regulates extracellular exchange and mediates fertilization. Lipid composition of the sperm plasma membrane is a major determinant of mobility characteristics, cold sensitivity, and overall viability. Species differences in lipid composition of the sperm plasma membrane is a key factor for freezability of the sperm. In several mammalian species, especially ruminants up to 60 % of phospholipid bound the total fatty acids of cells are long-chain polyunsaturated fatty acids (LCPUFAs) especially, DHA. Presence of these LCPUFAs is assumed to impart greater fluidity (less order) within the plasma membrane due in large measure to the presence of the many double bonds. These specific physical characteristics may give membranes greater resistance to damage arising from the formation of ice crystals. DHA may contribute to the membrane fluidity required for the bending sperm tails. Mammalian spermatozoa are sensitive to lipid peroxidation, due to the phospholipid content of sperm membranes with their high PUFA sidechains. Seminal plasma provided some protection against peroxidation via its constituent antioxidants. However, dilution of semen reduces antioxidant availability for sperm. On the other hand, supplementing semen extender with PUFAs during cryopreservation increases ROS production. Therefore, including an antioxidant when adding PUFAs to semen extender is beneficial. The objective of our studies were to investi-

gate combined effects of omega-3 fatty acids (FA) and α-tocopherol (vitamin E, VE) on fresh and post-thaw sperm quality in most important ruminant species including cattle, sheep and goat.

**Materials and Methods:** Two series of experiments were conducted to investigate *In Vivo* and *in vitro* effects of supplementation of omega-3 FA and VE on fresh or post-thaw sperm quality in bull, ram and buck. For *In Vivo* trials, animals were fed diets supplemented with omega-3 FA (fish oil) or/and VE or without any supplements (control) for 2 months. Semen samples were collected, frozen and thawed biweekly. For *in vitro* trials, semen samples were collected and diluted with extender supplemented with different levels of omega-3 FA and VE, and then frozen and thawed. The fresh and post-thaw quality of semen including motion characteristics and viability was evaluated, and FA composition of sperm lipid was analyzed by GC in both trials.

**Results:** The results of *in vivo* trials indicated that combined feeding omega-3 FA and VE improved fresh semen quality in each 3 animal species. Use of VE promoted the results in goat. Feeding FA also increased post-thaw sperm motion characteristics in bulls. The results of *in vitro* trials showed that using FA or/and VE increased motility and viability of frozen-thawed sperm. The best results were observed in groups that received combination of FA and VE. The DHA content and the ratio of omega-3:omega-6 FA of sperm lipid was increased in treated-groups in both trials.

**Conclusion:** Feeding omega-3 FA in ruminants could enhance fresh semen quality, and α-tocopherol promoted this effect. Moreover, supplementation omega-3 FA accompanied with α-tocopherol during cryopreservation improved post-thaw quality of bovine, ovine and caprine sperm. These effects were apparently mediated by alteration of sperm lipid composition.

***Keywords:*** Semen, Omega-3 Fatty Acids, Vitamin E, Cryopreservation, Ruminants

# Ethics and Reproductive Health

## I-14: Therapeutic Abortion Law in Iran: Past, Present and Future

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Although the definition of abortion is pregnancy loss under 20 weeks of gestational age, in Islamic law, termination pregnancy in any gestational age is considered abortion "SEGHT JANIN". Also only "induced abortion" has legal validity, and other kinds of miscarriage (recurrent, missed,…) have no legal or ethical issue.

In Iran, Islam is the base of the law. Article 4 of Iran constitution says: "All civil, penal financial, economic, administrative, cultural, military, political, and other laws and regulations must be based on Islamic criteria." And also according to the article 167, in case of the absence of the law, judges have to deliver their judgment on the basis of authoritative Islamic sources and religious scholars' decrees (fatawa). So, every law is originated from decrees.

In 1997, supreme leader Ayatollah Khamenei, released a decree to allow abortion of fetuses with Beta thalasemia major. This decree began a procedure leading to the first "therapeutic abortion regulation" released on 2002 by Iran forensic medicine authority. Then in 2005, "Therapeutic abortion law" was passed by parliament accepting the induced abortion under some circumstances. The law says: "Therapeutic abortion with confirmation of three specialists and confirmation of the forensic medicine authority of fetal abnormalities or fetal mental disorders as burden (HARAJ) for the mother or mother's disease which puts her life in danger, before ensoulment (4 months) with the mother's consent is acceptable and no legal responsibility is on the operating physician." Although the law has solved so many problems of pregnant women, it seems to be incomplete and does not cover all the situations. The main lack of the law is the definition of "burden" and weather it is subjective or objective. It seems that in the law, the burden is considered "objective", so, three specialists and forensic medicine authority must approve the situation and permit the abortion. But here we have "problem of criteria". There are no criteria upon which we can verify the burden on someone and till now, there is no official executive guideline for this law. So, for pregnancy by rape or incest, with huge stigma and burden on the victim, nobody gives the abortion permission. Another lack of the law is the limitation of abortion to just under the four months of gestational age (ensoulment), even if the life of the mother is in danger. Although currently pregnancy is terminated in any gestational age in the case of the threat of mother's life, but according to the law after four months it is completely illegal. The future of this law must include the definition of burden and its criteria and also deletion of the ensoulment limitation for when the mother's life is in danger.

The therapeutic abortion law could solve many problems in Iran, but it seems that the law is not complete and must be revised.

***Keywords:*** Abortion, Therapeutic Abortion, Law, Iran, Ensoulment

## I-15: Ethical Issues in Sex Selection and Family Balancing

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Sex selection for non-medical reasons is a major controversy in around the world. Here in this paper, we presented the ethical arguments about sex selection for non-medical reasons and discuss about them in Islamic context. Significant arguments about sex selection relate to sexism and sex discrimination, sex dispropor-

tion, human dignity, playing God, psychological pressure on unselected children and other ethical issues. Main responses includes: parental autonomy, compare with their authority in using contraceptive methods or cosmetic surgery; utilitarianism and consequentialism that practically prefers sex selection that abortion; sex ratio can be protected by some legislations. Islam underscores the will of God in any human action so no one can play God and unlike Christians, the vision of Islam to the pre-implantation embryo is not a human. Our conclusion from religious, legal and ethical survey is: sex selection in family balancing type is acceptable in Islamic context with the following conditions: only for married couples, only one successful time, not for the first child not for same sex. Detail counseling should be performed with the couple about the risks and success rate. If both genders exist in family, sex selection should not be performed. If the selected child dies with any reasons, another attempt is acceptable. Sex selection clinics should act under supervision of health care authorities.

***Keywords:*** Sex Selection, Family Balancing, Ethics, Legal, Islam

# Female Infertility

## I-16: Quality of Life in Patients with Endometriosis

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

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

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

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

Even with advance surgery and medical treatment there is a real chance of recurrence of the disease and this subject should be discussed with the patient. She should be advised about this chronic disease that potentially affect her quality of life and should be informed about the potential complications of the disease and medical or surgical treatments. Coping with endometriosis as a chronic disease is an important component of management. Psychiatric consult may be helpful in those patients with intractable pain and those with depression following to the disease.

## I-17: The Immunological Aspect of Implantation Failure

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The success of reproduction, although gradually increasing over the years, many couples also had been left frustrated following repeated failed attempts. Clinicians who treated unsuccessful couples often face a challenge.

The probable causes of repeated IVF failures classified as: reduced endometrial receptivity, embryonic defects or multifactorial causes. Intrauterine and endometrial integrity abnormalities such as thin endometrium, altered expression of adhesive molecules and immunological factors like anti sperm (ASA), Anticardiolipin (aCL), Lupus anticoagulant (LA), anti-phosphatidylserine (aPS) , anti-phosphatidylethanolamine (aPE), and antinuclear antibody (ANA), anti DNA, anti zona and anti ovarian (AOA) antibodies, thrombophilia, decreased expression of endometrial integrins, increase of natural killer cells activities and imbalance of cytokine networks (balance between IL-12 and IL-18) , may decrease endometrial

receptivity, whereas chromosomal and genetic abnormalities of the male sperm or female ovarian defects, embryonic aneuploidia or zona hardening are embryonic reasons for the failure of implantation. By the way Endometriosis, non fixed stimulation protocols and hydrosalpinges may adversely influence both . It will be discussed all aspects of implantation failure specially endometrial and immunological reason .

***Keywords:*** Immunology ,Implantation Failure, Endometrium

## I-18: Should All Women with PCOS-Related Infertility Be Treated with Insulin Sensitizing Drugs?

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Interest in the role of insulin sensitizing drugs (ISDs) as a means of reducing compensatory hyperinsulinemia in the hope of improving metabolic and reproductive functions in women with PCOS has grown measurably over the past decade. Metformin is believed to lower fasting serum insulin levels in insulin-resistance.

For the management of ovulatory infertility in the non-obese PCOS population (BMI < 30kg/$m^2$), there is moderate-quality evidence demonstrating that metformin monotherapy improves the odds of ovulation and chance of achieving clinical pregnancy.

There is moderate-quality evidence demonstrating the absence of reproductive benefit when metformin is combined with CC therapy in the non-obese population of women with PCOS-related subfertility. CC-alone therapy remains the mainstay pharmacological therapy for this group.

For obese women (BMI ≥ 30 kg/$m^2$) with PCOS-associated subfertility, there is low-quality evidence showing the failure of metformin monotherapy to improve reproductive endpoints. In view of the considerable side effect profile, metformin monotherapy may not be recommended for fertility management in this group of women.

There is moderate-quality evidence to support a beneficial effect of metformin in combination with CC therapy in increasing the likelihood of ovulation and clinical pregnancies. For women with CC-resistant subfertility, there is moderate-quality evidence to support that metformin co-treatment increases ovulation rates. There is also low-quality evidence demonstrating that metformin/CC combination therapy may be associated with higher live births than laparoscopic ovarian drilling. Women with CC-resistant PCOS may be given the benefit of a trial of medical ovulation induction using combination therapy prior to committing to the more invasive and expensive alternative of LOD.

For women with PCOS undergoing IVF/ICSI treatments, there is moderate evidence to support the failure of metformin co-administration to improve the clinical outcomes of live births, clinical pregnancies, or miscarriages. There is moderate evidence demonstrating a significant reduction in the risk of OHSS with metformin co-treatment, when hCG is used to trigger final oocyte maturation.

## I-19: Techniques and Technologies for Embryo Transfer: Does It Really Matter.

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The learning objectives of this presentation are to understand the dynamics involved in the process of ET, evaluate the evidence for/against common practices and techniques and develop a standardized ET process in view of supporting evidence.

Gametes and embryos are handled with extreme care at every step of the Laboratory process. ET is the least sophisticated step of the *in vitro* Fertilization process, making it the most vulnerable. The working hypothesis is that designing a Standardized Embryo Transfer Approach is expected to maintain higher pregnancy rates and lower adverse effects.

Experimental findings suggest that positioning of the patient while keeping the uterine fundus at the highest point in the sagittal cross-section above the horizon, placement of the catheter tip at mid cavity about 2.0 cm from the fundus and delivery of the load over a course of 10 s or more, may be associated with the desired outcome.

Evidence supports: a definite proof of benefit for the use of soft embryo transfer catheters and ultrasound guidance; a limited proof of benefit for mid-uterine position of catheter tip, acupuncture, use of hyaluronic acid , shortening of the loading discharging interval time of embryos and mechanical closure of the cervical canal following ET; and no proof of benefit for the use of mock transfer prior to ET, antibiotic administration, full bladder, removal or flushing of the cervical mucus, use of fibrin sealants and bed rest following ET.

## I-20: The Future of Medical Education: from The Classroom to i-tunes

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Medical training has been lately the subject of intense scrutiny. The knowledge transfer approach has shifted focus on the trainee as an active participant in the education process. The traditional view that learning stems from the transmission of knowledge, has recently been challenged. Although controversial, some suggest that a student can maximize this learning process when educators tailor educational activities to complement each trainee's learning style. Recognition of these different learning styles within individuals and educational systems therefore is expected to improve the efficiency of the learning process, promote long-term retention, and application of newly acquired knowledge. This view undermines the conventional approach to education that

assumes that learners are uniform in processing and organizing information.
Classroom-based post-graduate training is a dominant feature of academic health-related disciplines. These activities range from local institutional meetings to large international congresses. In theory, these meetings aim to disseminate and advance medical knowledge. Although these are worthy goals, there is virtually no evidence supporting the utility of most large conferences if we exclude small-group brainstorming gatherings. In the cyber era in which information can be shared instantly free from time and space restrictions, the contribution of the classroom-based meeting to the dissemination and advancement of science becomes less relevant. It is therefore reasonable to exert more efforts in the direction of exploiting the advantages offered by new information technologies, mainly web-enhanced computer-aided learning.

## I-21: The Selective Vitamin D Receptor Agonist Elocalcitol Reduces Development of Endometriosis and Formation of Peritoneal Adhesion in A Mouse Model

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**Background:** Endometriosis is a chronic disorder characterized by the presence of endometrial tissue outside the uterus. Endometrial cells from retrograde menstruation implant on peritoneal surfaces and elicit an inflammatory response, associated with angiogenesis, fibrosis, neuronal infiltration, and anatomical distortion. Affecting an estimated 176 million women worldwide, the condition is still an unmet clinical need since an optimal drug that allows for pain management and continued attempts to conceive does not exist. An ideal treatment should not only eradicate lesions but also prevent post-surgical recurrences and eliminate peritoneal adhesions Since both eutopic and ectopic endometrium express the vitamin D receptor (VDR), and VDR agonists are endowed with anti-proliferative, anti-inflammatory, and anti-fibrotic properties, we evaluated the effect of elocalcitol, a VDR agonist with low calcemic liability, in a mouse model of experimentally induced endometriosis.
**Materials and Methods:** Endometriosis was induced by injection of syngeneic endometrial tissue fragments into adult Balb/c female mice. Mice were administered with elocalcitol (100 µg/kg) or vehicle orally, once a day, starting at different times after disease induction, and sacrificed at day 15 or 21. Peritoneal lesions and adhesions were evaluated by an operator blind to the experiment. Lesion extent was evaluated by weighting of dried lesions. Adhesion extent was evaluated by taking into account the number and the extent of adhesion sites. Cell adhesion assaye were performed on ECM-coated plates. Peritoneal macrophages were isolated and analysed for cytokine secretion by specific ELISA assays. Differences between groups were compared by Student's paired t test or the Kruskal-Wallis test as appropriate. Probability <0.05 was considered as statistically significant.
**Results:** Mice with induced endometriosis were administered with elocalcitol (100 µg/kg) or vehicle orally, once a day, for different times. In this model, elocalcitol reduced total lesion weight up to 70% upon treatment for one week before and two weeks after disease induction. Peritoneal adhesions were not detected in elocalcitol-treated mice. Interestingly, a therapeutic effect was also observed on already established lesions. Elocalcitol was shown to reduce the capacity of mouse endometrial cells to adhere to collagen. In addition, a decreased state of peritoneal inflammation in treated mice was demonstrated by the inhibition of macrophage recruitment and inflammatory cytokine secretion.
**Conclusion:** The VDR agonist elocalcitol inhibits lesion development in a validated mouse model of endometriosis, and exerts a protective effect on both the implantation and organization of transferred endometrial tissue. The realistic objective to suppress, rather than eliminate implant growth, can be achieved with an anti-inflammatory drug such as elocalcitol that may represent a safe treatment in limiting the growth of pre-existing lesions and treat recurrences. Further experiments using primate models as well as clinical trials will be helpful in evaluating the therapeutic potential of elocalcitol in women with endometriosis. Based on the results of this study, a possible translation into the clinical setting would be to administer elocalcitol during the perimenstrual and menstrual phase of the cycle. In this phase, all the potential activities of the compound (inhibition of inflammation, inhibition of endometrial cell adhesion, inhibition of lesion organization) could be exerted with the maximal efficacy.
***Keywords:*** Endometriosis, Inflammation, Adhesion, VDR Agonist

## I-22: Management of Endometrioma

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Endometrioma is defined as an ovarian pseudocyst arising from growth of ectopic endometrial tissue, which progressively invaginates the ovarian cortex . Nearly17-44% of women with endometriosis also have endometriomas. Whereas detection of peritoneal endometriosis and adhesions typically requires laparoscopic assessment of the pelvis, endometriomas can be reliably diagnosed by transvaginal ultrasound scan . The expanding ovarian cyst may cause pressure atrophy of the ovarian tissue or affect the normal vascularization of the ovary. Nakahara et al. (1998) found a higher incidence of apoptotic bodies in the ovarian membrana granulosa of patients with endometriosis than that of a control (male factor infertility) group. The incidence of apoptotic bodies correlated with the stage of endometriosis but was significantly higher in women with endometrioma. It has also been suggested by some investigators that oocyte quality

may be affected by endometrioma.
Currently, there is insufficient data to clarify whether the endometrioma-related damage to ovarian reserve precedes or follows surgery. In conclusion, the standard management of endometrioma in subfertile women before IVF remains controversial owing to the insufficient evidence to suggest superiority of one treatment strategy over another. A large, well-designed, adequately powered multicenter RCT that would compare the effects of surgical removal with expectant management of endometrioma on ovarian performance and pregnancy outcomes in women undergoing IVF is clearly overdue. Until such a trial is conducted and definite conclusions can be drawn, the management of women with endometrioma before IVF should be individualized. All the therapeutic options, including conservative, medical, or surgical treatment, as well as the advantages and disadvantages should be fully discussed with the patient. Any decision for surgery should be carefully considered and balanced against the risks, especially in women with previous adnexal surgery or women with suboptimal ovarian reserve. If the woman opts for surgical treatment, she should be appropriately counseled about the potential risks of reduced ovarian function after surgery, including the remote possibility of oophorectomy.
The realities mentioned above about the diagnosis and treatment of endometrioma besides the existence of some evidence to suggest that untreated endometriosis may resolve spontaneously in up to a third of women, and the fact that Subfertile women with endometrioma comprise a small group among a heterogeneous population of women who suffer from endometriosis urges a comprehensive evidence based discussion about four groups of endometriotic patients:
1. Management of endometioma in patients with pelvic pain
2. Management of endometioma in asymptomatic patients
3. Management of endometioma in subfertile patients
4. Management of endometioma prior to IVF.

## I-23: Fertility Preservation and Ovarian Stimulation in Cancer Patients

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Cancer is not uncommon and no longer considered to be an incurable disorder. 10% of cancer cases occur under the age of 45. There is a remarkable improvement in treatment and survival rates. Today women have been delaying initiation of childbearing because the incidence of most cancers increases with age. Delayed childbearing results in more female cancer survivors. As a consequence there is an increase in the number of patients surviving cancer interested in fertility preservation. There are strategies aiming to preserve fertility in women with different types of cancers these include oocyte and embryo cryopreservation, cortical and whole ovary cryopreservation, ovarian transplantation, ovarian transposition, and GnRH agonist protection. Embryo or oocyte cryopreservation is the most preferred option for fertility preservation in cancer patients, due to its higher success rates. And other modalities are more experimental.
Malignancy affects other tissues throughout the body and can result in a variety of complications during ovarian stimulation. Ovarian stimulation protocol and gonadotropin dose requires an individualized approach. There are mixed reports about how cancer patients respond to the ovarian stimulation but it seems risk of inadequate response leading to cycle cancellation is higher in these patients. In patients with diminished ovarian reserve higher doses of gonadotropins required. Different simulation protocols are proposes, majority of them treated with a GnRH antagonist. Some cases, owing to the urgency of the cancer treatment should not to wait for the next menstrual cycle, so random-start stimulation protocols have been suggested. During ovarian stimulation, a potential risk that the higher Estrogen evels may have adverse affect on the Estrogen -sensitive tumors. This lecture highlights the new stimulation protocols and strategies aiming to reduce time constraints and emphasizes management considerations to reduce complications.

## I-24: Hysteroscopic Complications

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Hysteroscopic complications are infrequent. Major complications include:
1. Uterine perforation that is the most common complication of operative hysteroscopy (0.8 - 1.6 %). The perforation rate is less during diagnostic hysteroscopy.
2. Bowel or bladder injury which is rare, but may occur in association with uterine perforation or as a result of use of electrical current.
3. Cervical lacerations which can occur, particularly in women with cervical stenosis in post menopausal patients, or pre operative use of GnRha. Misoprostol is a promising cervical ripening agent used before hysteroscopy in premenopausal women, but its role in postmenopausal women is yet to be determined
4. Excessive fluid absorption that is related to distending media vary according to the patient status and the media used.
5. Embolism (air or carbon dioxide) that can occur with any hysteroscopic technique and can cause cardiovascular collapse.
6. Hemorrhage that is common causes of bleeding and are operative sites bleeding, uterine perforation, and cervical laceration. Continuous bleeding can be treated by placing a Foley catheter in the uterine cavity and then distending the bulb with 15 to 30 mL of normal saline.
7. Electrosurgical injury that is thermal effects of electrical (or laser) energy and can cause injuries to the uterine cavity, as well as bowel, urinary bladder, and large pelvic vessels. One must be cautious if coagulating in the tubal recesses. Electrode insulation defects can also cause thermal injury.
8. Infection which its risk after operative hysteroscopy is low.

9. Dissemination of tumor in which iatrogenic positive peritoneal washing does not increase mortality.

## I-25: Individualized Controlled Ovarian Stimulation (iCOS)

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**Background:** With the recent development of recombinant gonadotropins (FSH and LH), it has become possible to further adjust the stimulation protocol according to the expected needs of the patient. In this respect, the possible beneficial role of exogenous LH activity supplementation for stimulated ART cycles has received increasing attention. According to the two-cell, two- gonadotropin theory, both FSH and LH are required for normal folliculogenesis in humans. LH stimulates the production of androgens in the theca cells, which in turn are aromatised to estradiol by the granulosa cells under the action of FSH. However, at a follicle size of 8-10 mm in normogonadotropic women, the granulosa cell also acquires LH receptors in addition to the FSH receptors, already present. Once LH receptors are expressed in the granulosa cell, LH is able to regulate both steroidogenesis and growth of the follicle; thus, from this moment on FSH function can to a large extent be replaced by LH activity.

**Materials and Methods:** During recent years an increasing body of scientific evidence has raised the question whether the endogenous LH level achieved after down-regulation with either GnRHa or GnRH antagonist is really optimal for all patients, or whether sub-groups of patients exist who might benefit from exogenous LH supplementation.

Several studies have until now addressed the effect of LH activity supplementation. The results of these studies indicate that two subgroups of normogonadotropic patients: patients > 35 years of age and patients with an initial sub-optimal response to FSH only preparations seem to benefit from modifications of the stimulation protocol in terms of exogenous LH activity supplementation. Possible biological reasons for a beneficial effect of LH activity supplementation in these sub-groups will be discussed as well as molecular, structural and functional differences between LH and hCG.

Finally, the importance - or not of late follicular progesterone rise during COS will be debated.

**Conclusions:** Age and LH gene polymorphisms and are some of the factors known until now to influence the ovarian response after COS. LH supplementation in sub-groups seems to improve the ovarian response and the reproductive outcome. Ovarian response to stimulation with FSH is a polygenic trait and the future scenario of ART will include pharmacogenetics in order to define the specific needs of gonadotropins to secure the most optimal ovarian response.

## I-26: GnRHa Trigger State of the ART - Towards the OHSS Free Clinic

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Human chorionic gonadotropin (hCG) has been used as a surrogate for the mid-cycle LH surge for several decades. Due to structural and biological similarities with LH, hCG binds to and activates the same receptor - the LH/hCG receptor. However, despite the fact that hCG effectively secures final oocyte maturation and ovulation, its use as a surrogate for LH has got several drawbacks - first and foremost a sustained luteotropic effect, facilitating ovarian hyperstimulation syndrome (OHSS).

Recently GnRH antagonist protocols for the prevention of a premature LH surge were introduced, allowing final oocyte maturation to be triggered with a single bolus of a GnRH agonist (GnRHa). GnRHa is as effective as hCG for the induction of ovulation, and in addition to the LH surge a FSH surge is also induced, resembling the natural mid-cycle surge of gonadotropins. The first prospective randomized studies reported a poor clinical outcome when GnRHa was used to trigger final oocyte maturation in IVF/ICSI, due to a luteal phase deficiency, despite standard luteal phase supplementation with progesterone and estradiol.

As GnRHa triggering of final oocyte maturation possesses advantages over hCG triggering in terms of a reduced, if not eliminated risk of OHSS, the retrieval of more mature oocytes, and a higher patient convenience, the challenge has been to rescue the luteal phase. The development of the present protocol for luteal phase rescue after GnRHa trigger, employing a so called modified luteal phase support will be presented. The paramount aim has been to improve pregnancy rates after GnRHa trigger without increasing the OHSS rate.

Although fine tuning of the luteal phase support is still possible, GnRHa triggering is now a valid alternative to hCG trigger with potential benefits.

MC questions

GnRHa trigger elicits a surge of gonadotropins with duration of:

1. 48 hours
2. 20 hours
3. 24-28 hours
4. 40 hours
5. < 20 hours

GnRHa trigger may be used in:

1. All patients
2. PCOS patients only
3. At a smaller follicular size than HCG trigger
4. Patients transferred without a modified luteal phase support
5. All patients except the hypo/hypo patient.

## I-27: IVF Scenario in India

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The development of assisted reproductive technologies (ARTs) in India began with efforts to create "test tube babies" with *in vitro* fertilization in the 1980s. But rather than commit public funding to advance such research, the Indian government withheld the use of government funds for research on human embryos, largely as a by-product of the debate over population control. India with a population of 1.2 Bn and ticking also represents underline a major problem of infertility and it is quite paradox that country with large amount of population also has a large section of people who does not have a child. ARTs encompass various procedures ranging from relatively simple intra uterine insemination (IUI), IVF, ICSI, IMSI, Cryo Preservation and Vitrification. Surrogacy which is not a technique but n arrangement is also included under the umbrella term of ARTs.

In India there is an unprecedented and unregulated growth of ART clinics providing IVF procedures over the years within the framework of medical tourism. IVF is the latest addition. Low cost, easy access and easy availability and economical prices of IVF drugs has fueled further growth. India is estimated to be doing currently 80000 cycles per year. To be more precise it is estimated that 70000 to 85000 Ovum pick up and 90000 to 95000 as embryo transfer and it is expected that the growth of IVF in India is growing at the rate of 10-15% every year. The estimated number of clinics in India is almost 500 and going up every day and with such high growth and potential in IVF segment has attracted close attention of the Government. Also easy availability of Surrogate mother, gamete donors have made India a favoured destination for various IVF procedures especially Surrogacy . The resulting surge of the ART Industry in the country has posed a number of ethical, legal, social and moral dilemmas and the Government is working towards bringing regulation in form of all ART regulation bill and rules-2008 which is drafted by Ministry of Health and Family welfare and the Indian Council of Medical Research. It is important and welcome step in the direction. New technology introduced by one clinic is quickly offered by others as a matter of survival. But unlike other areas of medicine, in which new therapies are developed after controlled research in humans, ARTs often are introduced directly from the lab as clinical services for patients. Data are collected as patients are treated with untested new approaches, creating the only area of medicine where patients come for treatment but in reality pay for the privilege of being research subjects. The irony is hard to ignore:

The research protection policies applied elsewhere in medical research were driven by efforts to prevent exploitation of the vulnerable—yet patients confronting infertility are often the most vulnerable.There are a few straightforward ways to bring assisted reproduction into the fold. Research in assisted reproduction should receive the same sort of approval and oversight as government funded research. Such policies are long overdue. Bringing ARTs into the open will better serve patients and improve research oversight.

## I-28: Risk Factors of Endometriosis in Iranian Infertile Women

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**Background:** Endometriosis as one of the most common health problems of women during their reproductive age, affects 2.5-3.3% of women. Endometriosis may be asymptomatic or associated with dysmenorrheal symptoms, dyspareunia, pelvic pain, abnormal uterine bleeding and infertility. The objective of present study was to determine the risk factors related to endometriosis among Iranian infertile women.

**Materials and Methods:** In this case control study, infertile women who referred for laparoscopy to two infertility clinics in Tehran-Iran were enrolled. According to laparoscopy results,infertile women were divided into case and control groups. Control group consists of women who had normal pelvis in laparoscopy while women who had endometriosis considered as case group. The case group was divided into 2 subgroups: Mild (stage I and II of endometriosis) and severe (Stage III and IV). A questionnaire consists of demographic, menstrual and reproductive characteristics was completed for each one of the patients. The significant level was defined as p value less than 0.05.

**Results:** In present study, 403 infertile women were studied. Among them, 250 subjects (62%) had endometriosis (case group) and 153 (38%) had normal pelvis (control group). This study revealed that age, duration of infertility, BMI, duration of menstrual cycle, abortion history, dyspareunia, pelvic pain and family history of endometriosis are the independent predictive factors for any type of endometriosis. In addition, it was shown that education, duration of infertility, BMI, amount and duration of menstrual bleeding, menstrual pattern, dyspareunia, pelvic pain and family history of endometriosis are the independent predictive factors of severe endometriosis.

**Conclusion:** Demographic, menstrual and reproductive characteristics of infertile women could predict any type of endometriosis and also severe ones .

***Keywords:*** Endometriosis; Infertility; Risk factors; Laparoscopy

## I-29: The Effect of Coasting Duration on Oocytes Retrieved in ART Cycle

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**Background:** Coasting is one of the most common strategies which has been proposed for preventing ovarian hyperstimulation (OHSS) in overstimulated cycles. However, the debate on the effect of coasting duration (days) on ART cycles outcome is ongoing. This study aimed to assess the influence of coasting duration in oocytes number and quality and fertilization rate in patients with male factor infertility (normal female) and PCOS.
**Materials and Methods:** In this prospective cross-sectional study of IVF/ICSI cycles, using long protocol, seventy -seven patients undergoing coasting (fifty-three women with male factor infertility and twenty-four PCOS patients) were evaluated at Royan Institute between July 2011 and December 2012. Coasting started when the estradiol level was more than 3000 pg/ml in the presence of at least 15 follicles with size of 14 mm. The results were analyzed according to the coasting periods of 1 to 4 days. The Statistical comparisons were performed using one- way ANOVA, Kruskal-wallis, Chi-square and Fisher's exact tests.
**Results:** There were no statistical between-group differences in age, BMI and hormonal parameters. There was a statistically significant decrease in the number of oocytes retrieved and MII oocytes in coasting periods of 1 to 4 days in normal female patients. No significant differences were observed in the number and quality of oocytes retrieved with regard to coasting days in PCOS patients.
**Conclusion:** In normal female patients, prolonged coasting may affect the number and quality of oocytes, anticipating fertilization rate.
***Keywords:*** Coasting, Ovarian Hyperstimulation Syndrome, IVF/ICSI, Outcome, PCOS, Male Factor Infertility

## I-30: Luteal Phase Support in Frozen-Thawed Embryo Transfer Cycle

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Cumulative pregnancy rate has been significantly increased since frozen-thawed embryo transfer was applied in ART cycles. This method has become an essential part of IVF/ICSI treatment. Luteal phase support has been proven to be associated with higher rate of live birth rate. Human chorionic gonadotropin (HCG), and progestrone have been successfully used for luteal phase support in ovarian stimulated ,fresh embryo transfer. Giving luteal phase support in frozen- thawed embryo transfer is still a debate. Since frozen-thawed embryo transfer is performed either in natural or pituitary suppressed cycle, it is important to determine the necessity of luteal phase support. For natural cycle in which ovulation is documented and corpus luteum exist, there is no physiological basis for luteal phase support.

## I-31: Prevalence of Metabolic Syndrome in Women with Polycystic Ovary Syndrome

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**Background:** Metabolic syndrome is a disorder which is composed of several metabolic abnormalities: glucose intolerance, dyslipidemia, obesity and hypertension. PCOS which is characterized by menstrual irregularities and hyperandrogenism, is a common endocrinological disorder in female reproductive age. The prevalence of the syndrome is 5-10%. Insulin resistance is a common feature of the PCOS (60-65%), both obese and non-obese women. Insulin resistance and hyperinsulinemia are also the important pathophysiology of MS. Recent data have shown that the prevalence of MS among PCOS women is 43-46%, which is higher than that reported for age-matched women from the general population. The aim of the present study was to determine the prevalence of MS in Iranian women with PCOS using the NCEP-ATPIII criteria.
**Materials and Methods:** This cross-sectional study was conducted in 2012. 624 women with PCOS who consecutively attended the reproductive endocrinology and infertility unit of Royan institute were enrolled in this study. The rotterdom criteria was used for the diagnosis of PCOS. Women aged>40 and who used contraceptive drugs at 3 months prior the study, excluded from the study. NCEP ATPIII criteria for DX of MS: 3 of the following disorders: 1- hypertension: BP≥130 mm/hg and/or current antihypertensive therapy. 2-dyslipidemia: triglyceride level≥150 mg/dl and/or HDL level<50 mg/dl. 3-obesity: waist circumference≥88cm. 4-FBS≥100mg/dl.
**Results:** The prevalence of MS (3 criteria) was 19.7%. 1/5 of PCOS patients had abnormal glucose metabolism. Dyslipidemia is the most frequent metabolic disorder in PCOS patients (>70%).
**Conclusion:** Metabolic syndrome was no more frequent in Iranian infertile PCOS patients than in Iranian normal population.

## I-32: New Treatment in PCOS

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Polycystic ovary syndrome (PCOS) is now recognized

as an important metabolic as well as reproductive disorder conferring substantially increased risk for type 2 diabetes. Affected women have marked insulin resistance, independent of obesity.
Prolonged (6 months) medical therapy for hirsutism is necessary to document effectiveness . Many drugs used for the treatment of hirsutism are not FDA approved for this indication.
No effective treatment for alopecia is known. Anti-androgens should not be used without effective contraception. Flutamide is of limited value because of its dose-dependent hepatotoxicity. Drospirenone in the dosage used in some OCs is not antiandrogenic. The best medical therapy and long of treatment for hirsutism is unclear. Measurement of serum androgens is fraught with error but is the best estimate we have for hyperandrogenism. Overall, the benefits of OCPs outweigh the risks in most patients with PCOS. Women with PCOS are more likely to have contraindications for OCP use than normal women. In the absence of other risk factors, there is no evidence that women with PCOS are at increased risk of cardiovascular disease (CVD) with OCP treatment compared with normal women. There is no evidence for differences in effectiveness and risk among the various progestogens and when used in combination with a 20 versus a 30 mg daily dose of estrogen. OCPs do not negatively affect subsequent fertility. There is no definitive evidence that the type of OCP determines efficacy of hirsutism control.
Statins can reduce the concentration of total testosterone, Total Cholesterol, TG and LDL. However, it cannot be concluded that statins have long-term benefit. Efficacy and safety of newer drugs for treatment of type2 DM in PCOS (including GLP-1 agonists) is unclear.
The efficacy of bariatric surgery and its long-term effect is unclear.

### I-33: Management of Diminished Ovarian Reserve

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The standard goal of all fertility treatments is the improvement of pregnancy rates in infertile patients. Within the last 20 years, ovulation induction has contributed to the success of assisted reproduction techniques e.g. *in vitro* fertilization (IVF) and embryo-transfer (ET). The efficacy of these techniques depends on a personalized protocol of controlled ovarian hyperstimulation (COH) and adequate oocytes recruitment. The response to common ovarian stimulation protocols is not always as expected. Failure to respond adequately to standard protocols and to recruit adequate follicles is called ' poor response ' in these patients although it is highly correlated with age. However, in younger women it might be associated with advanced endometriosis or prior ovarian surgery, gonadotoxic chemotherapy, ovarian radiation, pelvic infection and certain genetic conditions (including mosaic turner syndrome, FMR1 pre-mutation carriers) which are common causes of diminished ovarian reserve (DOR).
Several tests have been suggested but none of them has significant value to predict DOR. Furthermore, an accurate diagnostic test of low ovarian reserve would help the clinicians to use the most suitable COH protocol and resulted in declining the cost and psychological decay. However, the ideal stimulation for diminished ovarian reserve still is great challenge for the clinician and patient.

## Genetics

### I-34: Pharmacogenetics of Reproductive Medicine

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Adverse drug reactions (ADRs) are a major problem in drug therapy and drug development. Inter-individual genetic differences can have significant roles in determining an individual's susceptibility to ADRs. The rapid development of techniques in the area of genome analysis has put the scientific community in a power position and facilitated identification of new pharmacogenomic biomarkers that can provide predictive tools for improved drug response and fewer ADRs. Such biomarkers mainly originate from genes encoding drug-metabolizing enzymes, drug transporters, drug targets and human leukocyte antigens.
Understanding pharmacogenetic (PG) differences in drug response and tolerability has been an important area of research in personalized medicine, but the clinical utility of PGs testing has not been established very well. Identification of genetic polymorphisms due to single nucleotide polymorphisms is the most common approach.PG studies are committed to selecting the best therapy for every patient with a minimum risk of complications. Furthermore, these studies allow the development of clinical tests based on the presence of profiles of biomolecules and other biological markers useful for routine diagnosis.
Since genetic variations play an important role in reproductive medicine, pharmacogenetics studies open a new field to modify and develop the treatments of infertile couples. For instance, the application of PG to assisted reproductive techniques (ART) will help clinicians to improve the efficacy of hormone treatments that are being routinely applied during ART protocols. As an example, FSH- and estrogen-receptors are genetic markers involving controlled ovarian hyperstimulation as clinical studies have demonstrated that the p.N680S polymorphism of the FSH-receptor gene determines the less ovarian response to FSH stimulation in patients undergoing IVF. Consequently, pharmacogenetics can assist physicians with prescribing medicine to achieve the controlled ovarian stimulation.

## I-35: NRY Haplotype Analysis: towards A Better Understanding of The Genetic Basis of Spermatogenic Failure

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It has been established that the Y chromosome carries genes required for spermatogenesis and male fertility. For many decades worldwide screening for gene identification has been conducted in research laboratories. However, it has been a difficult process in identifying such genes (i.e. causative mutations) which could explain the phenotypic variation and could be potentially used as markers for diagnostic purposes. Since the genetics of Y-linked male infertility is still vague and that the AZF regions on Y chromosome have experienced duplication events resulting in repetitive sequence, we discuss a long-range haplotype-based analysis of azoospermic patients in order to identify modal haplotypes strongly associated with azoospermia. The haplotypes will be based on standard UEP and STR markers in the non-recombining region of the Y chromosome (NRY). This approach has the potential of developing a diagnostic DNA screening test for azoospermia.

## I-36: Genetic Aberrations in Early Development: The Origins and The Fates

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Genetic aberrations are commonly seen in human preimplantation embryos. Non-disjunction and premature division of a chromosome are common in both meiosis and mitosis divisions. The expected result for meiotic aneuploidies is full aneuploidy in the later stages whereas mosaicism is the most frequent event in the cleavage and blastocyst stages. The main causes for mosaicism are post-zygotic events during early mitotic divisions of the embryo most particularly at the cleavage stage because of genome activation by third cell division. Segmental chromosome aneuploidies following chromosome breakage, blastomeres fusion and errors in cytokinesis are frequent events in the cleavage stage. Changes in culture conditions and hormonal stimulation protocols could affect chromosome segregation. Diploid-aneuploid mosaicism is the most frequent abnormality observed in preimplantation stage. The analysis of a number of cells cannot guarantee omission of all mosaic embryos; hence, embryos selected by preimplantation genetic diagnosis at the cleavage or blastocyst stage could be partly abnormal. Several reasons have been proposed for self-correction of aneuploidies during later stages of development including primary misdiagnosis, allocation of the aneuploidy in the trophectoderm, cell growth advantage of diploid cells in mosaic embryos, lagging of aneuploid cell division, extrusion or duplication of an aneuploid chromosome, and the abundance of DNA repair gene products. Differentiation is known as the barrier for eliminating mosaic embryos by death and/or decreased division of abnormal cells, there would be selection based on the type and rate of mosaicism in each cell line. However some mosaicisms, such as copy number variations could be compatible with live birth and might result in differentiation advantage. The genetic difference between discordant monozygotic twins provides evidence for such mosaicisms. There are three outcomes for mosaic embryos following differentiation: abortions, birth defects and healthy newborns. Self-correction may rarely occur as a result of the advantage that diploid cells have for survival and division. More information will be discussed in this presentation.

## I-37: The Necessity of Genetic Screening in Premature Ovarian Failure and Diminished Ovarian Reserved Patients

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Variability in the subfertile patient population excludes the possibility of a single approach to controlled ovarian stimulation (COS) covering all the requirements of a patient. Modern medical science has made great advances in the understanding and the development of new drugs, treatment options and quantitative methods that can identify single patient characteristics. Factors that reduce follicle or defect in the follicle growth stimulating mechanism defined as numerous complication factors that they can cause premature ovarian failure (POF) or diminished ovarian reserve (DOR). According to reports several genetic factors considered to cause these conditions. Genes on the X-chromosome and autosomal genes are detected in these disorders too. FMR1 gene that is on X-chromosome is one of the important genes which is related to POF and also DOR. Premutation in this gene are more common in these patients. Subsequently, screening for FMR1 premutations is recommended for the routine work-up for any woman presenting with POF and DOR. The reason for this is these women need to be informed if they are at risk of having a child with fragile X syndrome. In addition, the identification of a family in which the fragile X repeat site is expanded can lead to the identification of other female family members at risk of transmitting fragile X syndrome. Presently, even though the biological response to any given drug may be influenced by hundreds of genes, progress is being made in the identification of specific genetic variances such as SNPs that can predict the safety and effectiveness of certain drugs in individual patients. For example, there are evidences that GDF-9 c.G546A to be correlated with POF, poor ovarian stimulation and *in vitro* fertilization outcomes in women with DOR. Another possible reason for this hyporesponder population is that they may have a genetic predisposition to a reduced sensi-

tivity to FSH which may also be caused by a genetic variation and polymorphisms of the LH, the FSH receptor or the LH receptor. Moreover, a multigenic model including specific ESR1 and ESR2 genotype patterns may partially explain the poor response to FSH. Besides, the ER-alpha gene polymorphisms may be associated with idiopathic POF too. Thus, genetic testing of women showing reduced sensitivity to FSH may assist in tailoring subsequent treatment.
Finally, in the future, genetic screening may allow an individual patient's response to stimulation during COS to be predicted based on genotype. If a patient's genetic profile also diminishes her response to fertility treatment, the failure to consider the genotype when designing the treatment consequently leads to a suboptimal treatment strategy.

## I-38: Genome Instability and DNA Damage in Male Somatic and Germ Cells Expressed as Chromosomal Microdeletion and Aneuploidy Is A Major Cause of Male Infertility

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**Background:** Sperm chromatin insufficiencies leading to low sperm count and quality, infertility and transmission of chromosomal microdeletion and aneuploidies to next generations can be due to exposure to environmental pollutions, chemicals and natural or manmade ionizing radiation. In this project which has continued for more than 10 years and is unique in many technical aspects in Iran and in the region, genome instability induced by exogenous DNA damaging agents in sperm or spermatogenic cycle, the correlation of DNA damage with chromosomal microdeletion and aneuploidy, sperm chromatin alterations in failed fertilized oocyte's, chromosomal alterations in preimplantation embryos and localization of DAZ microdeletion on sperm nuclei were studied. Correlation of DNA damage induced during spermatogenesis leading to chromosomal aneuploidy and micronuclei in mouse preimplantation embryo is shown.
**Materials and Methods:** To do these, various cytogenetic methods such as metaphase analysis, micronucleus assay, sperm chromosome study using Golden hamster zona free oocytes, fluorescent in situ hybridization (FISH) and primed in situ labeling (PRINS) was used. Sperm DNA damage was assessed using the alkaline comet assay and CA3 staining. Impact of DNA damage and chromosomal abnormality in sperm on *in vitro* fertilization and pregnancy outcome is also studied. Real time PCR was performed for three markers (SY 1206, SY 1197, SY 579) for testing copy number variation before and after irradiation. For evaluation of DAZ gene on Y chromosome in sperm nuclei a combined Primed in situ labeling (PRINS) and fluorescence in situ hybridization (FISH) technique was used for the first time.
**Results:** Results indicate that sperm DNA damage in fertile and subfertile patients increased with increasing severity of male infertility and is well correlated with chromosomal aneuploidy especially sex chromosomes. Copy number variations of studied markers in AZFc region (microdeletion and duplication) after exposure to radiation increased with a dose dependent fashion ($p<0.001$). Correlation of DNA damage and chromosomal aneuploidy with *in vitro* fertilization and pregnancy outcome is also shown. Using FISH-PRINS technique showed that DAZ microdeletion on sperm nuclei can be easily evaluated and shown that situation of DAZ microdeletion in somatic and germ cells might not be always similar.
**Conclusion:** A direct correlation between protamine deficiency and sperm DNA damage was found for all subfertile patients studied and also sperm DNA damage is well correlated with chromosomal aneuploidy especially sex chromosomes. DNA damage might be involved in the process of malsegregation of chromosomes. This study indicates that genomic instability in infertile men could probably contribute to the development of an impaired reproductive capacity. Irradiation of gonads during spermatogenesis may lead to unstable chromosomal aberrations and probably stable chromosomal abnormalities affecting pairing and disjunction of chromosomes in successive preimplantation embryos expressed as MN. Increased frequency of induced microdeletion and duplication in infertile men compared with normal might be attributed to the deficiency in repair systems and the genetic factors involved in incomplete spermatogenesis of infertile men. Therefore, evaluation of DAZ microdeletion on sperms of male candidates for ICSI is necessary instead of simply using somatic cells for DAZ evaluation.
***Keywords:*** Male Infertility, Genome Instability, DAZ Microdeletion, DNA Damage, Aneuploidy, Sperm, Leukocytes, Exogenous DNA Damaging Agents

## I-39: Search for Genetic Causes of Male Infertility

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**Background:** We are convinced that better infertility treatment will only be achieved with a better under understanding of the molecular mechanisms specific to each patient. To that effect we want to indentify genes involved in male infertility.
**Materials and Methods:** We screened cohorts of infertile men to identify the cause of their infertility.
**Results:** Our team has identified and caracterized several genes implicated in the genesis of two form of male

infertility: macrozoospermia and globozoospermia. We showed that mutations in AURKC were responsible for macrozoospermia and that a homozygous deletion of the DPY19L2 was found in approximately 70% of globozoospermia patients. Before the characterization of these two genes the microdeletion of the Y chromosome were the only genetic defects described to alter spermatogenesis. We showed that AURKC deletions were are approximately as frequent in infertile men of North African descent as the Y chromosome microdeletions. We showed that Dpy19l2 is part of a new family of proteins permiting to establish a link between the nucleus and different organelles in the cytoplasm.

**Conclusion:** The strategy of homozygocity mapping and exome sequencing has allowed us to identify several genes responsible for different infertility phenotype. We are convinced that many more genes will be identified in the near future.

***Keywords:*** Male Infertility, DNA Sequencing, Spematogenesis

## I-40: Exploring New Frontiers in Human Y Chromosome Proteome Project

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The major goal of the Chromosome-Centric Human Proteome Project (C-HPP) is to systematically map the entire human proteome with the intent to enhance our understanding of human biology at the cellular level. However, this goal may be hindered by the lack of quality observations of given proteins due to absence of expression in a given tissue, very low abundance, and expression only in rare samples. We applied a cell-based approach to analyze the expression of genes located on male specific region of human Y chromosome (MSY). We analyzed the expression pattern of MSY genes at transcript and protein levels during differentiation of human embryonic stem cell and human neuron-committed teratocarcinoma cell lines to dopaminergic neurons. We observed that several MSY genes/proteins previously known as testis specific were highly upregulated during differentiation. Further interesting results were observed when human embryonic and adult stem cells were differentiated to other cell types. We also present data showing that suppression of MSY gene combined with shotgun quantitative proteomic approach of resulting cells can enhance our insights about MSY gene function. Our results highlight the importance of utilizing cell-based approach particularly stem cells and their derivatives to enhance the quality of observations for MSY proteins and to increase our understanding about their eminent biological roles in specific target cells.

## I-41: Non Invasive Prenatal Genetic Diagnosis; Current Status and The Future

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Discovery of cell free fetal DNA in 1997 has deeply changed the outlook of prenatal diagnosis approaches as most of the clinically established screening tests are not sensitive/specific enough while the current practical diagnostic tests are also invasive in their nature. The most common prenatal screening test is routinely practiced for the diagnosis of Down syndrome (DS) which includes a 10% failure in diagnosis of pregnancies with DS, whilst 19 from 20 positive-screened are also falsely positive. In addition, definite diagnosis of DS pregnancies can be achieved by invasive procedures of amniocentesis and or CVS which will contain a risk of 0.5 to 1% for fetal loss. Non-invasive prenatal genetic diagnosis (NIPNGD) relies on genotyping of the fetus rather than phenotypic evaluations and is done through separation of fetal cells or nucleic acids from maternal blood. In the last 15 years, an increasing number of researches were done on NIPNGD which in turn have brought it to the edge of approval for clinical application at least in some prenatal conditions like DS. Nowadays, NIPNGD methods are broadly under investigation to determine their applicability in various fetomaternal conditions such as fetal sex determination, aneuploidies, single gene disorders, microdeletions and microduplications, Rhesus incompatibility, fetal growth restrictions, preeclampsia, preterm labor and so on. These approaches are mostly based on detecting fetal nucleic acids in maternal blood as the number of intact fetal cells and the methods for their separation from maternal blood are quietly limited. The two important obstacles that have hindered the use of cell free fetal DNA in NIPNGD are the very low ratio of fetal DNA in maternal blood (3 to 6%) and difficulty in distinguishing fetal DNA from maternal DNA. These difficulties are mostly affecting the result of conventional methods such as PCR and Sanger sequencing. Sophis-

ticated and highly throughput new approaches such as array CGH and next generation sequencing (NGS) are promising to be able to overcome these drawbacks. At the present, NGS is going to be clinically applicable for screening trisomic pregnancies with 100% sensitivity and specificity. It is predictable that these methods can be extensively applied in the clinic for non-invasive prenatal diagnosis of various genetic conditions in the future.

# Reproductive Imaging

## I-42: Chorionic Villus Sampling (CVS): An Invasive Method for Prenatal Diagnosis

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**Background:** Chorionic villus sampling(CVS) has a major role in prenatal diagnosis . It is an invasive diagnostic method in completion of primary prenatal screenings. This article is a report of our 8 years experience of CVS in Mashhad.
**Materials and Methods:** We have done 1511 cases of invasive diagnostic procedure including 723 CVS which were included in our study and also 788 cases of amniocentesis. The gestational ages of the patients whom CVS were done for them were between 10 to 22 weeks based on the LMP. All CVS's were done by 19.5 G needles under Ultrasonographic real time guide using freehand method by a radiologist.
**Results:** Mean gestational age at the time of procedure was 12.5 weeks. The most common cause of CVS application was due to beta thalassemia (81%) followed by karyotype (7%), spinal muscular atrophy(werding Hoffman syndrome) (3.3%), Congenital adrenal hyperplasia (2.7%), Duchene muscular dystrophy (3%), and hemophilia, achondroplasia,paternity test, cystinuria, PKU in remaining cases. Needle insertions into placenta were done for 1 time in 517 patients (71.5%), 2 times in 163 patients (22.5%), 3 times in 38 patients (5.2%) and 4 times in 5 patients (0.7%). Any gestational complications within 1 months of CVS were considered as the complication of our procedure.As the complication of the CVS there were 13 cases of spotting without miscarriage (1.8%) and 5 cases of miscarriage (0.7%) including 3 cases of fetal death (0.4%) and one case of abortion (0.1%).
**Conclusion:** CVS is considered as an invasive diagnostic method in early pregnancies but it's benefits outweighing the complications in all cases that it is indicated.
***Keywords:*** Chorionic Villus Sampling, Ultrasonography, Pregnancy, Invasive Diagnostic Methods

## I-43: Advanced Ultrasonic Techniques in The Assessment of Early Human Development

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**Background:** To evaluate the role of 3D, 4D and color Doppler ultrasound (US) in the assessment of early human development.
**Materials and Methods:** 380 women with uncomplicated early pregnancy between 5 and 14 weeks were evaluated by 3D, 4D and color Doppler US.
**Results:** Regression analysis revealed exponential rise of the gestational sac volume with gestational age throughout the first trimester. An exponential rise of the yolk sac volume was noticed between gestational week 5 and 8, followed by gradual increase of the yolk sac volume between 8 and 10 weeks. After reaching the plateau from 10 to 11 weeks, yolk sac volume started to decrease. 3D US was used to study nuchal translucency in 120 patients between 10 and 14 weeks of gestation. Multiplanar imaging allowed appropriate mid-sagittal section of the fetus and clear distinction of the nuchal region from the amniotic membrane in all the examined patients. This enabled us to obtain nuchal translucency measurements in 100% of cases. Rotation of the embryo and close scrutiny of the volume allowed systematic review of anatomic structures such as cord insertion, limb buds, cerebral cavities, stomach and bladder.
**Conclusions:** 3D US is advantageous for studying normal embryonic and/or fetal development, as well as providing information for families at risk for specific congenital anomalies by confirming normality. 3D US imaging complements pathologic and histological evaluation of the developing embryo rising a new term: 3D sonoembryology. It is expected that interesting data on fetal behavior will be collected with introduction of 4D sonography.

## I-44: Scientific and Religious Controversies on The Beginning of Human Life- What Does 3D/4D Sonography Offer?

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One of the most controversial topics in modern bioethics, science, and philosophy is the beginning of individual human life. In the seemingly endless debate, strongly stimulated by recent technologic advances in human reproduction, a synthesis between scientific data and hypothesis, philosophical thought, and issues of humanities has become a necessity to deal with ethical, juridical, and social problems. Furthermore, in this field there is a temptation to ask science to choose between opinions and beliefs, which neutralize one another. The question of when human life begins requires the essential aid of different forms of knowledge. Here we become involved in the juncture between science and religion, which needs to be carefully explored.
Modern bioethics and science are strongly concerned for the respect of human life at both ends of its existence (birth and death), but other sciences (eg. Phi-

losophy, technology, psychology, sociology, law, and politics) consider the beginning of human life according to different points of view. However, bioethical topics like this one cannot be treated from only one perspective (eg. Biological, philosophical, or religious) because conclusions might be not good enough or reductive. This reality should be regarded in all its richness: An embryo gives a biologist and a geneticist substance for consideration, but because we are talking about the beginning of human life, it requires philosophical-anthropological consideration and confrontation with theology; in its protection we have to include ethics and law. In experiencing and investigating social behavior, other disciplines, such as the history of medicine and sociology, have to be included.

It is hard to answer the question when human life should be legaly protected. At the time of conception? At the time of implantation? At the time of birth? In all countries (except Ireland and Liechtenstein) juridical considerations are based on roman law. Roman civil law says that the fetus has right when it is born or if it is born-nasciterus.

Few countries agree with definition of beginning of human personality the time of conception. The majority does not grant legal status to the human embryo *in vitro* (i.e., during the 14 days after fertilization). Thus, even in the absence of legal rights, there is no denying that the embryo constitutes the beginning of human life, a member of the human family. Therefore, whatever the attitude, every country has to examine which practices are compatible with the respect of that dignity and the security of human genetic material.

The question when a human life begins and how to define it, could be answered only through the inner-connecting pathways of history, philosophy, medical science and religion. It has not been easy to determine where to draw the fine line between the competence of science and methaphysics in this delicate philosophical field. To a large extent the drawing of this line depends on one's fundamental philosophical outlook. The point at which human life begins will always be seen differently by different individuals, groups, cultures, and religious faiths. In democracy there are always at least two sides, and the center holds only when the majority realizes that without a minority democracy itself is lost. The minority in turn must realize its best chance lies in persuasion by reason and thoughtfulness rather than fanaticism.

3D/4D sonography offers new possibilities in visualizing beginning of human life virtually from conception.

## I-45: Increased Nuchal Translucency at 11-14 Weeks of Gestation As A Marker for Adverse Pregnancy Outcomes

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**Background:** To evaluate the association of increased fetal nuchal translucency( NT) and adverse pregnancy outcomes.

**Materials and Methods:** In a prospective study, 2221 women who were conceived after Assisted Conception were investigated by sonography independent of multiplicity between 11 and 13 weeks' gestation. We performed 2899 NT examinations during the study period(21 March 2010 to 21 March 2013). All cases with an increased NT (>95$^{th}$ percentile for a given crown rump length (CRL)) were referred to amniocentesis and karyotyping, genetic counseling, and fetal echocardiography. Maternal and fetal data (NT, caryotype, pregnancy outcome) and infant follow-up of fetuses were analyzed.

**Results:** The NT was abnormal in 41 (41/2899=1.14 %) fetuses .The mean age of participant was 32.6 ± 4.72 (24-43years) and the mean duration of infertility was 5.71±4.2.

Thirteen fetuses underwent first-trimester fetal reduction for multiplicity. Six out of 28 remained fetuses ( 24%) detected with abnormal karyotypes either by amniocentesis or fetal pathology. Three cases of Down syndrome and two cases of Turner syndrome confirmed by amniocentesis and one case of triploid chromosomal disorder reported after fetal pathology. Elective terminations of pregnancy due to multiple anomalies occurred in two cases. The loss of fetuses due to intrauterine fetal demise (IUFD) occurred in six cases. Overall adverse pregnancy outcomes were reported in 15 cases ( 53%). It is noteworthy that adverse pregnancy outcomes also occurred for eight fetuses with normal amniocentesis(2/8= 25%).Thirteen cases of normal infants were born finally(13/28= 47%).

**Conclusion:** First-trimester increased NT thickness is associated with an increased risk of subsequent pregnancy complications and adverse pregnancy outcome. The need for prenatal screening is extra important in the case of assisted conception due to more concern over specific anomalies, multiplicity and poor outcome pregnancies. In cases of increased NT, parents should be offered a detailed fetal evaluation (genetic sonography) at 18-22 weeks of gestation. Triple Test, amniocentesis, fetal echocardiography and follow-up after birth are necessary. In the case of normal follow-up scans fetuses could have a good prognosis for a normal early childhood.

## I-46: Choroid Plexus Cyst

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CPCs are a relatively common finding during the second trimester sonography.

when a choroid plexus cyst is identified, the presence of structural malformation and other sonographic markers of aneuploidy should be assessed with a detailed fetal anatomic survey performed by an experienced person. if no other sonographic abnormalities are present, the CPC is considered isolated.

given the considerable improvements in imaging technology and aneuploidy screening in recent years, the risk of aneuploidy especialy the trisomy 18 in the setting of isolated CPCs is now believed to be very low.

## I-47: Obstetrical Doppler

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Accurate assessment of gestational age, fetal growth, and the detection of fetal and placental abnormalities are major benefits of sonography. Color Doppler can be used to assist in the identification of vascular architecture, detection of vascular pathology and visualization of blood flow changes associated with physiologic processes and disease states.

The clinical applications of obstetrical Doppler are Intrauterine Growth Restriction, diagnosis of fetal anemia and twin-twin transfusion syndrome.

The use of Doppler during antenatal fetal surveillance has involved assessment of (1) the umbilical arterial and venous flow velocity waveforms, (2) the fetal cerebral circulation, and (3) the fetal venous circulation, in particular the ductus venosus.

Doppler studies of the fetal circulation in intrauterine growth retardation and hypoxia have demonstrated increased resistance to flow in the umbilical arteries and redistribution in the fetal circulation with reduced resistance and increased velocity in the internal carotid and middle cerebral artery and the opposite in the descending thoracic aorta.

Assessment of placental function using umbilical artery Doppler velocimetry:

Placental insufficiency can be quantified based on the reduction of end-diastolic Doppler flow velocity into (1) reduced enddiastolic flow velocity, (2) absent end-diastolic flow velocity, and (3) reversed end-diastolic flow velocity.

Use of middle cerebral artery velocimetry to detect fetal hypoxia:

an increase in $pCO_2$ or a reduction in $pO_2$ will cause an increase in fetal cerebral arterial Doppler end-diastolic flow velocity, likely related to cerebral vasodilatation. This phenomenon has been described as the “brain sparing” effect.

Use of Doppler to detect fetal anemia:

The MCA-PSV is an accurate predictor of severe fetal anemia in pregnancies complicated by red cell alloimmunization.

Use of uterine artery Doppler to detect maternal complications of pregnancy:

The uterine artery Doppler reflects fetal response to stress of hostile intrauterine environment (pregnancy-induced hypertension, re-eclamptic toxemia).

Fetal venous flow:

The umbilical vein, ductus venosus and the inferior vena cava are the main areas of interest in the investigation of venous blood return to the fetal heart.

Pulsatile umbilical vein flow and back flow in ductus venosus and IVC are indications advanced cardiac decompensation.

# Oral Presentations

## Andrology

### O-1: Intravaginal Bee Honey and Royal Jelly Versus Intrauterine Insemination for Infertility Due to Asthenozoospermia

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**Background:** Intrauterine insemination (IUI) is a simple treatment method for infertility reasoned by asthenozoospermia. However, the pregnancy rates are, in general, not satisfactory. The purpose of this work was to evaluate another simple method for "sperm enhancement" that entailed the midcyclic intravaginal use of Egyptian bee honey and royal jelly (H/RJ) as compared to IUI.
**Materials and Methods:** A crossover study of 99 infertile couples affected by asthenozoospermia. One group used midcycle pericoital vaginal applications of H/RJ and the other underwent a standard intrauterine insemination (IUI) procedure, for 3 cycles or until conception occurred for both groups. After a washout period of 2 months, the couples for whom no pregnancy occurred were crossed over.
**Results:** A total of 553 cycles were analyzed. There were 23 (8.1%) and 7 (2.6%) pregnancies per cycle, respectively, in the H/RJ and the IUI groups, and the difference was statistically significant ($p<0.001$).
**Conclusion:** Using H/RJ intravaginally might be a simple and reasonably effective method of treating asthenozoospermia.
***Keywords:*** Infertility, Asthenozoospermia, Treatment

### O-2: Study The Effect of Oral Zinc Supplementation on Enzymes of Urate Pathway in Spermatozoa of Patients with Asthenospermia

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**Background:** The generation of reactive oxygen species (ROS) in the male reproductive tract has become an actual concern because of their probable noxious effects, at high levels, on physical properties of sperm quality. ROS are extremely reactive oxidizing agents, which a member of the class of free radicals. It was found that uric acid can act as an essential water soluble antioxidant and therefore has a possible function in protection of spermatozoa against oxidative damage.
**Materials and Methods:** Semen samples were obtained from 60 fertile and 60 subfertile men with asthenozoospermia between July 2011 to July 2012, from couples who consulted the infertility clinic of the Babil hospital of maternity (Hilla city/ IRAQ). The subfertile group consist of the patients which treated with zinc sulfate, every participant took two capsules of zinc sulfate per day for three months (each one 220 mg). Semen samples were obtained (before and after zinc sulfate supplementation). After liquefaction seminal fluid at room temperature, routine semen analyses were performed. The levels of uric acid (UA), 5--nucleotidase (5--NU) activity, adenosine deaminase (ADA) activity and Xanthine oxidase (XO) activity are measured in the spermatozoa and seminal plasma of patients with asthenospermia and healthy subjects.
**Results:** Compared with healthy controls, uric acid levels were found to significantly decrease in seminal plasma and spermatozoa of patients with asthenozoospermia. Zinc supplementation elevates uric acid levels in seminal plasma of asthenozoospermic subjects to normal value. This study recorded highest decrease in seminal 5'-NU activity in asthenospermic patients. The decreased activity of this marker enzyme exposed spermatozoal damage from stress induced oxidative damage possibly causing disruption in membrane function and integrity. The values of ADA were significantly higher in asthenospermic specimens as compared to the controls. The values of XO were significantly higher in asthenospermic specimens as compared to the controls. The zinc supplementation induced decrease of XO activity is of particular interest. Volume of semen, progressive sperm motility percentage, and total normal sperm count were increased after zinc sulfate supplementation.
**Conclusion:** Zinc treatment decreases asthenozoospermia through several mechanisms such as reduction of oxidative stress and apoptosis.
***Keywords:*** Asthenospermia, Uric Acid, 5'-nucleotidase, Adenosine Deaminase, Xanthine Oxidase

### O-3: Testosterone and Vitamin E administration Up-Regulates Hsp70 Protein Expression in Varicocele-induced Rats; Correlation with Leydig Cells Steroidogenesis and Germinal Cells RNA Damage

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**Background:** Varicocele (VCL) impacts the testicular tissue partly by down-regulating the antioxidant status accomplished with reducing the intra-testicular endocrine potential. It has been reported that the HSP70 families play a critical role in spermiogenesis. Therefore, current study was aimed to evaluate the protective effect of exogenous testosterone and vitamin E administration on VCL-induced damages on testicular tissue.
**Materials and Methods:** Thirty mature male rats were

divided into five control-sham and test groups (N=6 rats for each group). The test groups underwent to an experimental varicocele. Group VCL alone (varicocele-induced, for 60 days), group VCL+testosterone (45 µg intraperitoneally, for 60 days), group VCL+vitamin E (150mg/kg, orally, for 60 days) and group VCL+testosterone+vitamin E. Simple laparotomy was conducted in control-sham group. The Western blotting and immunohistochemical analyses for Hsp70 protein were performed. The total RNA and total protein levels were evaluated. The Epifluorescent and fluorescent analyses were conducted to evaluate the germinal cells RNA damage and leydig cells biosteroid activity, respectively.

**Results:** The Hsp70 protein expression significantly ($p<0.05$) up-regulated in treated groups. Qua, the VCL+testosterone+vitamin E group showed the highest level of Hsp70 protein beside higher detected immunohistochemical spots in germinal cells. The animals in all treated groups showed remarkably ($p<0.05$) higher total protein and normal RNA contents versus VCL alone group. The higher biosteroidogenesis was observed in VCL+vitamin E and VCL+testosterone+vitamin E groups leydig cells.

**Conclusion:** Our data showed that testosterone by promoting the testicular endocrine function and the vitamin E by up-regulating testicular antioxidant capacity could inhibit the VCL-induced damages.

***Keywords:*** Varicocele, Testosterone, Vitamin E, Hsp70, RNA Damage

## O-4: Testicular Gametogenic and Steroidogenic Activities in Chlorpyrifos Insecticide-Treated Rats: A Correlation Study with Testicular Oxidative Stress and Role of Antioxidants Enzymes Defense Systems and Herbal Antioxidants Therapeutic Approaches to Ameliorate Male Infertility: Animal Studies in S.D. Rats

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**Background:** In public health and agriculture, estrogenic organophosphate insecticide / pesticide - Chlorpyrifos (CPF) is the most toxic synthetic agrochemicals purposefully added in environment causing threat to reproduction and results a number of acute and chronic poisoning events. Eventually CPF induces testicular oxidative stress and damages due to production of potentially destructive reactive oxygen species (ROS) which exceeds the bodies own natural antioxidant defenses, resulting in cellular damage and male infertility. Objective goal is to evaluate whether herbal antioxidants vitamins have some protection on CPF- induced oxidative stress and testicular gametogenic and steroidogenic alterations.

**Materials and Methods:** The experimental methods are to evaluate adverse effects of CPF on rat testes, spermatogenesis cell cycles, sperm counts, biochemical changes in lipid peroxidation and endogenous antioxidant enzymes profiles including serum hormones levels and as well as to assess ameliorating role of herbal antioxidants of plant fruits and leafs ( e.g. Capsicum and Aloe marmelose and Cannabis plant ) including Quercetin which are administered in pre, post and combination treatments.

**Results:** At sub-lethal acute doses, significant levels of increased lipid peroxidation and reduced antioxidant enzymes and reduction in plasma levels of testosterone and FSH and LH hormones along with significant shrinkage of seminiferous tubules and changes in germ cells at stage VIII of spermatogenesis are reported. But at chronic doses, protective effects are noticed. Moreover, on administrations of herbal antioxidants, revival of serum testosterone and FSH and LH revival of testicular hormones, gametogenic activities and $\Delta 5$, 3β-hydroxysteroid dehydrogenase and 7β-hydroxysteroid dehydrogenase with concomitant reduced level of lipid peroxidation and increased level of endogenous antioxidants enzymes defense systems (AOE ) are seen.

**Conclusion:** Present report reveals that herbal therapy acts as antidote to pesticides possibly by triggering of natural defense mechanism inherently present in all living systems through corrective measure of antioxidant enzymes defense system and pituitary gonadotropins hormones feedback mechanisms.

***Keywords:*** Estrogenic Pesticide- Chlorpyrifos, Rat Testes as Mammalian Model Systems, Oxidative Stress, Male Infertility, Various Herbal Therapies as Reproductive, Biomedicine

## O-5: Does Antioxidant Therapy Adds any Benefit in Improvement of Sperm Parameters to Standard Inguinal Varicocelectomy? A Randomize Case-Control Study

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**Background:** Varicocele occurs in approximately 15 to 20% of the general male population and it is the most common cause of poor semen production and decreased semen quality. Clinical studies have show that varicocele surgery can improve semen quality. The aim of this study was to evaluate if antioxidant therapy adds any extra benefit to standard inguinal varicocelectomy in improvement of sperm parameters.

**Materials and Methods:** We prospectively evaluated changes in sperm parameters in patients with varicocele before and after varicocelectomy . 100 men with varicocele were enrolled in this study and divided into 2 groups. Fifty men underwent inguinal varicocelectomy and second group underwent varicoceletomy and received L- carnitine 250 m 3 times / day for 6 months. World Health Organization semen analysis was assessed, before, 3 and 6 months post surgery.

**Results:** There was 50 patients with mean age of 27.57 (18-41y) in surgery group and 50 patients in surgery plus drug group with mean age of 26.69 (18-42). 64.6% of surgery only group and 615% of surgery plus drug

group surgery were married. Mean sperm count in surgery group before and three and six months after surgery was 46,000,000 ,96,947,000 and 150,444,000 and in surgery drug group 120, 235, 300, 122, 177, 600 and 146, 333, 300. There was not any statistically significant difference between two groups in terms of sperm count 3 and 6 months after surgery. Motility a ,b and normal morphology was compared between two groups that there was not any sign statistically significant difference except the normal morphology three months after surgery ,that was higher in surgery group (21.48 vs. 12.11% p value 0.04). Mean of motility a,b in surgery group was (6.83, 24.5, 3.688, 6.88 and 4.87, 31.77%). Presurgery and 3,6 months after surgery and in surgery drug group was 3.68, 20.43 and 6.28, 29.70 and 8.56, 31.63%. Normal morphology in surgery group was 23.82, 21.35, 22.48% and in surgery drug group was 16.41, 12.11, 21.66%. Within surgery group there was statistically significant improvement in sperm count before ,3 and 6 months after surgery (p value = 0.00, 0.01, 0.03) but in surgery plus drug group improvement was significant only between 3 and 6 months after surgery (p value = 0.15, 0.015 , 0.02). In terms of motility and morphology in surgery group there was significant improvement in morphology 3 and 6 months after surgery and in surgery drug group the only significant change was in motility a,b before and 3 months after surgery.

**Conclusion:** These results show that varicocelectomy improves semen parameters but adding antioxidant therapy to this procedure doesn't add any extra benefit in terms of improving semen parameters quality.

***Keywords:*** Varicocele, Sperm Parameters, Antioxidant Therapy

### O-6: Occupational Heat Exposures and Male Infertility

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**Background:** In humans, as in most mammals, spermatogenesis is temperature dependent. Experimental studies in animals and humans have demonstrated the role of exogenous heat exposure in male infertility. Several occupational and environmental exposures and toxins have known or suspected deleterious actions to male reproductive function. The main objective of this study was to determine the frequency of occupational categories of men who attended an infertility clinic, and to evaluate the differences in the semen quality parameters among occupational categories.

**Materials and Methods:** This cross-sectional study was conducted on 1382 males who were referred to the Royan Institute for treatment of infertility in order to evaluate the effects of certain occupations on infertility. The participants were divided into several categories according to their occupations and evaluated by means of a questionnaire for duration of infertility, BMI, sperm count, percentage of normal sperm morphology and percentages of sperm with class A and class B motilities. Descriptive statistics, analysis of variance, and correlations were conducted using SPSS 16.0 for Windows.

**Results:** The means and standard errors of age, infertility duration and BMI of the participants were 33.89± 5.95 years, 6.23 ± 0.14 years and 26.06 ± 5.04, respectively. In this research , 47.4% of participants reported that they had a stressful working environment . Exposure to occupational physical hazards including heat, vibration, ionizing radiation and non-ionizing radiation were reported in 42.8%(n=498), 17.6% (n=205), 0.3% (n=4) and 39.1%(n=455) of the participants, respectively.The highest and lowest mean percentages of sperm with class B motility were seen in the Electronics group (25.94± 2.5) and the Transportation group (20.26 ± 1.07). Drivers are thought to be at greater risk of infertility because long periods of sitting can increase testicular heat, as the testicles are insulated by the thighs whilst a man is seated. Our findings in agreement with a number of other researches.

**Conclusion:** Our findings support the results of previous studies regarding the association between occupation and sperm motility, particularly in the transportation category in which sedentary work is a common hazard. Further research is necessary to evaluate the observed associations in this study.

***Keywords:*** Male Infertility, Occupational Categories ,Occupational Heat Exposure, Semen Quality, Environmental Exposures

## Animal Biotechnology

### O-7: Oocyte Quality Determines Bovine Embryo Development after Fertilization with Hydrogen Peroxide-Stressed Spermatozoa

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**Background:** Exposure of gametes to specific stressors at sublethal levels can enhance the gametes' subsequent performance in processes such as cryopreservation. The aims of the present study were to investigate the effects of sublethal $H_2O_2$ stress on bovine sperm motility, DNA integrity, and the role of the oocyte diameter on subsequent embryo development and embryo quality in terms of apoptosis.

**Materials and Methods:** In the present study, bovine spermatozoa were subjected to $H_2O_2$ for 4 hours at 100, 200 and 500 µM levels: computer-assisted sperm analy-

sis (CASA) and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay were used for evaluation of subsequent sperm motility and DNA integrity, respectively.

**Results:** Exposure of spermatozoa to $H_2O_2$ did not affect sperm motility but DNA integrity was negatively affected by 500 µM $H_2O_2$ compared to mock-exposed spermatozoa, while both motility and DNA integrity were affected compared to control spermatozoa. Nevertheless, insemination of oocytes with spermatozoa exposed to 200 µM $H_2O_2$ increased fertilization, cleavage and blastocyst rates ($p<0.05$). Furthermore, we showed that the higher blastocyst yield after fertilization of oocytes with sperm exposed to 200 µM $H_2O_2$ was related to the oocyte diameter, with large/medium oocytes yielding higher blastocyst rates, while small-diameter oocytes consistently failed to develop into blastocysts.

**Conclusion:** In conclusion, the results indicate that exposure of spermatozoa to 200 µM $H_2O_2$ before sperm-oocyte interaction may enhance *in vitro* embryo production in cattle. However, this increased embryo production is largely dependent on the oocytes' intrinsic quality.

***Keywords:*** Oxidative Stress, DNA Damage, Total Cell Number

# Embryology

## O-8: Endocrinological and Morphological Evaluation of Vitrified-Warmed Ovarian Tissue

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**Background:** Our aim was to evaluate the recovery rate of ovarian function and the activity of hypothalamus-hypophiseal-gonadal axis following transplantation of vitrified ovaries.

**Materials and Methods:** Five weeks-old female Wistar Rats were distributed randomly in two experimental groups; Fresh Transplanted, Vitrified-Transplanted and 8 weeks-old female Wistar rats were considered as control group. Also, bilateral Ovarectomized rats were used for hormonal assessment as a sham group. In gonadectomized fresh transplanted group, excised right ovary was immediately autotransplanted under the cervical skin and in gonadectomized vitrified-transplanted group; excised ovarian tissue was vitrified by combination of EG and DMSO (in V1and V2, 7.5 and 15% of both cryoprotectants was used respectively) and after performance of 3step warming, transplanted at the same site. In both transplantation groups, 3 weeks after transplantation, blood was collected by cardiac puncture and serum Gonadotropins, Estradiol (E2) and Progesterone Concentration was determined by direct immunoassay by use of ELISA kit. Also, in all experimental groups, follicular count was evaluated by using histological analysis.

**Results:** Although serum E2, progesterone and gonadotropins concentrations in the fresh-transplanted and vitrified-transplanted groups were as the same as the control, serum E2 and progesterone level was increased and Gonadotropins level was decreased significantly as compared to the bilateral Ovarectomized group. In addition, preantral, antral and corpus luteum (CL) rate had increased in fresh-transplanted and vitrified- transplanted group in comparison to the control groups.

**Conclusion:** It could be concluded that although ovary Vitrification and autotransplantation reduced the rate of small sized follicles (primordial and primary), vitrified-transplanted ovarian tissue can resume its function and this was proven properly by hypothalamus-hypophiseal-gonadal axis activity resumption.

***Keywords:*** Vitrified-transplanted, Ovarian Tissue, Hypothalamus-Hypophiseal-Gonadal Axis, Immunoassay

## O-9: A Multi-Regional Study on New Approaches to Investigate The Quality of Human Sperm - Including DNA Fragmentation, Proteomics and Metabolomics

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**Background:** Preliminary data has also shown that there is less fragmented sperm in 2nd and 3rd ejaculates compared to first ones which could be a major factor in determining the pregnancy outcome. Assessing this factor objectively and relating it to other parameters in sperm quality in this study could result in new prediction criteria for the pregnancy outcome.

**Materials and Methods:** As one of the goals, this study will focus on analyzing the seminal fluid from the semen sample using NMR and Mass Spectrometry in research for a correlation between the results of these methods and the DNA fragmentation, Kinetic parameters and finally implantation rates and pregnancy outcome.

**Results:** As another goal in this study we plan to assess whether the second consecutive sample obtained within 12 hours of the first sample has a lower DNA fragmentation rate which will be very important in lowering the number of failed implantations and repeated abortions.

**Conclusion:** Collecting and analyzing the samples from different locations (different countries and continents) using the SCA (Sperm Class Analyzer, Microptic, Spain) which has eliminated the inter technician variation and provides quantitative data and a means to assess samples at the exact same scale, will provide a comparison of the average sperm statistics and also the quality of consecutive sperm samples in these different locations. This would specially prove to be of utmost importance in determining and endorsing a global scale for the computer assisted sperm analysis results which seem to be taking over the old visual (subjective) analysis of sperm among the laboratories and clinics worldwide.

## O-10: Study of Structure and Follicular Maturation, Angiogenic and Apoptotic Genes Expression of Rat Ovarian Tissue After Vitrification and Autotransplantation

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**Background:** Ovarian tissue cryopreservation and autotransplantation is the only fertility preservation way for prepubertal girls and situations that cytotoxic treatment is urgent.
**Materials and Methods:** The ovaries of premature (5 weeks old) rats were autografted under cervical skin after vitrification. 3 weeks later, transplanted ovaries were removed from the body and structure of ovary, expression changes of follicular maturation, apoptotic and angiogenic genes were investigated. Also the level of gonadotropins (LH and FSH) and steroid hormones (estradiol, progesterone and testosterone) were evaluated in blood serum. Finally, caspase-3 protein expression was studied with immunohistochemistry.
**Results:** This study has shown that fresh and vitrified rat ovaries display restoration of hormonal cycle and ovarian function after autotransplantation. Primordial follicles in both of the fresh and vitrified ovaries can recruit and develop to maturation stage during autotransplantation in cervical subcutaneous region. Also the results showed autotransplantation of vitrified-warmed rat ovaries may survive follicles in an optimum survival rate (74.32%).
**Conclusion:** VIV group (combination of EG + DMSO with sucrose) due to the better primordial follicular preservation and more survivability is still better suited for vitrification of whole ovarian tissue of rats. Both of the fresh and vitrified primordial follicles can recruit and develop to maturation stage under skin of the cervical region. Also this study has shown that fresh and vitrified rat ovaries display restoration of hormonal cycle and ovarian function after transplantation but not close to control group.
***Keywords:*** Whole Ovary, Vitrification, Autotransplantation, Rat

## O-11: Critical Role of Hyaluronan System in Pre-Implantation Embryo Development and Establishment of Pregnancy

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**Background:** Hyaluronan (HA) is a structural component of extracellular matrix synthesised by HA synthases HAS1-3, which produce HA of different molecular sizes with distinct biological functions associated with reproductive processes. Hyaluronidase (HYAL) cleaves the HA into biologically active small fragments which are known to regulate cell proliferation through CD44 receptor signaling. HA is currently used a supplement in embryo transfer media as a “glue” to improve embryo attachment. We have determined developmental pattern of expression and critical role of HA system in pre-implantation embryos. We have also investigated the effect of HA or HA-synthesis inhibitor on endometrial receptivity and embryo attachment.
**Materials and Methods:** Pattern of HA production and expression of HA system (HAS1-3, and HYAL2) in the embryos at different stages of development and also HYAL2 in the oviduct was assessed. Effects of HA sizes including HA fragments produced by HYAL2 on development to blastocyst stage and blastocysts quality of *in vitro* produced bovine and ovine embryos (*in vitro* and *in vivo*) were analysed in the presence or absence of HA synthesis inhibitor methylumbelliferone (4MU) and involvement of CD44 and MAPK signaling in HYAL-2 promoted blastocyst rate was investigated. In addition, the effect of HA on embryo implantation was determined in an *in vivo* study using sheep and uterine infusion of Saline (control), HA (0.5 mg/ml) or 4MU (HA-synthesis inhibitor; 1mM) on day 14 of pregnancy (3 days before embryo attachment). Animals were then euthanized on day 17 when the uteri were fixed for histological and immunohistochemical analysis.
**Results:** Immunostaining for HA was detected at all stages embryos to blastocyst. HAS1-3 mRNAs were expressed at all stages of embryos and showed specific pattern. Interestingly, HYAL2 mRNA expression was detected in the oviduct at early luteal phase but was only detected in the embryos at morula and blastocyst stages. In contrast to 4MU which inhibited blastocyst formation, HYAL2 improved blastocyst rate *in vitro* and increased embryo cell numbers ($p<0.05$). Addition of Anti-CD44 antibody or MAPK inhibitor abrogated the positive effects of HYAL2 on blastocyst rates ($p<0.05$). HA infusion resulted in failure of embryo, whereas all embryos in the 4MU infused group were attached on day 17. In contrast to HA infusion, the 4MU infusion inhibited HA synthesis in the endometrium and blocked mucin-1 (MUC-1) production, but increased adhesion molecules osteopontin (OPN) and CD44 expression at the uterus-embryo interface.
**Conclusion:** HAS and HYAL2 are differentially expressed in different stages of early embryo development and HYAL2 supplementation is required to maximize blastocyst yield and improve its quality *in vitro*. Presence of high molecular weight HA in the uterus inhibits embryo attachment and its removal may enhance pregnancy rate.
***Keywords:*** Hyaluronan, Hyaluronidase, Embryo Development, Implantation

## O-12: Duration of Enzymatic and Mechanical Denudation: Have any Effect on Fertilization Rate and Embryo Development?

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**Background:** Intracytoplasmic sperm injection (ICSI) is the choice option for treatment of couples with severe male infertility. Recent studies indicate that the ICSI procedure or the technique itself cannot increase chromosomal abnormalities observed in born children. It maybe result of the Materialss used during ICSI procedure such as PVP and hyalorunidase. Activation of the oocytes might be caused by pipetting procedure and exposure to the hyalorunidase. The microinjection technique has been completely standardized, but some other procedures such as oocyte denudation still need more attention.
**Materials and Methods:** In the present study, 358 oocytes from 58 cycles were evaluated. Cumulus and corona cells were removed in two pipetting steps: first in a medium containing hyaluronidase, and then in a medium without enzyme. We recorded the exposure time of oocytes to hyaluronidase and mechanical pipeting duration (without enzyme). Oocytes were put in a single droplet of G1 media., Procedures were performed by a single embryologist in 6 month. Post-ICSI fertilization rate and blastomere number as well as embryo quality were assessed.
**Results:** No statistically significant differences were found between the exposure time of oocytes to hyaluronidase with blastomere number, embryo quality and fertilization rate, but duration of mechanical pipetting had prominent effect on fertilization rate (p=0.001), embryo quality (p=0.02).
**Conclusion:** Our result showed that mechanical denudation had adverse effect on subsequent oocyte fertilization potential. It is possible that, these effects are caused by suboptimal situation of oocyte out of incubator or mechanical effect due to pipetting. So, to achived the best result of ICSI procedure, we should minimize the time of oocyte denudation.
*Keywords:* Oocyte Denudation, Fertilization Rate, Embryo Development

## O-13: A Marked Animal-Vegetal Polarity in The Localization of Na+,K+-ATPase Activity and Its Down-Regulation Following Progesterone-Induced Maturation

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**Background:** Polarized cells are key to the process of differentiation. Xenopus oocyte is a polarized cell that has complete blue-print to differentiate 3 germ layers following fertilization, as key determinant molecules (Proteins and RNAs) are asymmetrically localized. The objective of this work was to localize Na+, K+-ATPase activity along animal-vegetal axis of polarized Xenopus oocyte and following their progesterone-induced maturation (M-phase of the cell cycle) and their role in polarity.
**Materials and Methods:** Enzyme-specific electron microscopy phosphatase histochemistry techniques were used.
**Results:** The Na+, K+-ATPase is the most critical and important protein. The stage-VI Xenopus oocyte has a very distinct animal-vegetal polarity with structural and functional asymmetry. This polarity begins to develop in late stage III with accumulation of pigment granules towards the animal hemisphere. In this study, we have shown the expression and distribution pattern of Na+,K+-ATPase activity in stage-VI oocytes, and following progesterone-induced maturation. Using enzyme-specific electron microscopy phosphatase histochemistry, [3H]-ouabain autoradiography, and immunofluorescence cytochemistry, we have shown that Na+,K+-ATPase activity is mainly confined to the entire animal hemisphere as bell (the Nucleus/Germinal Vesicle is present in the animal hemisphere). The polarized distribution of Na+,K+-ATPase activity begins to develop in late stage III and continues through to stage VI. Electron microscopy histochemical results also show this polarized distribution persists following progesterone-induced maturation (leading to M-phase), and it becomes gradually more polarized towards the animal pole. The time course following progesterone-induced maturation suggests that there is gradual down-regulation of Na+, K+-ATPase activity leading to germinal vesicle breakdown (GVBD). By GVBD, the Na+, K+-ATPase activity is completely down-regulated due to endocytotic removal of pump molecules from the plasma membrane into the sub-cortical region of the oocyte. This study provides the first direct evidence for a marked asymmetric localization of Na+, K+-ATPase activity in any vertebrate oocyte. Here, we propose that such asymmetry in Na+,K+-ATPase activity establishes and maintains polarity in Xenopus oocytes due to extracellular and intracellular ionic and electrical gradients. Also such asymmetry in stage VI oocyte and their down regulation following progesterone-induced maturation plays a key role in the active state of the germinal vesicle in stage-VI oocytes and chromosomal condensation after GVBD. This work will entail 1. What are the key Na+ dependent genes expressed in cell? 2. What role Na+,K+-ATPase plays in cell cycle? 3. Does down-regulation of Na+,K+-ATPase in M-Phase phosphorelate key enzymes involved in cell cycle? 4. What is the level of Na+,K+-ATPase activity in uterine cancer and if alpha subunit of the protein acts as receptor for progesterone?
**Conclusion:** At the resolution of electron microscopy and light microscopy, Na+,K+-ATPase activity is found to be localized in the animal hemispheres of polarized Xenopus oocytes and it is completely down regulated following progesterone induced maturation.
*Keywords:* Na+, K+-ATPase, Oocyte Polarity, Progesterone-Induce Maturation, Xenopus Laevis

## O-14: Zona Pellucida Birefringence and Meiotic Spindle Visualization of Human Oocytes Are Not Influenced by *In Vitro* Maturation Technology

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**Background:** *In vitro* maturation (IVM) is a promising treatment option for certain infertile women. Nowadays, with the aid of the Polscope, it has become possible to evaluate zona pellucida (ZP) and spindle as parameters of oocyte quality. The goal was to investigate the relationship between the presence of the meiotic spindle and ZP birefringence with morphology of the *in vivo* and *in vitro* matured human oocytes.

**Materials and Methods:** The oocytes were obtained from stimulated ovaries of patients undergoing ICSI (intra cytoplasmic sperm injection). Germinal vesicles (GV; n=47) and metaphase I (MI; n=38) oocytes underwent *in vitro* maturation (IVM) using maturation medium supplemented with FSH+LH. They were checked for maturity 24-40 hours after culture. With aid of Polscope, the presence of spindles and ZP birefringence were assessed in both *in vivo* (n=56) and *in vitro* (n=56) matured oocytes. In addition, the morphology of each matured oocyte was evaluated using inverted microscope. The morphologic characteristics of oocytes were categorized to intracytoplasmic and extracytoplasmic abnormalities.

**Results:** The rate of IVM in GV and MI oocytes was 59.6% and 73.7%, respectively (p=0.25). There were insignificant differences for ZP birefringence and meiotic spindle between the *in vivo* and *in vitro* MII (metaphase II) oocytes. In sub-analysis, the rates of morphologically abnormal oocytes had no significant differences between two groups, except for irregular shape (p=0.001), refractile body (p=0.001) and fragmented polar body (p=0.03), which were higher in *in vitro* matured oocytes. In *in vivo* matured oocytes, the oocytes with intracytoplasmic and both abnormalities showed significantly higher low birefringent ZP (p=0.007and p=0.02, respectively). There was no relationship between the morpho-abnormality of oocytes and spindle detection.

**Conclusion:** Clinical IVM is a safe technology for keeping the maturation and integrity of oocytes high. Also, application of non-invasive Polscope is recommended in IVM program for detection of the most suitable oocytes for ICSI.

***Keywords:*** IVM, ZP Birefringence, Spindle, Oocyte Morphology, Polscope

## O-15: Clinical Grade Isolation and Cryopreservation of Human Testicular Cells

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**Background:** More than a decade work in preservation of male fertility by spermatogonial stem cell (SSC) transplantation in animal models as well as new discoveries about the nature of human SSCs has prompted an investigation into the possibility of an effective clinical-grade procedure for isolation and cryopreservation of human testicular cells.

**Materials and Methods:** Clinical-grade reagents, validated equipment, and protocols consistent with cGTP/cGMP standards were used in developing a procedure suitable for the safe and effective isolation and cryopreservation of human testicular cells. These procedures were designed to be compliant with the relevant FDA regulations. Flow Cytometry in conjunction with specific markers for germ cells (VASA), SSCs (SSEA-4) and testicular somatic cells (LHR) were used to determine the cellular composition before and after cryopreservation.

**Results:** The procedure proved to effectively isolate and cryopreserve testicular cells from hormone-treated adult patients undergoing sexual reassignment. Our results clearly showed that important cell populations are not only preserved but enriched by the cryopreservation process. Sterility tests show that the cryopreserved cells and the thawed cells processed by our protocols under cGTP and some cGMP conditions were free from any microbial contamination.

**Conclusion:** Cryopreservation of testicular cells isolated from fresh tissue is favorable to cryopreservation of testicular tissue because it accommodates cell analysis, quality control, and sterility testing. Further studies are needed to determine whether these findings in adult testicular tissue from sexual reassignment patients can be generalized to young male patients prior cancer therapy.

***Keywords:*** SSC, Fertility Preservation, Cryopreservation, Testis Cells

## O-16: Na+/K+-ATPase Alpha1 Isoform Mediates Ouabain-Induced Expression of Cyclin D1 and Proliferation of Rat Sertoli Cells

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**Background:** Novel roles for the interaction of cardiotonic steroids to Na+/K+-ATPase have been established in recent years. The aim of the present study was to investigate the intracellular signaling events downstream the action of ouabain on Na+/K+-ATPase in Sertoli cell obtained from immature rats. Treatment of Sertoli cells with ouabain (1 μM) induced a rapid and transient increase in the extracellular signal-regulated kinase (ERK1/2 or MAPK3/1) and phosphatidylinositol 3-kinase (PI3K)/serine-threonine protein kinase (AKT) phosphorylation. Also, ouabain upregulated the expression of cyclin D1 and incorporation of [methyl- 3H]thymidine, both of which were dependent on MAPK3/1 but not AKT intracellular cascade, as shown by pretreatment with MEK (MAP2K1/2) inhibitor U0126 and PI3K inhibitor wortmannin, respectively. Moreover, the effect of ouabain on these proliferation parameters was completely prevented by phospho-CREB (cyclic AMP response element-binding protein)/CREB binding protein (CPB) complex inhibitor KG501 and only partially by NFκB nuclear translocation inhibitor SN50. Pretreatment with estrogen receptors antagonist ICI 182,780 showed that MAPK3/1 activation by ouabain does not involve this receptor. The Na+/K+-ATPase α1 isoform, but not α4 was detected in Sertoli cells, suggesting that ouabain effects in Sertoli

cells are mediated via α1. Taken together, these results show a rapid ouabain action in the Sertoli cells, which in turn can modulate nuclear transcriptional events essential for Sertoli cell proliferation in a critical period of testicular development. Our findings are important to understand the role of ouabain in the testis and its possible implications in male infertility.

**Materials and Methods:** Primary cultures of Sertoli cell were obtained from 15-day old male Wistar rats and incubated in the absence (control) or presence of ouabain for detection of cyclin D1, phospho- and total extracellular signal-regulated kinase (ERK1/2 or MAPK3/1) and phosphatidylinositol 3-kinase (PI3K)/serine-threonine protein kinase (AKT) by Western blot. In some experiments the cells were pretreated for 30 minutes with the estrogen receptor antagonist ICI 182,780, MAP2K1/2 inhibitor U0126, PI3K inhibitor wortmannin, disruptor of the CREB:CBP complex KG501 or translocation inhibitor of the NFκB active complex into the nucleus SN50. For proliferation assays, incorporation of [methyl-3H]thymidine into cell DNA was estimated in Sertoli cells.

**Results:** Treatment of Sertoli cells with ouabain (1 microM) induced a rapid and transient increase in MAPK3/1 and PI3K/AKT phosphorylation. Also, ouabain upregulated the expression of cyclin D1 and incorporation of [methyl-3H] thymidine, both of which were dependent on MAPK3/1 but not AKT intracellular cascade, as shown by pretreatment with U0126 and wortmannin, respectively. Moreover, the effect of ouabain on these proliferation parameters was completely prevented by phospho-CREB (cyclic AMP response element-binding protein)/CREB binding protein (CPB) complex inhibitor KG501 and only partially by NFκB nuclear translocation inhibitor SN50. Pretreatment with estrogen receptors antagonist ICI 182,780 showed that MAPK3/1 activation by ouabain does not involve this receptor. The Na+/K+-ATPase alpha1 isoform, but not alpha4 was detected in Sertoli cells, suggesting that ouabain effects in Sertoli cells are mediated via alpha1.

**Conclusion:** Taken together, these results show a rapid ouabain action in the Sertoli cells, which in turn can modulate nuclear transcriptional events essential for Sertoli cell proliferation in a critical period of testicular development. Our findings are important to understand the role of ouabain in the testis and its possible implications in male infertility.

*Keywords:* Na+/K+-ATPase, Ouabain, Rat Sertoli Cells, Cell Signaling

## O-17: Effect of Kisspeptin on Testicular Tissue and Accessory Sex Glands, Seminal Vesicle and Prostate, in Prepubertal Rats

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**Background:** Hypothalamus-derived kisspeptins are critical regulators of reproduction in nearly all mammalian species including the humans. These small peptides mediate their actions through the GnRH loop system. How kisspeptins regulate gonadal maturation in sexually immature male mammals remains elusive. To address this, kisspeptin was administered as subchronic (12 days) twice daily i. p. doses at three different dosage regimens: 15 pmol (10 pg), 1.5 nmol (1 ng) and 1.5 μmol (1 μg), to prepubertal male Sprague Dawley rats (PND 35).

**Materials and Methods:** Effects on spermatogenesis, secretion of testosterone and pituitary gonadotropins, the LH and FSH, DNA parameters and histomorphology of testicular tissue and accessory sex glands, seminal vesicle and prostate, were studied. Major scientific approaches applied were; radioimmunoassay, light and electron microscopy, DNA extraction, electrophoresis and morphometrical measurements. Spermatogenesis was studied histologically at stage VII of the spermatogenic cycle. Data were analyzed statistically.

**Results:** Results showed that at end of the treatments plasma FSH levels were not altered in any of the treatment groups. LH and testosterone concentrations were reduced in the 1 ng ($p < 0.05$) and 1 μg kisspeptin groups ($p < 0.01$), while no significant change was observed at 10 pg dose. At 1 ng and 1 μg kisspeptin doses, testicular parameters that decreased significantly were mainly: the number of type A spermatogonia ($p < 0.05$; $p < 0.01$), preleptotene spermatocytes ($p < 0.05$), pachytene spermatocytes ($p < 0.01$; $p < 0.001$), step 7 spermatids ($p < 0.05$; $p < 0.001$), elongated spermatids and daily sperm production ($p < 0.05$; $p < 0.001$), while at 10 pg dose the decrease was non-significant. Histomorphology showed scant round and elongated spermatids, intratubular vacuolizations, multinucleated giant cells and atrophied germinal epithelium. Ultrastructure evidenced vacuolated mitochondria in Sertoli cells, involuted acrosome, degenerated and vacuolated Leydig cells and thin basal laminae. Seminal vesicle and prostate weights decreased significantly ($p < 0.01$) at 1 μg kisspeptin dose. The epithelial height of secretory acini of seminal vesicle and prostate decreased at 10 pg ($p<0.05$), 1 ng and 1μg doses ($p < 0.001$). Histological observations demonstrated dilated lumen and decrease in epithelial folds and height of epithelial cells. Ultrastructure showed disorganization of the organelles involved in the secretory process such as dilatation of the endoplasmic reticulum, disorganization of the Golgi complex and decrease in the number of secretory granules in principal cells with kisspeptin treatment. Percent DNA damage was significant in all tested doses.

**Conclusion:** The present findings indicate that subchronic kisspeptin administration does not lead to an early maturation but instead it may act as a suppressor of pubertal maturation during non-pubertal states.

*Keywords:* Kisspeptin, Seminal Vesicles, Histomorphology

## O-18: Different Leukocyte Concentrations in Normal and Abnormal Human Semen and Its Correlation with Sperm Intracellular Reactive Oxygen Species, Lipid Peroxidation and DNA Fragmentation

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**Background:** According to World Health Organization guideline (WHO 2010), seminal leukocyte concentration over 1×106/mL is a threat for sperm quality because of their role in reactive oxygen species (ROS) production, lipid per-oxidation (LPO) and later DNA fragmentation (DF). However, this threshold is equal for all semen samples without attention to their qualities. Therefore this study was setup to evaluation of leukocytes concentration in subgroups of human semen and its relationship with intracellular ROS, LPO and sperm DNA fragmentation.
**Materials and Methods:** Ninety-nine semen samples were divided to normal (n=39) and abnormal (n =60) groups. Each group was classified again based on seminal leukocytes concentration include of categories A: ≥0 to ≤0.25 × $10^6$/mL; B: ≥0.25 to ≤0.5 × $10^6$/mL; C: ≥0.5 to ≤0.75 × $10^6$/mL; D: ≥0.75 to ≤1.0 × $10^6$/mL, E: ≥1 × $10^6$/mL). LPO, intracellular ROS, and DNA fragmentation (sperm chromatin structure assay) were studied in all groups and subgroups.
**Results:** Based on Data analysis, in normal group, LPO increased significantly in leukocyte ≥ 1×$10^6$/mL ($p<0.01$) and other parameters did not show any important association with different leukocyte concentrations. Meanwhile, LPO in abnormal group rose significantly in leukocyte concentrations ≥0.75 ($p<0.01$). Also, significant percentage of high DNA stainable (HDS) was observed in class ≥ 1 × $10^6$/mL ($p<0.01$). Intracellular ROS did not show any important correlation in this group too.
**Conclusion:** In normal semen samples, leukocyte concentration ≥1×$10^6$/mL seems as an initial stage of LPO damage; whereas in abnormal semen samples, this point is over 0.75× $10^6$.
***Keywords:*** Human Sperm, Leukocyte, Lipid Peroxidation, Intracellular ROS, DNA Fragmentation

## O-19: Comparison of Pre-Antral Follicle Culture Development during 2 Dimensional and 3 Dimensional Culture Systems

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**Background:** Setting up an *in vitro* follicle culture system that resembles *in vivo* ovary condition has high value in research. Additionally, expression evaluation of folliculogenesis involved genes could lead us to the designing of better culture system.
**Materials and Methods:** ovaries of 12-day-old female NMRI mice were removed, 100-130 µm pre-antral follicles were mechanically isolated from fresh ovaries and cultured for 12 days in two-dimensional (2D) and three-dimensional (3D) culture systems. Alginate hydrogel (0.8%) was chose as a matrix for 3D culture and α-MEM medium that supplemented with FSH, LH, ITS, Activin and FBS was consisted as a Culture medium. Follicles survival rate, growth and also quantitative expression of oocyte maturation genes (Gdf9, Bmp15, Bmp6) were analyzed after 24 hours and 12 days of culture.
**Results:** In 2D culture system, a higher survival rate was observed in comparison with 3D culture system ($p<0.05$). During culture period, significant follicle diameter increase was observed. oocyte maturation genes relative expression indicated non significant higher expression of three mentioned genes in 3D culture system than 2D system.
**Conclusion:** As 3D culture system maintains oocyte maturation m-RNA reserve better than 2D culture system, it seems that improving of 3D culture condition could cause better follicle survival, development and growth.
***Keywords:*** Pre-antral Follicles, Three-Dimensional Culture System, Oocyte Maturation Genes

## O-20: Human Ovarian Tissue Xenotransplantation Application in Drug Discover

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**Background:** Reproductive toxicity studies (RTS) represent an important part of pre-clinical safety evaluation of any drug development process for human consumption. It is mandatory requirement that RTS must be completed before any drug can be administered to women of child-bearing age. Therefore, at first segment of toxicity check the toxic effect of the drug on fertility must be tested on non-clinical studies. In line with it, segment II and III are performed to detect effects on embryonic and post-natal development.
**Materials and Methods:** Two random agents of AX384 and CR21 were received for evaluation blindly. We used our previously introduced human-to-SCID mice (dorsal muscle) ovarian tissue xenotransplantation model as a potential for preclinical RTS. Therefore, xenotransplanted animals were let to recover for the duration of four weeks and proposed dose of each agent was administered using proposed route of administration. Animals in each study group were sacrificed at different time points after drug administration and the grafts were recovered (No=50±6) for further evaluations. Samples were processed for immunonohistochemical evaluated. Number

of existing and apoptotic ovarian follicles (using Active caspase-3) was counted in each sample. Follicles were also collected using laser capture microdissection for microarray-based gene expression profiling.

**Results:** While a single dose administration of AX384 presented a sever toxicity demonstrated by follicular loss evaluated on day 7 (8.1 ± 1.2 vs. 16.4 ± 0.8 %, $p<0.01$) and apoptotic follicles in general (12 ± 5.3 % vs. 3.5 ± 2.2 %, $p<0.05$), CR21 had very moderate follicular toxicity effect ($p>0.1$). From 9100 targeted analyzed genes, 112 were discriminated in samples collected one week after one dose administration of AX384 and 53 genes in CR21 group. In general Discriminating genes were mostly those involved in stress responses, apoptosis, cell cycle control and DNA repair response.

**Conclusion:** Our experiment approved the ability of human ovarian tissue xenografting model as a new *in vitro* testing approach to predict chemical toxicity in drug development procedure. However, further studies are still ongoing till obtaining adequate data to establish a standard protocol.

***Keywords:*** Human Ovarian Tissue, Xenotransplantation, Toxicity Test, Drug Discovery

## O-21: Sperm Chromatin Condensation, DNA Integrity, and Apoptosis in Men with Spinal Cord Injury

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**Background:** To evaluate the effect of cord injury on sperm parameters and DNA chromatin status.

**Materials and Methods:** Data were collected from men referred to Research and Clinical Center for Infertility, Yazd, Iran.Thirty infertile men with the presence of any level of spinal cord injury (SCI) were compared with 30 healthy donors with definite fertility and normal sperm parameters. Sperm chromatin integrity was assessed using aniline blue (AB), chromomycin A3 (CMA3), toluidine blue (TB), and acridine orange (AO) assays. The rate of apoptotic spermatozoa was evaluated with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) staining.

**Results:** Sperm concentration, motility, and morphology in men with SCI were significantly decreased compared with control group ($p < 0.05$). In addition, with regard to cytochemical staining and TUNEL test, the rate of reacted spermatozoa was increased significantly in SCI group when compared with the controls ($p<0.05$). The majority of AB, TB, AO, and CMA3-reacted spermatozoa were higher than the "cut-off" value in men with SCI, as were the number of apoptotic spermatozoa stained with TUNEL.

**Conclusion:** Results showed that SCI disturbs sperm parameters, nuclear maturity, and DNA integrity of spermatozoa. Therefore, the production of spermatozoa with less condensed chromatin and more apoptotic rate increases after cord injury and this may be one possible cause of infertility following SCI.

***Keywords:*** Infertility, Male, Chromatin Condensation, Apoptosis, Spermatozoa, Semen Quality, Spinal Cord Injuries, Cytochemical Tests

## O-22: Fertilization Rates Are Improved after IVF if The Corona Radiata Is Left Intact in Vitrified-Warmed Human Oocytes

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**Background:** Before human MII oocytes are vitrified they are usually denuded from their cumulus cells. In this study we wanted to investigate the effects of an intact corona radiata on the vitrification and fertilization of human oocytes.

**Materials and Methods:** The study comprised two different parts. In Part 1, 36 MII stage oocytes, from 6 patients, were randomly assigned into a control group, a group of vitrified-warmed oocytes without a corona radiata and a group of vitrified-warmed oocytes with an intact corona radiata. In each group of 12, 6 oocytes were used for evaluation of the zona pellucida solubility (hardening) and another 6 oocytes were used for the analysis of their ultrastructure. In addition, six polyspermically fertilized oocytes were used as positive controls for zona pellucida hardening. In Part 2, 16 patients in total produced 107 fresh and 98 vitrified-warmed oocytes, with or without an intact corona radiata. All oocytes were fertilized via conventional IVF and embryos were transferred according to our standard ET routines. The oocyte survival and fertilization rates, embryo quality and pregnancy and implantation rates were evaluated.

**Results:** There were no differences in oocyte survival, zona pellucida solubility (hardening) or the number of cortical granules between the vitrified-warmed and fresh oocytes. There were also no differences in the zona pellucida solubility and the number of cortical granules between vitrified-warmed oocytes with or without an intact corona radiata. However, the oocytes with an intact corona radiata had a higher fertilization rate after conventional IVF insemination. No differences were seen in the survival and cleavage rates, the percentage of highquality embryos or the clinical outcome.

**Conclusion:** Zona hardening and ultrastructural damage do not seem to occur in vitrified human oocytes. An intact corona radiata in vitrified-warmed oocytes retains their fertilization capacity in conventional IVF, but does not improve the embryo quality. Poor fertilizing capacities of vitrified-warmed oocytes without an intact corona radiata seem to have been due to the complete removal of the cumulus cells.

***Keywords:*** Vitrification, Cumulus Cells

## O-23: The Impairment of Reproduction in db/db Mice is not Mediated by Intraovarian Defective Leptin Signaling

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**Background:** To demonstrate whether leptin modulates reproduction by a direct effect within the ovary.
**Materials and Methods:** Adult littermate wild-type (WT) and diabetic (db) leptin receptor (LR) mutant female mice were matched for the allograft of the ovary to construct new genotypic models, respectively. WT mouse received only one ovary from a WT or a db/db mouse (WT Ov-WT,WT Ov-db), and db/db mouse received one ovary from a WT or a db/db mouse (db Ov-WT, db Ov-db). WT and db/db mice received one ovary from a WT mouse and another ovary from a db/db mouse (WT Ov-WT/db, db Ov-WT/db) or received two ovaries all from a WT mouse (db Ov-WT/WT).
**Results:** Both WT Ov-WT and WT Ov-db mice presented normal cycles, comparable serum E2 and FSH levels, and ovarian expressions of the Star, Cyp17, and Cyp19 mRNA, even with different ovary genotypes. In WT Ov-WT/db with hMG stimulation, db ovaries with LR mutation expressed higher Star, Cyp17, Cyp19, Jak2, Stat3, and Pias3 mRNA than in the basal state, whereas WT ovaries with intact LR expressed higher Star, Cyp17, and Cyp19 but divergently lower Jak2, Stat3, and Pias3 levels.
**Conclusion:** We confirmed that impairment of reproduction in intact db/db mice is not mediated by intraovary intact/defective leptin signaling even in face of a divergent modulation by gonadotropins.
***Keywords:*** Db/db Mice, Leptin Receptor, Reproduction, Ovary, Leptin Signaling, PCOS

# Ethics and Reproductive Health

## O-24: Perception of Motherhood Among Donor Oocyte Recipient Women: A Phenomenological Study

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**Background:** Despite the increased demand of donor oocyte treatment, little is known about the experience of donor oocyte recipient women and how they perceive motherhood especially in Iran, due to the cultural, social, and religious situation. The aim of this study is to recognize the recipient women's perception of motherhood.
**Materials and Methods:** Eleven oocyte recipient women participated in this study and their experiences was investigated. It was a qualitative study using phenomenological approach. The cases were chosen by purposive sampling among the women who have referred to Royan institute, Iran. In this study, the data are generated from transcription of taped interviews. Data analysis was in accordance with the procedure introduced by colaizzi (1978).
**Results:** Four main themes emerged from the in-depth interviews: 1. perceiving motherhood as a defective experience, 2. perceiving motherhood as no different experience, 3. perceiving motherhood as a possessive experience and 4. perceiving motherhood as an experience needs to have social approval. Some of the participants put the strong emphasis on genetic relation between mother and child, described this kind of experience as second hand motherhood and other women were perceived motherhood experience as a whole, because of pregnancy, childbirth and breastfeeding. Most of the women talked about trying to make the child their own for example by finding physical appearance similarities between themselves and the children. They gave descriptions of perceiving motherhood pending to have friends and relatives approval.
**Conclusion:** Motherhood through oocyte donation is not an evident phenomenon. According to the finding of the study, acknowledging recipient women's perception of motherhood highlights the importance of counseling, guiding, and supporting the infertile women during and after their treatments.
***Keywords:*** Motherhood, Perception, Oocyte Donation, Phenomenology

## O-25: Factors Affecting The Number of Grade A Embryos in Assisted Reproductive Technology

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**Background:** Infertility is one of the most devastating diagnoses for any woman or man to face, and assisted reproductive technology (ART) is a great gift of life given to these people. There are many factors influencing success rate of ART but quality of embryos is believed to improve this rate and therefore is a major challenge. This paper will focus on examining factors affecting the number of grade A embryos retrieved from infertile patients.
**Materials and Methods:** This was a retrospective study using the data of all ART patients who underwent ART in Royan institute during 9 months in 2010. As many patients had no grade A embryo, Zero Inflated Poisson (ZIP) regression model was fitted using STATA version 11 software to find the factors affecting the number of grade A embryos.
**Results:** Maternal age (b=0.066, p value=0.015) and

cleavages day (b = -0.756, p value = 0.002) were significant in ZIP regression model.

**Conclusion:** The most important factors affecting the number of grade A embryos retrieved are maternal age and cleavages day.

***Keywords:*** Grade A Embryo, Assisted Reproductive Technology, Zero Inflated Poisson

## O-26: The Association of Self Efficacy with Health Locus of Control and Psychological Distress in Infertile Women

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**Background:** Infertility as an important life stressor causes increased distress. The way of dealing with stress in individuals is different depending on the health locus of control. Also self- efficacy as an essential indicator of mental health determines how people cope with infertility. This study aimed to investigate the association of self efficacy with health locus of control and psychological distress in infertile women in 2012 in Mashhad, Iran.

**Materials and Methods:** This descriptive-correlational study was carried out on 205 infertile women who were selected using convenience sampling from Montaserieh Infertility Research Center in Mashhad. Infertile women's self-efficacy, psychological distress and also health locus of control were measured using valid and reliable self-structured questionnaires. Data were analyzed using statistical tests including Pearson and Spearman's correlation coefficient.

**Results:** There was a significant direct relationship between self-efficacy and two external health locus of control subscales including the role of physicians and other people (p=0.000). But an inverse relationship was seen between self-efficacy and chance subscale of external health locus of control(p=0.005). There was no relationship between self- efficacy and internal locus of control. A significant direct relationship was observed between psychological distress and two external health locus of control subscales including chance and the role of other people (p=0.000), but there was no relationship between psychological distress and the role of physicians and internal locus of control. There was a significant direct relationship between self-efficacy and psychological distress (p=0.000).

**Conclusion:** Considering that increased self-efficacy is associated with less psychological distress and self-efficacy itself is affected by external health locus of control, it is recommended to enhance self efficacy and also manage the level of external health locus of control using counseling programs in fertility clinics.

***Keywords:*** Self-Efficacy, Psychological Distress, Health Locus of Control, Infertility

## O-27: "Caring My Family Integrity": Couples' First Childbearing Experience in Urban Society of Mashhad, Iran

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**Background:** Due to the lack of information about the process of forming the choice for and timing of the first child from the participants' viewpoint, the aim of this qualitative study was to discover the process of fertile couples' experience of having their first child in the urban society of Mashhad, the center of Khorasan Razavi province in Iran.

**Materials and Methods:** In this Grounded theory study, 45 participants including 27 women, 12 men and 17 key informants were purposively selected. In-depth interviews were conducted at health centers, homes and workplaces until data saturation was achieved. Data analysis were carried out adopting Strauss and Corbin's mode of analysis through constant comparative method applying open, axial and selective coding using MAXqda software. Study rigor was verified via prolonged engagement, member validation of codes and peer debriefing.

**Results:** The core category that describes couples' experience of having their first child was "Preserving my family integrity". The process of the first time childbearing experience in couples included four interrelated stages of 1. gaining confidence about spouse's capability for being a parent, 2. evaluating circumstantial conditions to make a decision, 3. managing the course of having a child, and 4. parenthood role attainment.

**Conclusion:** Couples' decisions about their first childbirth are influenced by their mutual relationship and the results of their evaluation of a variety of circumstantial conditions. Both planned and unplanned events influence childbearing experiences. It is important to comprehend this four stage process and apply it in reproductive health care especially in family planning services delivery.

***Keywords:*** Couples, First Childbearing Experience, Fertility, Urban Society

## O-28: The Effect of Environmental Contaminants Endocrine-Disrupting PBBs and PBDEs on Reproduction and Fertility Outcomes

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**Background:** Polybrominated Biphenyls (PBBs) and Polybrominated Diphenyl Ethers (PBDEs) are semi-permanent Halogenated compounds that are mainly used in flame retardant. These contaminants as endocrine-disrupting associated with dysfunction of immune, endocrine and nervous systems. Influence various physiological processes such as menarche, birth weight, fetus growth and development, head circumference and gestational age during prenatal and postnatal periods.
**Materials and Methods:** In a cohort study including 1111 birth and 560 women, the association between PBBs with gestational age and birth weight was assessed. In this study participants were classified into 3 groups: under 10 (64), 11-16 and 17-42 years old. Measured PBBs levels were in the range of 0 to 1490 ppb and the mean of each group was 2, 3 and 2 respectively. Serum levels of PBBs were much higher in the younger age exposure. The result of this study show that mothers who have been exposed to these pollutants in younger ages (under 10 years old and before of puberty) were at higher risk for increasing birth weight; 16 gram more birth weight for every 10 ppb increasing in serum level of PBBs. This study shows no effect on gestational age.
**Results:** Studies shown the Half-life of PBBs is 10.8 years in humans. These contaminants are considered disrupting of immune, endocrine and nervous systems. The mechanism of these disrupting is binding to acyl hydrocarbon receptor (ah), cytochrome p450 gene expression and alternation the genes have key role in growth and development. Some of them act by interfering with the estrogen hormone.
**Conclusion:** In a study including 20 pairs of mother and child have seen that higher levels of PBDEs in breast milk has relationship with under mean birth weight (<3.05 Kg).
***Keywords:*** Polybrominated Biphenyls, Polybrominated Diphenyl Ethers, Fertility, Environmental Health

## O-29: Comparing The Relationship of Body Image with Sexual Function and Marital Adjustment in Fertile and Infertile Women

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**Background:** Fertility is one of the great events of human life which can affect the development of women's psychological personality. In contrast infertility is a reproductive crisis. Body image is one of the important concepts in women's mental health and due to its relationship with physical and emotional dimensions of women's life;it can predict their different behaviors, such as sexual behaviors. This study therefore aimed to compare the relationship of body image with sexual function and marital adjustment in fertile and infertile women in 2011 in Mashhad,Iran.
**Materials and Methods:** This comparative correlation study was carried out on 130 fertile women referred to urban health clinics and 130 infertile women referred to Montaserieh Infertility Research Center,Mashhad who were selected using cluster and convenient sampling, respectively.Research tools were consisted of valid and reliable demographic questionnaires including personal and fertility/infertility-related information, modified Younesi Body Image Questionnaire,ROSEN Female Sexual Function Index(FSFI),modified Spanier Marital Adjustment Scale(DAS)and general Health Questionnaire Goldberg and Hillier,which were completed by the subjects. Data analysis was carried out by SPSS(15.5) software using t-test, one way ANOVA,Spearman and Pearson correlation tests and regression.
**Results:** There was no significant difference between the mean score of body image in fertile and infertile women but, it didn't show significant difference between the mean score of sexual function (25.82 ± 4.34 versus 27.23 ± 3.8, p=0.006) and marital adjustment (107.89 ± 21.34 versus 113.8 ± 19.73, p=0.02) in fertile and infertile women.There was a direct correlation between body image with sexual function in fertile (p=0.03) and infertile (p<0.001) women. Also was found a direct correlation between body image with marital adjustment in both fertile women (p=0.001) and infertile women (p<0.001). However, there was no significant difference between the body image with sexual function and marital adjustment in fertile and infertile women.
**Conclusion:** Relationship between body image and sexual functioning and marital adjustment in fertile and infertile women reflects the attention to design and implement training programs and consulting to improve body image in cases of sexual dysfunction and marital adjustment.
***Keywords:*** Body Image, Sexual Function, Marital Adjustment, Fertility, Infertility Fertile and Infertile Women

## O-30: Socio-Cultural Correlate of Fertility Behavior of Civil Servants in Nigeria

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**Background:** Fertility is a complex phenomenon and depends on the normality of an array of important biological systems and mechanism. However, fertility preferences are important because actual fertility desired family size. Fertility behavior of Civil Servants i.e Gov-

ernment Workers are conditioned by both biological and social norms. The objective of the research is to examines the socio-cultural factors influencing fertility behavior of civil servants in three states of Southwest, Nigeria. Assess knowledge of contraceptive usage among civil servants. Examine sources of information, method adopted and decision as a determinant of fertility seeking behavior of civil servants in Nigeria. Explore factors influencing number of child birth.

**Materials and Methods:** The study use data from nationally- representative house-hold based survey from the National Bureau of Statistics (NBS) on fertility behavior of ages between years old. All 19-65 years old de facto resident in each sampled household were eligible for inclusion in the survey. The study adopted structured questionnaire to elicit information from 635 respondents from Lagos, Ibadan, Ekiti and Osun State. The result considered the socio-cultural factors that could determine fertility seeking behavior of civil servants in three major cities of southwestern, Nigeria.

**Results:** The result reveals that civil servants in 61% of civil servants in Lagos, Nigeria have less fertility preference while respondents in Ibadan had 31% fertility preferences and Ekiti were supported with 10% of fertility preferences all these are conditioned based on age, religion, occupation and their income level. In the same vein, respondents within the ages of 30 and 65years are consider numbers of child birth as important considering their monthly and daily expenses.

**Conclusion:** In the findings suggest a behaviour change program on family planning fashioned towards civil servant in the south western region of Nigeria should be organised. The study recommended new intervention strategies for fertility behavior for families with low income level.

***Keywords:*** Socio-Cultural, Fertility Behavior, Civil Servants

### O-31: The Impact of Embryo Scores on Pregnancy Rate in Mother's Clinic in Tabriz

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**Background:** One of the main problems in ART is low pregnancy rates. The factors involved in successful pregnancy include; 1. mother related factors such as the status of endometrial receptivity and hormonal status of the mother. 2. embryo related factors such as; number of transferred embryo, embryo scores including: number and size of the blastomeres, and their fragmentation. The aim of the present study is to study the impact of embryo scores on pregnancy rates.

**Materials and Methods:** For this purpose, the number of ICSIs which were carried out in the Mother's Clinic during 2012, the number of registered pregnancies and score of transferred embryos were collected. The association between different scores and pregnancy were studied.

**Results:** The study revealed that 700 ICSI were carried out and pregnancy rate were 26%. From these; 15% were single embryo transfer, 15% were 2 embryo transfer, 48% were 3 embryo transfer and 22% were 4 embryo transfere. Based on the symmetry of blastomere size 70% were A, 26% were B and 4% were C. According to the number of blastomeres 11% were 4 cell or less, 22% were 6 cell, and 67% were 8 cell embryos. Regarding blastomere fragmentation 56% were grade 1, 22% were grade 2, and 22% were grade 3.

**Conclusion:** It is concluded that the suitable scores that may result in pregnancy are: 3 embryos, A symmetry, 8 cell embryos, and grade 1 fragmentation.

***Keywords:*** Embryo Score, Pregnancy Rate, ICSI

## Female Infertility

### O-32: An *In Vitro* Model for the Use of Egyptian Bee Honey and Royal Jelly in Cases of Premature Rupture of the Fetal Membranes (PROM)

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**Background:** Cases of premature rupture of the fetal membranes (PROM) are very critical with high incidence of fetal mortality. Up till now, there is no definitive prophylaxis for these cases. We present an *in vitro* model objectively testing the effect of Egyptian bee honey (H) and royal jelly (RJ) on the mechanical properties of the fetal membranes.

**Materials and Methods:** Amnion (A) and amnion/chorion complexes (B) of fetal membranes were collected from 138 delivered women following normal labor and PL/PROM. Membrane pieces were treated with either H, RJ, H/RJ mixture (cases), or physiologic saline (controls). The membranes were subsequently evaluated by: a) a manometric device for their mechanical properties, and, b) histological examination for the collagen content.

**Results:** The tearing pressure and the elastic extension yield were significantly improved for both A (pressure of 105.7 with H and 146.5 for RJ versus 50.3 mm Hg for the controls; and elastic extension yield of 1.73 with H and 1.93 for RJ versus 1.46 cm for the controls); and B membranes (pressure of 190.7 mm Hg with H and 246.5 for RJ versus 121.2 mm Hg for the controls; elastic extension yield of 2.01 with H, 2.05 with RJ, versus 1.83 cm for the controls). Histological examination and image-analysis quantification revealed significantly increased collagen staining pattern, too.

**Conclusion:** Bee honey and royal jelly have positive effects on the mechanical properties of the fetal membranes. This may be through “collagen promoting action".

***Keywords:*** Fetal Membranes, Royal Jelly

## O-33: Association of Follicular Fluid Endocrine Changes with Oocyte Morphology and Quality in PCOS Patients Undergoing Metformin and N-Acetylcysteine Treatment

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**Background:** Polycystic ovary syndrome (PCOS) is a common cause of infertility often responsive to treatment with oral ovulation induction agents. PCOS patients are typically characterized by producing an increased number of oocytes that they are often of poor quality, leading to lower fertilization, cleavage and implantation rates. Recently data involve functional associations between endocrine/paracrine irregularities, metabolic dysfunction and changes in the intrafollicular microenvironment during folliculogenesis, follicle maturation in women with PCOS. In the present study, we have evaluated association of follicular fluid(FF) endocrine changes with oocyte morphology and Quality in PCOS Patients undergoing Metformin and N-acetylcysteine treatment.
**Materials and Methods:** A prospective randomized, placebo controlled experimental study was conducted in the IVF/ICSI Unit of infertility treatment center of the Qom. Total 80 cases between the age of 25 and 35 years of polycystic ovarian syndrome were selected for the study, 20 cases in each group were treated for six weeks with Metformin (500 mg three times daily), with N-acetyl cysteine (600 mg three times daily), with N-acetyl cysteine plus Metformin and with Placebo (Group Control). Before oocyte aspiration, follicular fluid was sampled and carefully collected from the first aspiration follicle of each ovary. The endocrine and hormonal parameters evaluated that including levels of fasting insulin, LH, E2, DHEA-S, TT, AMH, leptin and MDA in follicular fluid in each of the four patients groups.
**Results:** After treatment levels of follicular fluid leptin were significantly differences in the treatment groups compared with placebo group, and only levels of follicular fluid insulin and LH were significantly differences in the MET and NAC group compared with placebo group. Data showed that rates of immature oocytes, fertilization and embryo quality between exprimental groups were statistically significant differences. There were 47.4% of the MII oocytes that had abnormality in Placebo group, while there were 24.5, 34.9 and 39.7% of the MII oocytes that had abnormality in groups NAC, MET and MET+NAC groups, respectively. Also, there were significant correlation between of oocytes retrieved, oocyte quality and maturity, fertilization rate, embryo quality with endocrine variables of follicular fluid in PCOS patients.
**Conclusion:** In the present study, we suggest metformin and NAC promoted endocrine and hormonal levels, improvement of oocyte maturation and quality and ovulation of women with PCOS. Taking its lack of adverse effects into consideration, NAC can be regarded as an appropriate substitute for insulin- reducing medications in the treatment of PCOS patients.
***Keywords:*** Polycystic Ovary Syndrome, Metformin, N-acetylcysteine, Oocyte, Follicular Fluid Endocrine Changes

## O-34: Bipolar Disorder in Polycystic Ovarian Syndrome Women

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**Background:** Determining bipolar disorder in polycystic ovarian syndrome (PCOs) women.
**Materials and Methods:** One hundred and ten women with definite diagnosis of PCOS and one hundred and ten age-matched infertile women due to other reasons except for PCOS enrolled in this case-control study. Ten ml fasting venous blood sample obtained to check of fasting glucose, LH, FSH. Height, weight, Waist-to-hip ratio (WHR) checked by an expert technician. A psychiatrist with an experience near thirty years examined all 220 cases in order to determine depression and bipolarity in them.
**Results:** Mean age of each group participants were not significantly different while FBS, LH and LH/ FSH levels were significantly higher in PCOS patients. Eighty eight case were depressed in pco group while 96 were depressed in control group (p=0.03). Bipolar disorder were higher in pco ones in comparison with controls (8 vs. 0, p=0.004).
**Conclusion:** Psychiatric disorders should be considered in PCOS women.
***Keywords:*** PCOS, Bipolar Disorder, Infertility

## O-35: Evaluation of Vit-D3 and Glucoses Levels in Serum and Follicular Fluids of Infertile Patients Undergoing Microinjection and Their Association with The Outcome of The Therapy

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**Background:** The role of Vit-D3 in infertility has been investigated by few studies that have demonstrated the correlation of lower levels of Vit-D3 in follicular fluid and infertility. Additionally, it has been suggested the effect can be linked to lower levels of either glucoses or calcium of the follicular fluid due to lower levels of Vit-D3. Unless Vit-D3 deficiency is endemic in Iran, it is supposed that lower levels of Vit-D3 would decrease success rates of regular microinjection infertility treatments. Therefore, this study it is aimed to determine whether

VitD3 and Glucose levels of follicular fluid are related to failure of microinjection infertility measures in patients of Motahhary Hospital of Urmia.

**Materials and Methods:** This study was a prospective cohort one, which 76 infertile candidates of microinjection therapy enrolled and treated with standard long protocol. Both serum and follicular fluid samples collected and checked for levels of Vit-D3 and Glucoses within 36 hours after injection of HCG whilst follicles were larger than 17mm. Subsequently, clients examined for pregnancy by transvaginal ultrasonography.

**Results:** Based on this studies it is revealed although Vit-D3 deficiency is a common problem, it does not affects outcomes of microinjection in cases of Motahhary Hospital of Urmia (p=0.48). Additionally, it was noticed that levels of glucoses neither in the serum nor the follicular fluid are not related to levels of Vit-D3.

**Conclusion:** The latter findings of this study are in concordance with former researches in the country and are in contrasts with the previously published literature worldwide.

***Keywords:*** Vitamin D3, Glucose, Microinjection, Outcome, Infertility

## O-36: Detection of Fetal Major Structural Abnormalities with US in ART Patients during One Year

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**Background:** The aims were to determine the diagnostic accuracy of ultrasound sonography in detecting major structural anomalies on all patients who conceived during a year of infertility treatment [assisted reproductive technology (ART) or non-ART treatments] at the Royan Institute, and to study the outcome of cases with nuchal translucency (NT) ≥ 95th centile in the first trimester of pregnancy.

**Materials and Methods:** This prospective study was conducted to detect congenital malformations among 703 infertile patients using ultrasound sonography during the first and second trimesters at the Royan institute between 2008 and 2009. All treated patients who had a viable pregnancy on 7th weeks of gestation were included in this study. Prenatal ultrasound determined a total of 932 fetuses, including 509 singletons and194 multiples. All cases with an increased NT (>95th percentile) were referred to amniocentesis and karyotyping, genetic counseling as well as fetal echocardiography. All infants were examined by a pediatrician at birth, and follow-up visits for any major anomalies were scheduled during one year after birth. The obtained result was taken as the gold standard .In the case of major congenital anomalies fetal autopsy finding was taken as the gold standard.

**Results:** According to the results of this study, the finding of first trimester sonography revealed a sensitivity of 54.7%, a specificity of 99.6 %, a positive predictive value (PPV) of 55.1% and a negative predictive value (NPV) of 99.6 %. The result of second trimester sonography showed a sensitivity of 66.2%, specificity of 99.8 %, PPV of 74.3% and a NPV of 99.7%.This study also shows no elevated risk of major malformation between infants of infertile women conceived by ART.

**Conclusion:** Major structural anomalies in the fetus can be reliably diagnosed by a prenatal ultrasound screening.

***Keywords:*** Ultrasound, Major Congenital Anomaly, Assisted Conception

## O-37: HOXA10 Gene Expression in The Endometrium of Women with Repeated Implantation Failure

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**Background:** Repeated implantation failure (RIF) defined as 3 or more failure of pregnancy proceeding assisted reproduction and embryo transfer. Homeobox A10 (HOXA10) is one of the genes which take parts in endometrial decidualization during embryo implantation. In this study, endometrial expression of HOXA10 gene was compared between RIF and normal fertile women.

**Materials and Methods:** Endometrial samples were taken from 7 RIF and 10 normal fertile women. After isolation and culturing endometrial cells, mRNA was extracted and cDNA was synthesized. HOXA10 gene was assayed by quantitative real time PCR and compared between 2 groups. P values <0.05 were considered statistically significant.

**Results:** HOXA10 expression was lower in endometrial stromal cells of RIF patients compared to normal fertile women (p value<0.05).

**Conclusion:** Lower expression of HOXA10 in endome-

trial tissue could threaten the process of embryo implantation and results in repeated implantation failure.

*Keywords:* Homeobox A10, Repeated Implantation Failure, Endometrium

## O-38: Adopting Problem-Focused Coping Strategies Following Implementation of a Collaborative Counseling Program in Infertile Women Undergoing IVF

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**Background:** Infertility is deemed as a crisis, which typically involves both minor and major losses requiring different coping strategies to successfully adjust, adapt and preserve the emotional balance. Alternative ways has been recommended to cope with this problem in infertile couples. The present study was designed to examine the effect of collaborative counseling on problem-focused coping strategies in infertile women undergoing IVF in 2010 in Mashhad, Iran.

**Materials and Methods:** In this clinical trial, 60 women with primary infertility selected from Montaserieh Research Centre and were randomly allocated to intervention and control groups. Women in intervention group were counseled in five sessions with the participation of midwife, gynecologist and clinical psychologist. Control group were counseled routinely. Problem-focused coping strategies were measured at the beginning and embryo transfer day using modified Folkman and Lazarous Ways of Coping Questionnaire. Data were analyzed using statistical tests including t test and Mann- Whitney.

**Results:** A significant difference was seen between two groups in terms of adopting problem-focused coping strategies at the end of counseling program ($p<0.037$), i.e. intervention group used more problem-focused coping strategies. In terms of using various problem-focused coping strategies, the application of two strategies including 'planful problem solving' ($p<0.045$) and 'seeking social support' ($p<0.022$) was significantly different in two groups. But no significant difference was seen between two groups concerning making use of other problem-focused coping strategies such as 'accepting responsibility' and 'positive reappraisal'.

**Conclusion:** The use of some types of problem-focused coping strategies increases after conducting a collaborative counseling program for infertile women. So it is suggested to use collaborative counseling approaches in order to help infertile women to overcome infertility problems.

*Keywords:* Infertility, Collaborative Counseling, Coping Strategies, IVF

## O-39: Puerarin, A Selective Estrogen Receptor Modulator, Disrupts Embryo-Uterine Communication and Inhibits The Process of Implantation in Rats

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**Background:** Unintended pregnancy, abortion, and population explosion are the fundamental notions underlying the rationale for contraception. Emergency contraception (EC), the contraceptive measures that can be taken after sex to prevent pregnancy, is a popular form of reversible contraception; however the available ECs are mostly steroid-based, and therefore associated with steroidogenic untoward effects. Plant-derived estrogen-like compounds, the so-called phytoestrogens, act as non-steroidal selective estrogen receptor modulator. The n-BuOH fraction of the ethanolic extract of Pueraria tuberosa tubers possesses estrogenic activity and exhibits significant antifertility activity in laboratory animals. We explored the active principle(s) of the tuber extract with reference to contragestative effects in rats, and probed the underlying mechanism of action.

**Materials and Methods:** Air-dried tubers were extracted and purified using different methods of chromatography. Bioactivity-guided fractionation identified puerarin as the major constituent of the tuber extract that exerted pregnancy-terminating effects. Rats were orally administered with puerarin at ≥300 mg/kg/day for day 1-2 post-coitus (effective dose) that resulted in complete implantation failure. Rats receiving the vehicle (1% gum acacia) for the same duration served as control. For most of the investigations, rats were sacrificed on D5 at 18.00 hours. To examine whether puerarin exerted post- or pre-implantation failure, a section of rats from each group was subjected to pontamine sky blue reaction under light ether anesthesia. After 30 minutes, the uterine horns were dissected out; the numbers of blue spots produced in utero were taken as the presumptive sites of implantation. The utero-ovarian histoarchitecture was evaluated by staining with haematoxylin and eosin. Serum oestradiol and progesterone levels were measured by automated chemiluminiscence assay system. Embryos (D2-D4) were retrieved from both the control and treated rats and photographed under phase contrast microscope to note any morphological distortion. Uterine expression of leukaemia inhibitory factor (LIF), cyclooxegenase-2 (COX-2) and vascular endothelial growth factor (VEGF) were evaluated by indirect immunofluorescence assay, while expression of estrogen receptor α (ERα) and ERβ, progesterone receptor B (PRB) and PRAB mRNAs in uterine sections was quantified by real-time PCR.

**Results:** Oral administration of puerarin at 300 mg/kg/day for post-coitus day 1-2 adversely impacted the endometrial effects of estrogen by decreasing ERα to ERβ ratio and down-regulating PRAB expression. The consequent effect was down-regulated endometrial expression of a number of crucial implantation-mediating

factors including LIF, VEGF and COX-2 that ultimately deterred endometrial bed preparation and led to failure of implantation. Luteal function, as evaluated by corpus luteum morphology and circulating oestradiol and progesterone levels, however remained undisturbed. One major limitation of projecting puerarin as a prospective candidate molecule for development of contraceptive appears to be its high effective dose in rats. However, we have subsequently developed puerarin nanoparticles (PNP) that have good stability, enhanced intestinal absorption, and increased bioavailability after oral administration; and overall, PNP exerted anti-implantation effect at significantly lower dose levels. Investigations on contragestative potential of PNP and related studies are currently underway.

**Conclusion:** Development of non-steroidal EC is a global need that would significantly contribute to widen the menu of effective contraceptive options and impact the female reproductive health. The present findings bear presumptive evidence for a prospective role of puerarin, possibly in nano-particulate form, as the active molecule for future development of a non-steroidal emergency/post-coital contraceptive.

***Keywords:*** Emergency Contraceptive, Phytooestrogen, Estrogen Receptors, Nanoparticle

## O-40: Evaluation of Anti-Mullerian Hormone (AMH) Cut-Off Levels in Serum and Follicular Fluid in Patients with and without Endocrinological Risk Factor in ART

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**Background:** Assessment of AMH cut-off levels in serum and in follicle fluid (FF) for patients with (group 2, n=34) and without (group1, n= 96) endocrinological risk factors, such as ovarian hyperstimulation syndrome (OHSS) and polycystic ovary syndrome (PCOS), is clinically useful in optimising the treatment protocol.

**Materials and Methods:** AMH was analysed in serum and FF of 130 infertile patients on the day of follicular puncture (FP). Cut-off levels were evaluated for poor responders (n=21), with OHSS (n=19) and PCOS (n=15), by ELISA.

**Results:** In group 1 the AMH level in FF was higher than in serum (p<0.001). According to ROC-AUC, these results revealed for poor responders an AMH cut-off level of 0.61 ng/ml in serum and 1.44 ng/ml in FF, at a sensitivity of 82 % and a specificity of 77%. The pregnancy rate, at a cut-off level of < 0.61 ng/ml, was 12.2 % and at > 0.61 ng/ml, 41.5 %. Mean AMH levels in serum and in FF of patients with endocrinological risk factors were significantly higher than in normal patients in group 1 (p=0.001). OHSS patients revealed an AMH cut-off level in serum of 1.39 ng/ml and in FF of 2.38 ng/ml, at a sensitivity of 79% and a specificity of 74%. In patients with PCOS we found an AMH cut-off level of 4.0 ng/ml in serum and 6.7 ng/ml in FF, at a sensitivity of 92% and a specificity of 96%. We could optimise the treatment protocol according to the AMH cut-off level in serum of women undergoing ART.

**Conclusion:** AMH cut-off levels are an important predictor of poor response and a useful diagnostic criterion for OHSS and PCOS patients. They could be a useful marker for the application of appropriate stimulation protocols and IVF outcome.

***Keywords:*** AMH, Cut-off Level, Poor Response, Pregnancy, OHSS, PCOS

## O-41: Balancing Selected Medication Costs with Total Number of Daily Injections: A Preference Analysis of GnRH-Agonist and AntagonistProtocols by IVF Patients

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**Background:** During *in vitro* fertilization (IVF), fertility patients are expected to self-administer many injections as part of this treatment. While newer medications have been developed to substantially reduce the number of these injections, such agents are typically much more expensive. Considering these differences in both cost and number of injections, this study compared patient preferences between GnRH-agonist and GnRH-antagonist based protocols in IVF.

**Materials and Methods:** Data were collected by voluntary, anonymous questionnaire at first consultation appointment. Patient opinion concerning total number of s.c. injections as a function of non-reimbursed patient cost associated with GnRH-agonist (A) and GnRH-antagonist (B) protocols in IVF was studied.

**Results:** Completed questionnaires (n=71) revealed a mean ± SD patient age of 34 ± 4.1years. Most (83.1%) had no prior IVF experience; 2.8% reported another medical condition requiring self-administration of subcutaneous medication(s). When out-of-pocket cost for (A) and (B) were identical, preference for (B) was registered by 50.7% patients. The tendency to favor protocol (B) was weaker among patients with a health occupation. Estimated patient costs for (A) and (B) were $259.82 ± 11.75 and $654.55 ± 106.34, respectively (p<0.005). Measured patient preference for (B) diminished as the cost difference increased.

**Conclusion:** This investigation found consistently higher non-reimbursed direct medication costs for GnRH-antagonist IVF versus. GnRH-agonist IVF protocols. A conditional preference to minimize downregulation (using GnRH-antagonist) was noted among some, but not all, IVF patient sub-groups. Compared to IVF patients with a health occupation, the preference for GnRH-antagonist was weaker than for other patients. While reducing total number of injections by using GnRH-antagonist is a desirable goal, it appears this advantage is not perceived

equally by all IVF patients and its utility is likely discounted heavily by patients when nonreimbursed medication costs reach a critical level.

*Keywords:* GnRH-antagonist, IVF, Patient Cost, Health Economics

### O-42: Effects of Intrauterine Injection of Recombinant Human Chorionic Gonadotropin before Embryo Transfer on Outcome of *In Vitro* Fertilization/Intracytoplasmic Sperm Injection: A Randomized Clinical Trial

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**Background:** To evaluate the effectiveness of intrauterine injection of recombinant human chorionic gonadotropin (rhCG) before embryo transfer (ET).

**Materials and Methods:** In this randomized placebo-controlled clinical trial, a total number of 182 infertile patients undergoing their first *in vitro* fertilization/intracytoplasmic sperm injection (IVF-ICSI) were randomly assigned to receive 250 µg intrauterine rhCG (n=84) or placebo (n=98) before ET. The implantation and pregnancy rates were compared between groups.

**Results:** Patients who received intrauterine rhCG before ET had significantly higher implantation (36.9% vs. 22.4%; p=0.035), clinical pregnancy rates (34.5% vs. 20.4%; p=0.044) and ongoing pregnancy rate (32.1% vs. 18.4%; p=0.032) when compared to those who received placebo. The abortion (2.4% vs. 2.0%; p=0.929) and ectopic pregnancy rates (1.2% vs. 1.0%; p=0.976) were comparable between groups.

**Conclusion:** Intrauterine injection of 250 µg of rhCG before ET significantly improves the implantation and pregnancy rates in IVF/ICSI.

*Keywords:* Recombinant Human Chorionic Gonadotropin, Intracytoplasmic Sperm Injection, *In Vitro* Fertilization, Implantation Rate, Pregnancy Rate

## Genetics

### O-43: Nanosilver Impacts The Testicular Tissue by Reducing The GLUT I Expression in Leydig Cells; Correlation with Germinal Cells RNA Damage

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**Background:** Nanosilver (NS) contains silver nanoparticles to control infections delivered from bacteria. The NS compounds as well as being toxic to prokaryotes are highly toxic to mammalian cells. The present study was evaluated the effect of colloidal NS on leydig cells glucose transporter type I (GLUT I) expression, serum level of testosterone, germinal cells RNA damage and spermiogenesis process.

**Materials and Methods:** Twenty four mature male mice were divided into four groups (n=6) as test and control-sham groups. The animals in test groups received the colloid NS in doses of 0.5, 1 and 5 mg/kg and the animals in control-sham group received saline intraperitoneally, during 34 consecutive days. The immunohistochemical and Epi-fluorescent analyses were conducted to evaluate the GLUT I expression and germinal cells RNA damage, respectively. The serum testosterone level was assessed by RIA method. The spermiogenesis index was investigated.

**Results:** The GLUT I expression significantly ($p<0.05$) decreased in NS-administrated animals. Accordingly, the high dose received mice were manifested with lowest GLUT I on leydig cells. The serum level of testosterone decreased depending on dose. The germinal cells were exhibited with severe RNA damage accomplished with remarkable reduction in the percentage of seminiferous tubules with positive spermiogenesis index.

**Conclusion:** Our data suggest that NS partly by reducing GLUT I expression on the leydig cells membrane down-regulates the cells glucose intake. Therefore, the leydig cells loss their physiologic ability to synthesis testosterone. Ultimately, the induced impairment leads to severe RNA damage in germinal cells which negatively impacts the spermiogenesis process.

*Keywords:* Nanosilver, GLUT I, Testosterone, RNA Damage, Leydig Cells

## Reproductive Imaging

### O-44: Assessment of Endometrial Volume by Four-Dimensional Ultrasound As A Predictor of Pregnancy Outcome in IVF Patient Who Refer to Royan Institute

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**Background:** To investigate whether endometrial volume on the day of HCG (human chorionic gonadotropin) administration is a predictor of ART (assisted reproductive outcome) outcome

**Materials and Methods:** Two hundred and seven patients in ART cycle were included in this prospective study; In order to evaluate endometrial volume and its relationship to ART outcome. Endometrial volume ob-

tained from the sagittal, coronal and transverse plane of the uterus and it was evaluated in multiplaner 3D and multi-slice view mode.

**Results:** The endometrial volume was divided to 3 group <2cc (Group1), 2-4.5cc (Group2), >4.5cc (Group3). Positive pregnancy in group 1, 2 and 3 were 23.5%, 34.8% and 48.8% respectively.

**Conclusion:** There is no statistical significant relationship between endometrial volume on the day of hCG administration and predicting pregnancy outcome in ART cycle (According to chi-square test which was obtained by the data).However comparing 3 groups it has shown the better pregnancy rate in group 3(›4.5cc).

***Keywords:*** Endometrial Volume, Four-Dimensional Ultrasound, ART

# Poster Presentations

## Andrology

### P-1: The Impact of Gasoline Vapor Inhalation on Serum Levels of Testosterone in Male Rats

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**Background:** Studies have shown that gasoline vapor influences many physiological aspects of body. The present study was exerted to evaluate the effects of gasoline vapor inhalation on serum levels of testosterone in male rats.
**Materials and Methods:** Male Wistar rats were randomly divided into control, gasoline vapor receiving for 1hour, 2 hours and 3 hours/day. After 10 weeks, blood samples were collected using cardiac puncture method. Following serum collection, testosterone level was measured by radioimmunoassay method. Data were statistically analyzed and compared between groups using "one way- ANOVA".
**Results:** The results indicated that serum testosterone level was not significantly changed in animals receiving gasoline vapor for 1 hour per day; however, it was significantly decreased in gasoline vapor receiving animals (2 hours or 3 hours/day) compared with control animals ($p<0.01$).
**Conclusion:** Our findings indicate that chronic inhalation of gasoline vapor has inhibitory effects on testicular function leading to decreased serum level of testosterone.
*Keywords:* Gasoline, Testosterone, Rat

### P-2: Study The Effect of Oral Zinc Supplementation on Thiol Oxido-Reductive Index in Spermatozoa of Patients with Asthenospermia

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**Background:** There are several causes leading to male infertility, like oxidative stress, and nutritional deficiency of trace elements i.e., selenium and zinc. The Zinc is the following only to iron as the most abundant element in the body. Although, Zinc is found in red meat, white meat, fish, and milk; the World Health Organization (WHO) approximates that one-third of world population is deficient in Zinc.
**Materials and Methods:** Semen samples were obtained from 60 fertile and 60 subfertile men with asthenozoospermia between July 2011 to July 2012, from couples who consulted the infertility clinic of the Babil hospital of maternity (Hilla city/ IRAQ). The subfertile group consist of the patients which treated with zinc sulfate, every participant took two capsules of zinc sulfate per day for three months (each one 220 mg). Semen samples were obtained (before and after zinc sulfate supplementation). After liquefaction seminal fluid at room temperature, routine semen analyses were performed. The levels of reduced thiol (RSH), oxidized thiol (RSSR), thiol oxido-reductive index (RSH/RSSR), sulfhydryl oxidase activity (SHO), and glutathione peroxidase activity (GPx) were determined in the spermatozoa and seminal plasma of patients and healthy groups.
**Results:** Compared with healthy controls, thiol levels were found to significantly decrease in semen of asthenospermic patients. Furthermore while RSSR shows remarkable higher values, RSH/RSSR ratio was significantly lower relative to control. Zinc supplementation elevates reduced thiol to normal value level and thus improves the RSH/RS-SR ratio. GPx activities were found to be lower in seminal plasma of asthenospermic patients. Zinc supplementation elevates GPx activity to normal value. SHO activity was found to be lower in seminal plasma of asthenospermic patients. Zinc supplementation elevates SHO activity in seminal plasma of asthenozoospermic subjects to normal value. Volume of semen, progressive sperm motility percentage, and total normal sperm count were increased after zinc sulfate supplementation.
**Conclusion:** Zinc treatment decreases asthenozoospermia through several mechanisms such as reduction of oxidative stress and apoptosis.
*Keywords:* Asthenozoospermia, Male Infertility, Zinc Sulfate, Trace Elements

### P-3: Effects of Ethyl Pyruvate Against Methotrexate-Induced Testicular Injury

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**Background:** Recent advances showed that oxygen radicals and hydrogen peroxides are linked with the development of several pathological processes associated with chemotherapy, including adverse effects of antitumor drugs. Methotrexate (MTX) is an antifolate that is widely used in the treatment of rheumatic disorders and malignant tumors. The efficacy of methotrexate is often limited by severe side effects and toxic sequelae, where oxidative stress is noticeable.Ethyl pyruvate(EP), which is formed from pyruvate and ethanol, has been found capable of rescuing cells injured by oxidative stress. Here, we aimed to study the effect of EP in methotrexate-induced oxidative stress in testes of mice.
**Materials and Methods:** For this aim, 32 male mice of 4 weeks old were used in the study.The animals were divided into four groups randomly. Control, MTX (20 mg/kg ip once a week), EP (40 mg/kg/day ip) + MTX, and

EP. At the end of this period (30 days), they were sacrificed, and testis tissues were removed to be used in the analyses of catalase level.
**Results:** Catalase (CAT) level in testiculartissue of MTX group significantly ($p<0.05$) reduced after MTXadministration compared to control group and MTX +EP.
**Conclusion:** Our data suggests that MTX treatment induces oxidative tissue damage on the testicular tissue, asassessed by decreased CATlevels;EP may cause partially improvement CAT levels .
***Keywords:*** Methotrexate, Ethyl Pyruvate,Catalase

## P-4: Ethyl Pyruvate Against MTX- Induced Sperm Toxicity in Mice

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**Background:** Methotrexate, an immunosuppressive drug, is a folic acid antagonist. This inhibits synthesis of thymidylate, serine, and methionine, which disrupts synthesis of DNA, RNA, and protein and leads to cell death. Ethyl pyruvate, a commercial food additive, is a lipophilic ester derivative of pyruvate .In previous reports, that has been shown to ameliorate organ dysfunction in a variety of *in vivo* models.Our purpose is to evaluate the effect of ethyl pyruvate on sperm count in MTX treated mice.
**Materials and Methods:** 32 adult male mice weighing 26±2gm were divided in four groups: Control group (CG): received distilled water 0.1 ml/mice/day intraperitoneal (IP) for 30 days.Methotrexate group (MTXG): received methotrexate at a dose of 20 mg/kg once a week IP for 30 days.Ethyl pyruvate group (EPG): received ethyl pyruvate at dose of 40 mg/kg daily IP for 30 days. Methotrexate + ethyl pyruvate group (MTX + EPG): received methotrexate at dose of 20 mg/kg once a week IP concomitant with ethyl pyruvate administration.The animals were sacrificed with dislocation of the cervical vertebrae after receiving the last treatment. The Caudal sperm count was evaluated.
**Results:** The caudal sperm count was significantly ($p<0.05$) decreased in the MTX treated mice in comparison to control and MX+EP groups.
**Conclusion:** Considering the data, EPhas a protective effect onsperm count in mice receiving methotrexate.
***Keywords:*** Methotrexate, Ethyl Pyruvate, Sperm Count

## P-5: Assessment of Sperm Chromatin Status in Rat Experimental Varicocele

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**Background:** Varicocele is considered as a common cause of male infertility and can affect spermatogenesis. In addition, it also predisposed sperm to chromatin damage and morphological alternation. Other factors, such as environmental and genetic conditions also predispose sperm to these conditions. To answer this question, we aimed to evaluation of DNA fragmentation and protamine deficiency through induction of varicocele in animal model.
**Materials and Methods:** Twenty one Wistar male rats divided into three groups including left varicocele, control-sham and control groups. Left varicocele was induced by surgery. Two month after the surgery, all animals were scrutinized and sperm from bilateral caudal segment of epididymis were extracted and counted. DNA fragmentation and protamine deficiency were analyzed by acridine orange and chromomycin A3 staining, respectively.
**Results:** In the varicocele group, percentage of sperm DNA fragmentation and protamine deficiency were significantly higher in left compared to right ($p<0.05$). Also percentage of these parameters were significantly higher in the varicocele group than control-sham and control groups ($p<0.05$).
**Conclusion:** This study reveals that varicocele can effect sperm chromatin integrity and such a defects may be overcome by surgery.
***Keywords:*** Varicocele, Chromatin Integrity, Protamine Deficiency

## P-6: Pregnancy Rate after Varicocelectomy: Long-Term Outcome in A Large Number of Varicocele Individuals

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**Background:** Varicocele is commonly existed among infertile men and causes testis damage. Disorder of spermatogenesis was observed in varicocele individuals and leads to low quality sperm parameters and chromatin damage. Chromatin damage plays a critical role in pregnancy outcomes through *in vitro* or *in vivo* fertilization. Varicocelectomy considered as standard treatment of clinical varicocele. Improving pregnancy outcomes following varicocelectomy is under debate. Therefore, the aim of this study was to evaluate of sperm quality and pregnancy outcome after varicocelectomy in large population.
**Materials and Methods:** Microsurgical varicocelectomy was performed as part of patient management on 145 infertile men with grade II or III varicocele. Sperm parameters (concentration, motility and morphology) was assessed according to World Health Organization

(WHO, 2012) criteria and protamine content was evaluated with chromomycin A3 (CMA3) staining. In addition, spontaneous clinical pregnancy and miscarriage rates were evaluated over 12 months after surgery.

**Results:** Varicocele individuals (N=145) were divided into two groups based on pregnancy outcomes after varicocelectomy. One group included partners of 81 individuals whom became pregnant (pregnant group), while the other group included individuals whom partner did not became pregnant (non-pregnant group). In both groups, semen parameters and chromatin packaging significantly improve after varicocelectomy. The percentages of spontaneous cumulative pregnancies post-surgery were 33.1% (3 months), 42.06% (6 months), 46.2% (9 months), 48.9% (12 months), and 55.8% (after 12 months). Percentages of spontaneous cumulative miscarriage post-surgery also were 2.46% (3 months), 4.93%(6 months), 4.93% (9 months), 6.17% (12 months), and 6.17 % (after 12 months).

**Conclusion:** Varicocelectomy can improve semen quality and chromatin integrity in varicocele individuals but the differential factors between the individuals whose partners became pregnant in comparison to those that did not achieved pregnancy remains to be determined. Also these data sugget that if spontaneous pregnancy is not achieved within 6 12 month following surgery, assisted reproductive techniques (ART) are strongly recommended.

***Keywords:*** Varicocelectomy, Sperm Parameters, Chromatin Packaging, Pregnancy

## P-7: The Effect of 950 MHz Microwaves on Apoptotic Changes of Epididymis in Rabbits

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**Background:** Regarding the wide domain of microwaves applications in human life, yet its biologic impacts on human body cells are discussed. Therefore our goal is the investigation of mobile phone microwaves with frequency of 950 MHz on apoptotic changes of epididymis in rabbits.

**Materials and Methods:** 18 male New Zealand rabbits were randomly divided into 2 experiment group and 1 control group. Unlike control group rabbits, those of the experiment groups received simulated microwaves with the frequency of 950 MHz and output power of 3 and 6 watt for 2 weeks, 2 hours a day. After one week rest, the animals were killed by anesthesia and cell samples of their epididymis were provided, and the cells of epididymis were examined in terms of the number of apoptotic cells under optical microscope after being fixed and stained with TUNEL technique.

**Results:** The rate of apoptosis in epithelial cells of epididymis only in 6-watt group had a significant increase compare to the control group ($p<0.001$).

**Conclusion:** The current study showed that microwaves with the frequency of 950 MHz can have negative impacts on epididymis of rabbits by increasing the apoptosis.

***Keywords:*** Microwaves, Mobile, Epididymis, Rabbit

## P-8: Evaluation of Protective Effect of Satureja Hortensis Extract against Cyclosporine-A Induced Changes in Testicular Tissue; A Histomorphometrical Analysis

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**Background:** Cyclosporine-A (CsA) is an immunosuppressive drug used in organ transplantation. Side effects of CsA such as body weight loss and decreased fertility rate have been reported. Satureja hortensis is a therapeutic plant with antioxidant properties.

**Materials and Methods:** 24 adult male rats weighing 200±20 gr were divided into three groups (n=8): control group which received no treatment; CsA group which received 40/mg/kg/day CsA and CsA-S. hortensis group received 40/mg/kg/day CsA and 100 mg/kg/day aqueous extract of S. hortensis with oral gavage. Half of each group was sacrificed on 21st day of treatment and the other half on 45th day, and the testicular tissues were collected and fixed and stained with Haematoxylin and Eosin (H&E). The prepared slides were evaluated with light microscope.

**Results:** CsA significantly ($p<0.01$) decreased seminiferous tubules diameter, height of seminiferous epithelium (HSE) and number of Leydig cells compared to the control group. Furthermore, CsA increased number of mononuclear lymphatic cells in comparison with control group. S. hortensis in CsA-S. hortensis group partially improved the mentioned parameters.

**Conclusion:** Considering the data, S. hortensis may ameliorate CsA induced detrimental effects on testis.

***Keywords:*** Cyclosporine-A, Satureja Hortensis, Testis, Histomorphometry, Rat

## P-9: Effect of Lyophilized LDL and Different Levels of Clove Bud Extract on The Quality of Cryopreserved Ram Semen

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**Background:** Low density lipoprotein (LDL) of egg yolk

has been successfully used for stallion and bull semen cryopreservation. However, there is limited information to clarify the effect of LDL or lyophilized LDL on ram semen quality. On the other hand, during semen collection, dilution and cryopreservation we need to protect the spermatozoa from lipid peroxidation. Clove bud has polyphenolic compounds with powerful antioxidant properties. So the objective was to evaluate the cryoprotective effect of lyophilized LDL and different levels of clove bud extract added to ram semen extender.

**Materials and Methods:** Semen samples were collected from three mature Iranin rams (3-4 years old and 105 kg BW) by using an artificial vagina twice a week during the breeding season. The semen samples were diluted with extenders containing 15% egg yolk (as control) or 8% lyophilized LDL. The levels of clove bud extract added to the extender were 0, 35, 75, and 115 mg/ml.

**Results:** This study demonstrated that extender with lyophilized LDL had adverse effects on motility and viability of spermatozoa before freezing ($p < 0.05$), but there was no significant difference between the two group after thawing. Different levels of clove bud extract affect the sperm quality before and after cryopreservation ($p<0.05$).

**Conclusion:** It was concluded that 15% egg yolk had better performance than 8% lyophilized LDL in semen extenders in before freezing and the best level for clove bud extract was 75 mg/ml.

***Keywords:*** Lyophilized LDL, Plant Antioxidant, Semen Cryopreservation, Ram

## P-10: Ethyl Pyrovate Repopulate The Leydig and Sertoli Cells in Testis of Cyclophosphamide Treated Adult Mice

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**Background:** Increases in the survival rate of men treated with chemotherapeutic drugs and their desire to have children precipitate concerns about the effects of these drugs on germ cells. This study performed to evaluation of protective effects of ethyl pyrovate in oxidative stress induced by cyclophosphamide (CP) on testis.

**Materials and Methods:** Three groups (6 mice in each) of adult mice were used. Control group treated with normal saline via ip, and group 2 treated with CP 15 mg/kg/week,ip and group 3 treated with CP along with ethyl pyrovate 40 mg/kg/day,ip. After 35 days samples were took and fixed in 10% formal saline and paraffin sections were prepared and stained by H&E method. Mean distribution of Laydig and Sertoli cells were counted in 1 mm2 field in 5 regions of each slides by latticed objective device. All obtained data were analyzed by SPSS software in ANOVA and Duncan test.

**Results:** Results showed that the mean distribution of Leydig and Sertoli cells in group that treated with CP (9.27 ± 0.34 and 21.86 ± 0.81), significantly was lower than control group (18.99 ± 0.64 and 61.72 ± 3.17), but ethyl pyrovate ameliorate the oxidative effects of CP, and mean distribution of Leydig and Sertoli cells (15.57 ± 0.46 and 28.34 ± 0.6) significantly were more than CP treated group ($p<0.05$).

**Conclusion:** This study showed that ethyl pyrovate ameliorate the oxidative stress effects of CP on reproductive organ.

***Keywords:*** Ethyl Pyruvate, Leydig, Sertolicells, Cyclophosphamide, Mice

## P-11: Varicocele Improved Semen Quality and Chromatin Integrity in Normozoospermic and Non-Normozoospermic Varicocele Individual

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**Background:** Varicocele is related with poor semen quality and sperm chromatin integrity which possibility decreases the spontaneous pregnancy potential. Some of the varicocele individuals have normal semen parameters with failed pregnancy. It is also one of the most controversial issues in the field ofinfertility, especially regarding why, when and for whom varicocelectomy should be implemented. It is unclear that whether varicocelectomy is beneficial for varicocele individuals with normal sperm parameter. Therefore, we aimed to compare spontaneous pregnancy rate in normozoospermic and non-normozoospermic varicocele individual undergoing varicocelectomy.

**Materials and Methods:** Varicocele individual with grade II or III referred in Isfahan fertility and infertility divided tonormozoospermic group (n=81) and non-normozoospermic (n=64) groupsaccordingly World Health Organization guideline 2010. Cumulative spontaneous pregnancyafter varicocelectomy followed up during 12 month after varicocelectomy.

**Results:** Spontaneous pregnancy achievedup to 50% in normozoospermic group and 45 % in non-normozoospermic group.

**Conclusion:** Our study revealed that varicocelectomy has beneficial effect on normozoospermic and non-normozoospermic individual to achieve spontaneous pregnancy.

***Keywords:*** Varicocele, Varicocelectomy, Semen Parameters, Pregnancy

## P-12: Histological and Histometrical Studies of Rat Prostate Lobes Following Administration of Testosterone

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**Background:** The early development and normal

growth of the prostate gland, also development of benign prostatic hyperplasia (BPH) is an androgen-dependent functions. Testosterone plays a major role in the prostate gland. Rat prostate gland is one of the most widely used models of animals in investigation. The rat prostate gland consisted of five lobes; the ventral, anterior, dorsal, lateral type 1 and latteral type 2. The goal of present study was to reveal the influence of testosterone on histomorphological structure of different lobes of rat prostate gland.

**Materials and Methods:** For this purpose, 15 adult and healthy male rats in 3.5-4 months of age and weighing 250-300 g were selected and divided in three groups 1: control group, group2, received solvent of testosterone (almond oil) 10 mg/kg/day(SC), group3, received testosterone (SC) 10 mg/kg/day. The rats were kept in 12L 12D and 25°C temperature for 6 weeks. Then, rats were sacrificed, weighted and prostate glands were removed. The weight and volume of prostate were measured and fixed in buffer formalin 10%. Samples were taken from different lobes. The 5-6 µ sections were made using paraffin embedding method and were stained with H&E and PAS staining. The histometrical studies were done using Dino digital Lens and Dino soft ware capture1.

**Results:** The results showed that prostate/rat weight and prostate volume in the testosterone group were increased significantly compared to the other groups ($p < 0.0001$). Micrometry results indicated that the maximum effect of testosterone on the prostate gland were seen in ventral and anterior lobes.The parenchyma / stroma ratio ($p<0.05$), thickness of the epithelium ($p < 0.0001$), lumen diameter of secretary units and number of secretory cells ($p < 0.05$) were increased significantly in the ventral lobe of group received testosterone.

**Conclusion:** The results showed that different lobes have different response to T and ventral and anterior lobes are more sensitive to administration testosterone, which it must be consider in rat prostate investigation.

***Keywords:*** Histometrical, Rat Prostate, Testosterone

## P-13: Histomorphometric Changes of The Skin in Rats Following Administration of Testosterone and Nettle

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**Background:** Androgen plays role on body physiologic. Testosterone is one of the important androgen. It has been reported that testosterone can have effects on hair follicles, dermal fibroblasts and interfollicular keratinocytes *in vitro* conditions. The purpose of this study is to investigate the effects of testosterone and nettle on skin structures and sebaceous glands.

**Materials and Methods:** For this study, 20 healthy adult male wistar rats with average weight 250 to 300 g and approximately 3.5 - 4 months age were used. They were fed with standard diet. The rats were divided to four groups (5 rats in each group): the control group which the rat without injection, the testosterone group which the rats injected by testosterone with 10 mg/kg/day dose, nettle group which the rats injected by nettle extract with 50 mg/kg/day dose. The almond oils group with 10 mg / kg / day dose. All injections were performed subcutaneously. The rats were easy drawing with chloroform. After 42 days, samples were taken from head skin and the 5-6µ thickness sections were made by paraffin embedding method and were stained by H&E and Masson trichrome.The histomorphometrical studies were done using Olympus BX51 microscope and Olysia software.

**Results:** The results showed that the sebaceous glands increased significantly grew in the testosterone group than control group while histological changes were not observed between the control Group and nettles. Micrometrical results showed that thick of epidermis and dermis was increased in the testosterone group compared to other groups. Histological results showed that there are not difference in fibroblasts and fibers in the between group.

**Conclusion:** The sebaceous glands increased significantly grew in the testosterone group than control group while histological changes were not observed between the control Group and nettles

***Keywords:*** Histomorphometric, Skin, Rats, Testosterone, Nettle

## P-14: *In Vitro* Comparison of Different Dietary Omega-3/Omega-6 Fatty Acid Ratios and Vitamin E on The Rat Semen Quality

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**Background:** Beneficial effects of omega-3 polyunsaturated fatty acids (PUFAs) on the male fertility are widely described in the literature. However, there is little information about the effect of dietary n-3/n-6 ratios and Vit E on the male reproduction. The aim of this study was to evaluate the influence of dietary PUFAs on the rat sperm characteristics after eight weeks oil consumption.

**Materials and Methods:** Mature male Wistar rats (n=50; age 8 weeks) were divided into 5 experimental groups consisted of: Control (CTR; no additive); Vitamin E diet (Vit E; 2.5 times greater than NRC recommendations); n-6 group (0.5 ml/d sunflower oil+Vit E); n-3 group (0.5 ml /d fish oil+Vit E) and n-3+n-6 group (0.3 ml fish oil/d + 0.2 ml sunflower oil/d+Vit E). All animals were euthanized with ketamine-xylazine (KX) after 8 weeks treatment. Sperm samples obtained from the left distal cauda epididymis. Sperm quality and kinematic

parameters were measured by computer aided semen analyzer (CASA). Moreover, blood plasma lipoproteins parameters were measured. Finally, testis and livers were weighted at the end of period. Data were analyzied by SPSS16.

**Results:** The highest sperm concentartion was observed in n-3 group (73.2 × $10^6$/ml). Dietary omega-3 fatty acids and dietary Vit E 2.5% can improved progressive motility compare with other group ($p<0.05$). Interstingly, fish oil significanly improved motion patterns; so, straight line velocity (VSL), average path velocity (VAP), straightness (STR) and beat cross frequency (BCF) of sperm were the highest compared with all of other groups ($p<0.05$). A significant increase in the mean LDL-cholesterol level was observed in the n-3+n-6 group compared with all other groups ($p<0.05$).

**Conclusion:** In conclusion, originally the rat sperm contain high level of n-6 fatty acids; dietary omega-3 fatty acids may improve sperm quality. The manipulation of sperm parametres by dietary fatty acids in overall duration of spermatogenesis.

***Keywords:*** Omega-3 Fatty Acids, Omega-6 Fatty Acids, Vitamin E, Sperm Parameters

## P-15: Morphometrical and Histometrical Study of Protective Effect of Hydro Alcoholic Extract of Lepidium Sativum Seeds on Testis in Normal and Diabetic Male Rat

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**Background:** Clinical as well as experimental studies revealed impairment of spermatogenesis in diabetes. Diabetes leads to cell death due to increased oxidative stress. Lepidium sativum (LS) is a plant with antidiabetic effect.The aim of this study was to investigate the protective effect of LS on sperm parameter and testis morphology in adult male rat.

**Materials and Methods:** In this study male adult rat were divided into six groups. The control group received normal saline, Groups 2 and 3 received 50 and100 mg/kg LS mg/kg respectively. Group 4 received a single dose of 65 mg/kg STZ for induction of diabetes. Groups 5and6 received 50 and100 mg/kg LS respectively plus a single dose 65 mg/kg STZ. LS extract and normal saline was administered for 21 days. Animals were sacrificed 22 days after treatment and evaluations were made by determining of sperm count and sperm quality, histological study of testis and measuring of plasma testosterone level. Statistical analysis was performed using ANOVA and Tuckey test.

**Results:** LS -treated groups showed a significant decrease in the number of germinal cells of seminiferous duct ($p<0.01$) and sperm motility ($p<0.01$) compared with controls. Number of germ cells was reduced in LS -treated group and there was a significant difference with control groups. In both 50 and 100 mg/kg treated group there were no significant differences in morphologic and morphometric studies.

**Conclusion:** Administration of Lepidium sativum in doses of 50 and 100 mg/kg for 21 days has toxic effect on sperm parameter and morphology of testis.

***Keywords:*** Lepidium Sativum, Sperm Parameter, Diabetes

## P-16: Can Permeable Super Oxide Dismutase Mimetic Agents Improve The Quality of Frozen-Thawed Ram Semen?

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**Background:** This study was carried out to assess the effects of MnTBAP, a cell permeable antioxidant, on motility, membrane integrity, capacitation status and *in vitro* fertilization ability of frozen-thawed ram semen.

**Materials and Methods:** Fresh semen ejaculates were collected with artificial vagina from five rams, mixed and divided into five equal fractions, and diluted (1:20 v/v) with commercial extender, Bioxell®, containing 0 (control), 50, 100, 150 and 200 µM of MnTBAP. All diluted sperm suspensions were cooled to 5°C for 2 hours followed by transfer into 0.5 ml French straws before being stored in liquid nitrogen.

**Results:** The results showed that MnTBAP supplementation of extender improved ram semen quality in a dose-dependent manner. Accordingly, the extender supplemented with150 µM MnTBAP resulted in higher sperm motility and improved acrosomal membrane integrity compared to control. However, further supplementation (200 µM) with MnTBAP not only did not improve the results but inversely affected motility and membrane integrity. The results of *in vitro* fertilization (IVF) indicated that the presence of MnTBAP in semen extender has a marginal beneficial effect on developmental competence of inseminated oocytes, though this improvement was not significant.

**Conclusion:** This study demonstrated that semen extender supplemented with MnTBAP can reduce the oxidative stress provoked by freeze/thaw processes. Moreover, beneficial effect of 100 µM of MnTBAP on preservation of spermatozoa in a non-capacitated state post freezing, an important criterion for *in vitro* or *in vivo* fertilization, was observed. However, at 150 µM of MnTBAP, the harmful effects of cryopreservation on membrane integrity were decreased. Regarding to importance of non capacitated spermatozoa during IVF or artificial insemination, the optimum MnTBAP concentration appears to be 100 µM for commercial ram semen extender tested here.

***Keywords:*** Cryopreservation, Ram Semen, MnTBAP, Capacitation, *In Vitro* Fertilization

## P-17: Simultaneous Effects of Exposure to Microwaves and Noise on Male Rats' Sperm Parameters and Total Antioxidant Capacity

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**Background:** There is currently great concern about the possible adverse effect of microwave radiation from cell phones. In addition, noise is one of the physical pollutants of modern societies. The present study aimed to examine the separate and simultaneous effects of cell phone microwaves, noise, and their effects on sperm parameters and total antioxidant capacity in adult male rats.
**Materials and Methods:** An experimental study was conducted on 28 Wistar adult male rats (200-250 g). Randomly selected animals were divided into four groups; control (C), microwave (M), noise (N), and noise plus microwave (NM) groups. In all groups, a sperm analysis was performed based on World Health Organization (WHO) standards and the mean of the sperms' total antioxidant capacity was determined by a Ferric Reducing Ability of Plasma (FRAP) assay. The data were analyzed by a one way ANOVA statistical technique, followed by a Tukey's test using SPSS (version 16) software and $p < 0.05$ was considered significant.
**Results:** The findings of the study demonstrated that sperm viability and motility, in the exposure to cell phone waves group (group 2) and the simultaneous exposure to cell phone waves and noise group (group 4), decreased significantly compared to the control group ($p < 0.05$). Moreover, the total antioxidant capacity of sperm in all exposure groups decreased significantly compared to the control group ($p < 0.05$).
**Conclusion:** Exposure to cell phone waves can decrease sperm viability and motility in adult male rats. These waves can also lower rat sperms' total antioxidant capacity which results in oxidative stress. Exposure to severe noise levels can cause a significant decrease in the total antioxidant capacity of sperm in adult male rats, resulting in oxidative stress.
***Keywords:*** Cellular Phone, Noise, Oxidative Stress

## P-18: Effect of Royal Jelly on Oxidative Stress Marker in Testes Tissue in Streptozotocin-Induced Diabetic Rat

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**Background:** Chronic diabetes mellitus is accompanied with enhanced oxidative stress and reduce the activity of antioxidant defense system. Due to significant role of enhanced oxidative stress in development of testes damage in diabetices, this study was conducted to evaluate the effect of royal jelly on oxidative stress marker in testis tissue of diabetic rats.
**Materials and Methods:** In this experimental study, 21 male Wistar rats were divided into 3 groups: normal control, diabetic control and diabetic controls treated with royal jelly (100 mg / kg). In this study, diabetic was induced by intraperitoneal injection of streptozotocin (60 mg / kg). Royal jelly with a dose of 100 mg / kg body weight was given by gavage. The duration of treatment was 6 weeks. Tissue level of malondialdehyde in testes tissue were measured. Data were analyzed using ANOVA and Tukey tests.
**Results:** A significant increase in tissue level of malondialdehyde in diabetic rats were observed ($p<0.05$). diabetic controls treated with royal jelly significantly reduced the tissue level of Malondialdehyde.
**Conclusion:** Six weeks treatment of royal jelly (100 mg/kg bw) reduce oxidative stress indexes in testes tissue of diabetic rats.
***Keywords:*** Royal Jelly, Diabetes Mellitus, Testes, Oxidative Stress, Malondialdehyde

## P-19: Effect of Three Sperm Preparation Methods on Sperm DNA Integrity and Oocyte Fertilization Rate

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**Background:** Techniques of sperm preparation require centrifugation to separate spermatozoa from the seminal plasma. The centrifugation can induce damage to the spermatozoa and increase reactive oxygen species(ROS) productionspecially in leukospermic speciemens that are associated with impaired function and fertilizing capacity. However in ICSI, fertilization can occure with damage DNA sperm but normal sperm chromatin strcture has been directly correlated with the rate of oocyte fertilization and embryo development. Our aim is to compare the effect of Swim up, DGC (with centrifugation) and Swim out (without centrifugation) techniques on spem DNA integrity by sperm chromatin dispersion (SCD) test and the fertilization rate.
**Materials and Methods:** Semen sample was obtained from 207 patients referring to Montaserieh fertility and infertility center. 75 patients had leukospermia ($>5\times10^6$ Not/ml) . Semen was analysed according to World Health Organization (WHO) criteria. Each semen sample was divided into 4 equal portions (Raw, Swim up, DGC, Swim out). Aliquots of each semen samples was measured for DNA fragmentation by SCD test. A total of 1863 oocytes were retrieved from patients and were injected with the husbands، prepared sperm. Then fertilization rate was evaluated on pronuclei stage.
**Results:** For 207 patients, the level of DNA fragmentation in recovered sperms by 3 preparation methods was significantly lower than Raw semen ($p<0.05$). There was no significant difference in the percentage of DNA fragmentation and fertilization of recovered sperms by each of 3 methods($p>0.05$). For 75 leukospermic patients, sperm DNA fragmentation after 3 methods was improved significantly compared with Raw semen. Sperm DNA fragmentation and fertilization rate signifi-

cantly augmented in comparison with DNA integrity and Swimed up recovered sperms (p<0.05).

**Conclusion:** Detrimental effect of sperm preparation techniques on sperm DNA fragmentation is related to the initial semen quality. It seems that use of Swim out method in leukospermic patients due to elimination of centrifugation step, which could stimulate ROS production by leukocytos can separate sperm with higher DNA integrity and increases fertilization rate.

***Keywords:*** Sperm Preparation, DNA Fragmentation, ICSI, SCD Test, Oocyte Fertilization

## P-20: A Study on Walnut Effect on Reproductive System of Adult Male Rat

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**Background:** Diabetes mellitus is a degenerative disease that has deleterious effects on male reproductive function, possibly through an increase in oxidative stress. Walnut is known as an antioxidant that may be effective on decreasing free radicals. The aim of this study was to evaluate walnut consumption effects on testis and prostate in diabetic Wistar male rats induced by STZ.

**Materials and Methods:** Diabetes mellitus was induced by STZ (60 mg/kg) in wistar male rats. Rats were randomly divided in 5 groups (6 rats in each group) included: normal diet and healthy (sham), Diabetic by normal diet (control) and Diabetic by 6, 9 and 12% walnut (experimental groups) in their diet. Testis weight, prostate weight and seminiferous tubules diameters were evaluated for each groups.

**Results:** Blood sugar was significantly increased in three times of testing(p<0.001). Prostate and right testis weight significantly decreased in experimental and control groups compare to sham group (p<0.05). Left testis weight significantly decreased in experimental (6 and 9%) and control groups compare to sham group (p<0.05). In 12 % walnut, there was no significant difference compare to sham group. Seminiferous diameters in control group significantly decreased and in experimental groups no significant difference in compared to sham groups (p<0.05).

**Conclusion:** Our data showed that, walnut as an antioxidant source had a significant improvement effects on reproductive system as testis and prostate in diabetes-induced male rats.

***Keywords:*** Walnut, Diabetes, Streptozotocin, Infertility

## P-21: Protective Effect of Black Grapes Seed Hydroalcholic Extract on Sperm Parameters in Male Mice Treated with Fluoxetine

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**Background:** Fluoxetine (FLX) is an Antidepressant compound. FLX produces undesired side effects including anxiety, sleep disturbances, sexual dysfunction and gastrointestinal disturbances. The grape is a significant source of nutritional antioxidants, such as polyphenols, anthocyanins, flavonids as well as biologically active dietary components. Present study was performed to investigate the protective effect of antioxidant treatment with Black grape seed extract (BGSE) on FLX -induced sperm damage.

**Materials and Methods:** In this study 24 mature male mice were used. The animals divided into four groups as, control, FLX (20 mg/kg), FLX (20 mg/kg) + BGSE (100mg/kg) and BGSE (100 mg/kg) orally for 42 days . The caudae epididymides spermatozoa were obtained on day 42 in all groups. Sperm count, viability, maturity, motility and DNA double-strand breaks were examined.

**Results:** The results revealed that sperm count and sperm motility was significantly (p<0.05) decreased in FLX treated group in compared to other groups. Sperm viability and sperm maturity in FLX group was not significant statically between all groups. Sperm DNA damage increased significantly in FLX group in comparisons with other groups.

**Conclusion:** Fluoxetine can induce male infertility. Simultaneously administriation FLX and BGSE prevent negative effect of FLX on sperm parameters.

***Keywords:*** Fluoxetine, Black Grapes Seed, Mice, Sperm

## P-22: Gibberellic Acid Can Decrease Sperm Quality of Adult Male Rats

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**Background:** Little is known about Giberellic acid, a plant growth regulator used in many countries, on sperm parameters; so this study was conducted to evaluate its effects on sperm parameters.

**Materials and Methods:** A number of twenty seven adult rats were divided into three groups (n=9): control group (C), control-sham group (C-Sh) and treatment group (Tr). The C-Shgroupand the Tr group were respectively injected 1% methanol and 2mg/kg BW Giberellic acid (dissolved in 1% methanol)intraperitoneally, every other day. On days 15, 30 and 45 three rats from each group were evaluated for sperm parameters including total

sperm count, viability (Eosin Negrosin staining), maturity (Aniline Blue staining) and chromatin integrity (using Acridine Orange staining).
**Results:** There was no significant ($p<0.05$) difference between C and C-Sh groups in any of the parameters. Also no significant($p<0.05$) difference between Tr and the other two groups in sperm chromatin integrity and sperm viability but there was a significant decrease in total sperm count of Tr group on day 45 and a significant increase in sperm maturity of this group on days 30 and 45 compared to the other two groups.
**Conclusion:** The longer Gibberellic acid is used, the more its detrimental effects on sperm parameters couldtake place.
*Keywords:* Gibberellic Acid, Sperm Parameters, Intraperitoneally, Rat

## P-23: Evaluation of Relation between Sperm DNA Fragmentation with IUI Outcomes

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**Background:** Sperm chromatin integrity assessment has been suggested as fertility predictor. The aim of this study was to examine the relationship between the results of sperm chromatin structure assay (SCSA) and the outcome of intrauterine insemination IUI.
**Materials and Methods:** A total of 576 consecutive couples undergoing IUI were included and IUI was performed. SCSA results were expressed as DNA fragmentation index (DFI) before and after processing. Reproductive outcome parameters were biochemical pregnancy (BP), clinical pregnancy (CP) and delivery (D).
**Results:** The average of DFI before processing was significantly higher than those of DFI after processing ($p<0.001$). DFI was negatively correlated to sperm concentration, sperm total motility, type A and B motility and sperm morphology before processing and also, between DFI before and after processing.
**Conclusion:** SCSA is a useful method for prediction of the outcome of IUI.
*Keywords:* IUI Outcome, Sperm, DFI, SCSA

## P-24: The Effect of Non-Steroidal Drug in Male Rats' Fertility (An Experimental Design)

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**Background:** COX-2 selective inhibitor is a form of nonsteroidal anti-inflammatory drug (NSAID) that directly targets COX-2, an enzyme responsible for inflammation and pain, It is the main feature of celecoxib, In some patients that used celecoxib for a long time, unusual effect of this drug may be seen. The goal of this survey is assess the effect celecoxib on male-reproductive system functions.
**Materials and Methods:** In This experimental survey, we study on the effect of celecoxib on rat reproductive system, on spermatogenesis and the level of blood testosterone hormone. Histological studies and measuring of weight (testis, prostate, seminal vesicle and epididymis) and the level of blood testestron are done. 50 rat with 200-230 g. weight selected and compared in 5 groups. 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) for 15 days. In the end of 15 days heart blood sampling for measuring serum testosterone level accomplished after that reproductive systems separated and prepared for histological study.
**Results:** Result showed sertoli cells in control and case groups are differences. So that in case group (40 mg/kg) number of sertoli cells decreased due to decrease testestron level. This can cause production of abnormal sperms. significant differences are seen in the mean weight of prostate per body weight in case group (40 mg/kg) in compared with control group.
**Conclusion:** Use of high doses of celecoxib can decreased size and number of lydig cells and this is cause of decreased testosterone hormone.
*Keywords:* Selective Cox-2 Inhibitor, Mail, Infertility, Testosterone Hormone

## P-25: Effect of Olive Leaf Extract (OLE) on Rooster Sperm Viability During Storage for 48 hours at 4°C

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**Background:** This experiment was conducted to determine the effect of supplementation roosters' semen diluent with olive leaf extract (OLE) infusion on semen quality during *in vitro* storage at for 48 hours.
**Materials and Methods:** Semen was collected from 12 Ross-308 roosters (30 weeks of age) kept in separated floor pens. Treatment groups were as follows: T1, T2, T3, T4 and T5 that were contain 0, 50, 100, 150 and 200 µg/ml OLE, respectively.
**Results:** Spermatozoa viability was affected by experimental treatments, with the highest and lowest viability percentage of 74.4 and 68.6 in each treatments T3 and T5 respectively ($p<0.05$).
**Conclusion:** These results clearly show that supplementation of the diluent of roosters' semen with OLE can

improve semen viability when semen samples *in vitro* are stored at 4°C for 48 hours.

***Keywords:*** Olive Leaf Extract, Rooster, Sperm, Viability

## P-26: Comparison of Serum Testosterone Level between Men with and without Varicocele

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**Background:** Varicocele suggests that in addition to effects on spermatogenesis, varicocele also impairs testicular Leydig cell function with a consequential decrease in testosterone production; correction of the varicocele may result in improved serum testosterone levels.
**Materials and Methods:** We measured preoperative serum testosterone levels in 40 infertile men with varicoceles varicocele (grade III and II) and in 50 fertile men without varicoceles who served as a comparison group. The serum testosterone levels between groups were compared by age (range 23-46) that underwent varicocelectomy because of infertility. Patients were categorized into two age groups (Less than 35 years and More than 35 years).
**Results:** No statistically significant changes were noted in serum testosterone levels for any of the groups. The mean of serum testosterone levels before surgery in infertile men with varicocele and fertile men were respectively 569(212) vs. 572(223) ng/dl (p = 0.95). No statistically significant changes were noted in serum testosterone levels for any of the groups. This difference persisted when analyzed by age. After surgical varicocelectomy, mean serum testosterone levels were significantly increased compared with preoperative levels. The serum testosterone levels significantly increased after repair from 568 (212) to 703 (251) ng/dL (p = 0.004).
**Conclusion:** These findings suggest that varicocele is a significant risk factor for androgen deficiency and that repair may increase testosterone levels in men with varicocele and low testosterone levels.

***Keywords:*** Varicocele, Varicocelectomy, Testosterone, Androgen, Leydig Cell

## P-27: Assessment of the Contraceptive Effects of Cyclo Hexyl and Propylene Glycol on Histological Changes of Rat Seminiferous Tubules

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**Background:** According to highlighted role of men in family planning, the use of reversible methods of contraception with no adverse effects on androgen-dependent metabolic reactions is very important. Therefore, in order to achieve a conductor combination in the designing contraceptive drugs, in the study, the effect of two drugs: Propylene glycol and Cyclo Hexyl on histological parameters of seminiferous tubules was investigated.
**Materials and Methods:** This study was conducted on the 33 rat Sprogue-Dawley with weighing 250-300 g and least 100 days. Then they were divided into a group containing 13 mice and two groups containing 10 mice and were Received respectively 15 mg/kg of Propylene glycol and Cyclo Hexyl and normal saline as subcutaneous injection daily for 60 days. Next, samples of testicular fixative were stained by the usual method (H&E) and Histological parameters of seminiferous tubules were measured through stereometry and morphometry methods.
**Results:** Reduce the Diameter and Perimeter of seminiferous tubules in the group of Cyclo Hexyl had significant difference with the two other groups while there was not noticeable difference among the three studied groups in terms of seminiferous tubules number.
**Conclusion:** Cyclo Hexyl was more effective on Histological parameters of seminiferous tubules. Thus with eliminating the cardiovascular effects and Conservation the contraceptives properties, It can be used as a guide combination.

***Keywords:*** Cyclo Hexyl, Morphometry, Propylene Glycol, Seminiferous Tubules, Stereometry

## P-28: Protective Effect of Curcumin on The Damages Induced by Nicotine on Reproductive Parameters in Male Mice

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**Background:** Nicotine is a substance that can induce oxidative stress in the testes. And thus may reduce fertility in men. Antioxidant supplements such as curcumin can reduce oxidative stress and thus may increase fertility in men. Since the most active component responsible for the yellow color of turmeric and curcumin has anti-inflammatory and antioxidant properties. Therefore this study investigated the protective effect of curcumin against nicotine-induced damage on reproductive parameters in male mice explains.
**Materials and Methods:** In this study, 56 male mice received 8 + ethanol, ethanol + nicotine + curcumin saline and three doses of 10, 30 and 60 mg nicotine + curcumin (10, 30, 60) into Vdvz 2.5 mg of nicotine is injected intraperitoneally at 28 days was done. Later, the mice were anesthetized and dissected WHO sperm parameters were measured according to the method.
**Results:** Nicotine can significantly induce oxidative stress in the testes and other curcumin could significant-

ly reduces oxidative stress and enhances fertility.
**Conclusion:** Anti-inflammatory and antioxidant effects of curcumin results in infertility treatment is approved.
*Keywords:* Sperm, Oxidative Stress, Infertility,Curcumin, Nicotine

## P-29: Crocin Down-Regulated The Cyclophosphamid-Increased Follicular Atresia in Mice

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**Background:** Although the chemotherapeutic agents have been known for their cytotoxic effects on normal mammalian cells, these compounds are used widely for controlling the carcinogenic cells proliferation. Cyclophosphamide (CP), a well-known nitrogen mustard alkylating agent is used for autoimmune disorders and tumors treatment. Considering the inhibitory effect of CP on cell division, the ovary, a tissue with high rate of mitosis, can be considered as a probable target tissue for CP. Thus, present study was designed to evaluate the protective effect of Crocin on CP-induced follicular atresia.
**Materials and Methods:** Fifteen mature female albino mice were divided into three groups (N=5 in each group) as control and test groups. The test groups subdivided into two groups including; CP alone (15 mg/kg, once in week, ip.) and CP+crocin (200 mg/kg, daily, ip.). The control group received normal saline (0.1ml, daily, ip.). Following 21 days, the ovaries were dissected out and the follicular atresia in different stages was evaluated using serial sections. Moreover, oocyte denaturation, the granulosa cells (GC) dissociation and theca cells luteinization were evaluated as critical characteristics for atresia.
**Results:** Histological observations demonstrated that CP significantly ($p<0.05$) increased follicular atresia versus the crocin-administrated animals. Comparing the different types of atretic follicles showed that the CP severely ($p<0.05$) impacted the secondary and tertiary follicles. Meanwhile, the crocin-received animals manifested with remarkably ($p<0.05$) reduced atresia in different types of follicles.
**Conclusion:** Our data showed that the CP exerts its pathological impact partly by promoting atresia. Moreover, the crocin as a potent antioxidant agent could down-regulate the CP-increased atresia.
*Keywords:* Crocin, Atretic Follicles, Cyclophosphamide, Mice

## P-30: Evaluation of Environmental Health Effects of TCDDs and PCDFs on Reproduction of Municipal Waste Incineration Operators Outcomes

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**Background:** Municipal waste incineration are one of the largest resources of 2,3,7,8- tetrachlorodibenzo-p-dioxins (TCDDs) and Polychlorinated dibenzofurans (PCDFs) from incineration ends up in water and food cycle are taken up by fish and etc. These gaseous emission products tend to give rise to concern due to their carcinogenic potential, as well as their potential adverse health effects on reproduction.
**Materials and Methods:** The study was carried out to compare SPSS ver 16. , and Arc GIS, to put emphasis on the effects of residing in proximity to several municipal resources. The risk seems to be greater for human who worked near incinerators though it is very difficult to discriminate between the effects of the indoor air and those of other resources of municipal pollution.
**Results:** The major result was that the cancer risk form TCDDs emissions from the MWI was extremely low, i.e., about 1000-fold below that calculated for PCDFs emissions from other resources in the same area. The TCDDs emissions were reported to be in the range of 18.32 ng I-TEq/$m^3$.The study does not contain any data on the levels of TCDDs in ambient air, soil or in the blood or adipose tissue derived from residents of the various areas or from cancer patients.
**Conclusion:** We conclude that men who worked in indoor waste terminals have sperm counts less than half as high as their grandfathers had at the same age. In addition, the occurrence of cancer in the testicles has increased 3-fold to 4-fold during the past 45 years; and various birth defects of the male reproductive system have increased 2- fold to 4-fold during the same period. Thus breast cancer, testicular cancer, and defects of the male reproductive system. Trimming fat from meat and consuming low fat dairy products may decrease the exposure to TCDDs and PCDFs compounds.
*Keywords:* TCDDs, PCDFs, Waste, Incineration, Reproduction

## P-31: Dexamethasone and Vitamin E Up-Regulated The Varicocele-Reduced Hsp70 Protein Expression; Correlation with Testicular Tissue Inflammation and Antioxidant Status

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**Background:** The varicocele (VCL) impacts the testicular tissue both by enhancing tissue inflammation and by down-regulating the antioxidant status. On the other hand, in varicocele patient the critical role of Hsp70 in spermiogenesis is identified. Therefore present study was estimated to evaluate the protective effect of testosterone and vitamin E on VCL-decreased Hsp70 expression and on VCL-induced inflammation.
**Materials and Methods:** Thirty mature male rats were

divided into five control-sham and test groups (N=6 rats for each group). The test groups underwent to an experimental varicocele as Group VCL alone (varicocele-induced, for 60 days), group VCL+Dexamethasone (45µg intraperitoneally, for 60 days), group VCL+vitamin E (150mg/kg, orally, for 60 days) and group VCL+dexamethasone+vitamin E. The Western blotting and immunohistochemical analyses for Hsp70 protein were performed. The total RNA, total protein, tissue alkaline phosphatase ALP and acid phosphatase (ACP) levels were evaluated. The Epi-fluorescent and fluorescent analyses were conducted to evaluate the germinal cells RNA damage and leydig cells biosteroid activity, respectively.

**Results:** The VCL+dexamethasone+vitamin E group showed the highest level of Hsp70 protein beside higher detected immunohistochemical spots in germinal cells. The animals in all treated groups showed remarkably ($p<0.05$) higher total protein and normal RNA contents versus VCL alone group. The higher biosteroidogenesis was observed in vitamin E and dexamethasone-recieved groups leydig cells. Dexamethasone-received animals showed the lowest levels of ALP and ACP versus other groups.

**Conclusion:** Our data showed that dexamethasone by inhibiting inflammation and the vitamin E by up-regulating testicular antioxidant capacity could inhibit the VCL-induced damages.

***Keywords:*** Varicocele, Hsp70, RNA Damage, Inflammation, Dexamethasone, Vitamin E

## P-32: Histomorphological Study of Testicular Seminiferous Tubules Following Time Dependent Administration of Methylphenidate in Adult Rats

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

**Materials and Methods:** Ritalin was administrated to adult rats (10 mg/kg/day) in three experimental groups for periods of two weeks, 11 weeks and 11 weeks with one week interval between each two weeks. At the end of investigation, Histological studies were performed for evaluation of testicular reproductive function.

**Results:** Histological assessment of testicular tissue showed some alteration in morphology of spermatogenic cells of seminiferous tubules. These changes were disruption of spermatogenesis, decrease of the diameter of seminiferous tubules, depletion in the height of germinal epithelium, giant cell formation and the alteration of the shape of testicular tubules. Most of these changes were observed in long term treated group in comparison to other groups.

**Conclusion:** The findings of this study indicate that, the long term use of methylphenidate can adversely damage the male fertility due to impairment of normal spermatogenesis.

***Keywords:*** Histomorphology, Methylphenidate, Rats, Seminiferous Tubules, Testis

## P-33: Expression of Toll-Like Receptor 2-3 Genes in Human Sertoli Cells

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

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

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

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

***Keywords:*** Sertoli Cell, TLRs, Lectin, Cell Culture, Sorting Flowcytometery

## P-34: Effect of Glucose on Buffalo Epididymal Sperm Motion Characteristics, Viability and Cytoplsmic Droplets During 24 Hours Incubation

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**Background:** The recovery of sperm from the cauda epididymis may be the last chance to conserve, genetic Materials of valuable farm animals. Sperm utilize glucose as source of energy through glycolysis and oxidative phosphorylation to provide motility and movement. While endogenous glucose of the culture media has little potential to preserve sperm motility and viability for long period of time and it needs to replace or supplemented with exogenous glucose. So the aim of this study was to investigate the effect of adding glucose in culture medium on quality of buffalo epididymal spermatozoa during 24 hours incubation.
**Materials and Methods:** Thirty testes from 15 mature buffalo bulls were collected from Urmia slaughterhouse and transported to laboratory in cool condition. Spermatozoa were obtained from caudal epididymis and pooled together. The samples were diluted in tissue culture media (TCM) at concentration of 20×106 sperm/ml and divided to five groups containing glucose at concentration of 0, 2.5, 5, 7.5 and 10 mM. Sperm motion characteristics, viability and cytoplsmic droplet were evaluated by computer assisted sperm analyzer and one steps eosin-nigrosin stain respectively at 1, 6, 12 and 24 hours of incubation at 37 °C.
**Results:** Sperm motion characteristics, viability and cytoplsmic droplets reduced in time depend manner. There were no significant differences in motion characteristics until 6 hours incubation. While addition of 5 and 7.5 mM glucose in culture medium increased motility and velocity patterns at 12 and 24 hours incubation, compared to control group ($p<0.05$). Distal cytoplsmic droplets were decreased significantly with supplementation of 5 and 7.5 mM glucose at 6 hour of incubation ($p<0.05$). Addition of 5 mM glucose resulted highest sperm viability at 6, 12 and 24 hours.
**Conclusion:** Addition of 5 mM glucose to culture medium led to release cytoplsmic droplets and improves motility and viability of buffalo epididymal spermatozoa.
***Keywords:*** Buffalo, Cytoplsmic Droplets, Epididymal Sperm, Glucose, Motion Characteristics

## P-35: The Effect of Microwave on Morphometric Changes in Rabbit Prostate

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**Background:** Regarding to the considerable application of microwave in everyday life, their effects on biological tissues are still controversial.This study determines the effect of microwave on morphometric changes in rabbit prostate.
**Materials and Methods:** 18 New Zealand male rabbits were divided into three groups (control, 3 w and 6 w),the animals in the control group were not exposed to microwave while the other ones were exposed to microwave (950 MHz, intensity of 3 watt and 6 watt) 2 hours a day, for 2 weeks. After one week rest, the rabbits were sacrificed using anesthetic drugs and the prostate samples were prepared. After tissue processing, The heights of the villi, the thickness of the muscle layers and villi were measured. Finally, the data were analyzed statistically (SPSS, ANOVA, $p <0/05$).
**Results:** The result showed that the hights of prostate's villi in the 3 watt group (B) significantly decreased compared with the control group (A) and 6 watt group (C). It also showed a significant increase in group C compared to other groups (A & B, $p<0/001$). The thickness of villi only in 6 watt group, were significantly increased compared to control ($p<0/02$). The thickness of muscle layer in both 3&6 watt groups significantly increased compared to the control group ($p<0/05$).
**Conclusion:** The present study showed that the micro waves with a frequency of 950 MHz have negative effects on prostate morphometric changes of rabbit .
***Keywords:*** Cell Phone, Microwave, Rabbit, Prostate

## P-36: Morphometric Evaluation of Seminiferous Tubules in Aged Mice Testes after Melatonin Administration

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**Background:** Melatonin, the pineal gland hormone as a direct or indirect antioxidant and free radical scavenger, is involved in the process of both aging and age-related diseases. This study investigates the effects of melatonin on the histology of testicular seminiferous tubules in aged mice.
**Materials and Methods:** Twenty male, white mice, aged 16 months, that weighed 20-23 gr were equally divided into control and experimental groups. The experimental group was intraperitoneally injected with a daily single dose of 10 mg/kg melatonin for 14 days. The control group received only saline. Six days after the last injection, all mice were sacrificed and the testes were excised and processed for light microscope observation. In the morphometric study, we evaluated testicular seminiferous tubule parameters such as height of germinal epithelium, seminiferous tubule diameter, thickness of interstitial connective tissue and spermatogenesis index (SI). SPSS software and student's t-test analyzed all pa-

rameters to assess the significance of changes between control and experimental groups.
**Results:** Melatonin-treated mice had seminiferous tubules with a wide lumen lined by low height germinal epithelium. The interstitial connective tissue thickened significantly in the experimental group ($p<0.05$), tubular diameter and germinal epithelium height decreased significantly ($p<0.01$), and the SI reduced compared to the control group ($p<0.001$).
**Conclusion:** The results of this study showed the disadvantages of melatonin on seminiferous tubules of aged mice testes.
*Keywords:* Melatonin, Seminiferous Tubules, Morphology, Aged Mice

## P-37: Evaluating Orally Administration of Aquatic Extract of Saffron (Crocus Sativus L.) on Histomorphometric Changes of Testicular Tissue in Stress Induced Rats

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**Background:** Saffron consists of dried stigmas and top of the styles of crocus sativus. It has various applications such as sexual potential stimulant, anti spasm, anti depression, sedative ,anti inflammation and anti cancer.
**Materials and Methods:** In present study, 200 male adult rats divided to five groups with 10 rats per each cage and with four replicate for each group. Solvent of saffron was prepared by soaking 1 g of saffron in 40 ml of distilled water. The groups followed of: 1] control group (Normal saline 10 ml/kg, orally); 2] saffron (50 mg/kg, orally); 3] saffron (100 mg/kg, orally); 4] saffron (200 mg/kg, orally); 5] saffron (200mg/kg orally with excessive noise stress). For stress induction, a cassette player had playing excessive noise were used in front of cage by 0/5 meter distance with it. The testis tissue was sampled after passing of above time and section providing, was stained by H and E and then was observed by microscope.
**Results:** The seminiferous tubules diameter, seminiferous epithelial thickness and testicular capsule thickness significantly decreased after saffron administration compared with control group and this decreasing is more significant in stress condition. Interstitial tissue increased after saffron administration compared with control group and this increasing is more significant.
**Conclusion:** Our results showed seminiferous tubules diameter, seminiferous epithelial thickness, interstitial tissue and testicular capsule thickness were significantly changed after saffron orally administration in stress condition. These results can cause by reducing of serum testosterone concentration. It suggested the effect of saffron on histomorphometric changes at stress condition in human can be more investigation.
*Keywords:* Saffron, Histomorphometric Changes, Stress, Rat

## P-38: Effect of Saffron on Spermatogenesis Index in Stress Induced Rats

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**Background:** Saffron is widely used as a food flavor and has well known medicinal effects which have an effect on steroid and sexual hormones. The aim of present study was investigation of saffron orally administration on spermatogenesis index in stress induced rats.
**Materials and Methods:** In present study, 160 male adult rats divided to four groups with 10 rats per each cage and with four replicate for each group. Solvent of saffron was prepared by soaking 1 g of saffron in 40 ml of distilled water. The groups followed of: 1. control group (Normal saline 10 ml/kg, orally), 2. saffron (50 mg/kg, orally), 3. saffron (100 mg/kg, orally), 4. saffron (200 mg/kg, orally) and 5. saffron (200mg/kg orally with excessive noise stress). In point of spermatogenesis indexes which includes tubular differentiation index (TDI), spermatogenesis index (SI), repopulation index (RI) were studied.
**Results:** The results showed rats that received 200 mg/kg dosage of saffron without stress significantly decresed ($p<0.05$) RI, SI and TDI indexes compared to control group. In rats treated by 200mg/kg saffron with excessive noise stress RI, SI and TDI significantly decreased ($p<0.05$) Compared to rats received 200 mg/kg saffron without noise stress.
**Conclusion:** Our results showed RI, SI and TDI were significantly changed after saffron orally administration. These results can cause by reducing of serum testosterone concentration. It suggested the effect of saffron on spermatogenesis index at strees condition in human can be more investigation.
*Keywords:* Saffron, Spermatogenesis, Stress, Rat

## P-39: Effect of L-Carnitine on Serum FSH, LH and Testosterone Levels in Adult Male Rats

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**Background:** The benefit effect of L-carnitine is proposed for treatment of obesity as long time periods. So, in the present study, effect of L-carnitine on serum FSH, LH and testosterone levels in adult male rats is evaluated.
**Materials and Methods:** Rats were treated different doses of L-carnitine tartarate daily for 16 days, peritoneally. The control group was treated saline as vehicle. After 16 days, animals were anesthetized with ether and blood specimens were obtained from heart. Serum FSH, LH and testosterone were measured by RIA.
**Results:** The results showed that L-carnitine increased FSH, LH and testosterone levels in treated animals, significantly.
**Conclusion:** The present study indicated that L-carnitine could affect on sex hormones and reproduction system. So, usage of L-carnitine for treatment of obesity as long periods should be noticed carefully
*Keywords:* L-Carnitine, FSH, LH, Testosterone, Rat

## P-40: Melatonin Could Improve The Sperm Quality, Level of Antioxidant Enzymes and Lipid Peroxidation of Testis after The Combination of Swimming Exercise and Supraphysiological Dose of Nandrolone Decanoate in Rats

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**Background:** This study investigates the effects of melatonin on the sperm quality, the level of antioxidant enzymes (TAC) and malondialdehyde (MDA) after the combination of swimming exercise and nandrolone decanoate (DECA).
**Materials and Methods:** Two groups of male Wistar rats were treated for eight weeks as follows; group A consist of: CO (control), Sham, N (DECA), S (swimming) and NS (DECA plus swimming); and group B: Sham M (sham melatonin), M (melatonin), MN (melatonin plus DECA), MS (melatonin plus swimming), MNS (melatonin, DECA plus swimming).
**Results:** The motility of sperm was significantly improved in melatonin groups in comparison to N, S and NS groups ($p \leq 0.05$). The level of MDA of testes significantly increased and the level of TAC decreased in N and NS groups in compare with the control group. Sperm motility was inversely correlated with MDA and directly to TAC levels, respectively ($p \leq 0.05$). The left testes weight was decreased in N, NS and MNS groups and the right testes weight was decreased in N,S,NS,MS and MNS groups in compare with control group.
**Conclusion:** This study concluded that melatonin probably could improve the sperm motility and sex organs weight after the combination of DECA and exercise which results from increasing the level of TAC.
*Keywords:* Melatonin, Nandrolone Decanoate, Swimming, Testis, MDA, Rat

## P-41: Association Study of MICA*008 Gene Polymorphism with Chlamydia Trachomatis Infection in Infertile Men Reffer to Royan Institute

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**Background:** Chlamydia trachomatis(CT) is an obligate intracellular bacteria, requires living cells to replicate itself. CT infection can remain up to 4 years in the couple and affect their fertility. The relationship between CT and infertility is very important because most patients are asymptomatic and untreated. After infection with CT, NK activation signals begin through interactions of its receptors with molecules like MHC class I(MICA/B).
**Materials and Methods:** Seven hundred patients with poor sperm parameters were selected for primary screening and detecting the presence of Chlamydia. ELISA test was performed for the presence of anti-CT IgA in these patients' semen plasma. The Sperm's DNA was extracted in order to confirm the presence of Chlamydia. Chlamydia genome amplification was performed using specific primers. Among these samples, 62 patients were diagnosed with Chlamydia infection, in which 32 samples were symptomatic while the others were asymptomatic. Moreover 34 fertile men with normal spermogram and without any past history of CT infection were selected as controls. PCR-SSP method by eight primers were used to detect MICA*008 alleles.
**Results:** The results indicate that the frequency of MICA*008 allele was significantly higher in control group than that of infected patients with CT ($p<0.05$). Although there were no significant difference between the allele frequency of control and symptomatic patients ($p=0.193$), it was higher in control group than in idiopathic group ($p<0.05$). Moreover, the frequency of this polymorphism was significantly higher in asymptomatic patients than in symptomatic patients ($p=0.010$).
**Conclusion:** These results show that the presence of MICA*008 allele product may reduce the susceptibility of the host to be infected with CT and those with its protein have more resistant to Chlamydia infection. According to these results, MICA gene can be considered as a good candidate gene to determine the host potential genetic predisposition toward resistance against pathogens.
*Keywords:* Chlamydia Trachomatis, Gene Polymorphism, MICA Gene

## P-42: Protective Effect of Curcumin on Spermatogenesis Defects Induced by Dexamehasone

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**Background:** Exposure to glucocorticoids such as dexamethasone (Dex) leads to numerous changes in various biological systems including the reproductive system. The present study investigates the efficacy of curcumin (Cur), an active component of turmeric, to attenuate Dex-induced spetmatogenic defects in the mouse testicles.
**Materials and Methods:** A total of 32 male mice were divided into four groups (8 mice each). The first group served as control and received corn oil only for 25 days. Mice of the second group received Dex at the dose of 7 mg/kg body weight by i.p. injection for 7 days. Mice of the third group received curcumin dissolved in corn oil at a dose of 200 ml/kg body weight. The fourth group received Cur at the dose of 200 mg/kg for 25 days and Dex at the same dose of the second group was injected during the last 7 (18 to 25) days. Statistical significance was determined using one-way analysis of variance (ANOVA)followedby Tukey-Kramer multiple comparison tests. A p value <0.05 denoted the presence of a statistically significant difference.
**Results:** Testicular histopathology, morphometric analysis and immunohistochemistry assessments were performed for evaluation of the Dex and Cur effects on testicular germ cells. There was marked staining for bcl-2, an important antiapoptotic factor, expression with Cur+Dex compared with the Dex groups. Dex caused epithelial vacuolization, sloughing of germ cells, reduction of seminiferous tubule diameter, and significant maturation arrest ($p<0.05$). Cur+Dex treatments showed significantly prevented these histopathologic changes ($P < 0.05$).
**Conclusion:** Curcumin may improve the adverse effects of Dex in the mouse testicular tissue by inducing anti-apoptotic mechanism.
***Keywords:*** Curcumin, Dexamethasone, Apoptosis, Spermatogenic Cells

## P-43: Short Term Effect of Oxytocin on Testis under Ischemia Reperfusion Injury

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**Background:** The aim of this study was to investigate the short term protective effect of oxytocin on testicular ischemia reperfusion injury immediately after detorsion in a rat model.
**Materials and Methods:** Twenty adult rats were randomly divided into four groups: Control, Ischemia- Reperfusion (IR), Oxytocin and IR+ Oxytocin. Testicular ischemia was achieved by torsion of the left testis 720° clockwise for 2 hours. Two hours after ischemia, torsion was removed and reperfusion was performed. Immediately after reperfusion 0.03 µg/kg oxytocin were administered intraperitoneally to the IR+ Oxytocin group. Three hours after surgery left testis were removed and evaluations were made by Jhonson's score and ELISA for study of maturation of seminiferous tubules and assay of serum testosterone, FSH and LH levels respectively.
**Results:** Torsion of testis in IR group for two hours induced degeneration of germ cells, edema and blood vessels congestion in testis. A significant reduced Jhonson's score were detected in IR group in compare with controls ($p< 0.05$). However there was not significant statistical difference in serum levels of testosterone, FSH and LH. Administration of oxytocin in IR+ Oxytocin group immediately after reperfusion, improved testicular histology in compare with IR group without any effect on levels of abovementioned hormones.
**Conclusion:** Exogenous oxytocin in short term after testicular reperfusion has a protective effect on testis.
***Keywords:*** Oxytocin, Ischemia Reperfusion, Testis, Torsion

## P-44: Oral Gibberellic Acid Administration Impact on Testicular Histochemistry

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**Background:** Gibberellic acid (GBA) is a naturally occurring and commercially produced plant growth regulator which may cause a variety of effects including the stimulation of seed germination in some cases. Histochemical changes in testicular tissue including carbohydrates, lipids and alkaline phosphatase enzyme changes were evaluated.
**Materials and Methods:** Twenty seven mature male rats after one week acclimatization period were randomly divided into three groups. Group 1 served as control group and received no treatment; group 2 was control-sham group and received 1% methanol (which was career of Gibberellic acid) and finally group 3 received 10 mg/kg gibberellic acid. All administrations were with oral gavages (every other day). After sacrificing the rats on days 15, 30 and 45 of treatment period (3 rats per day per group) stained testicular slides were prepared. These stainings include Periodic Acid Shift (PAS) for carbohydrates, Sudan Black-B staining for lipids and Alkaline Phosphatase staining to evaluate alkaline phosphatase enzyme in testicular tissue.
**Results:** In present study revealed that level of carbohydrates in three first spermatogenesis decreased in GBA group in comparison with other groups otherwise level of lipids increased. Furthermore, level of cytoplasmic alkaline phosphatase enzyme in GBA group increased in

spermatogenesis cells (especially spermatocyte I cells).
**Conclusion:** According to obtained results, acid Gibberellic agent may have metabolic negative effects in spermatogenesis cells.
***Keywords:*** Gibberellic Acid, Histochemistry, Testicular Tissue, Rat, Oral Gavage

## P-45: Ameliorative Effect of Satureja Hortensis on Histological Alterations of Testicular Tissue Induced by Cyclosporine-A in Adult Rat

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**Background:** Cyclosporine-A(CsA) is an immunosuppressive polypeptide which is produced byTolypocladium inflatum gams and can cause infertility. Satureja hortensis or summer savory is a one year plant from Lamiaceae family which is used as a motive drug, anti-bloat, anti-infection and sexual power enhancer. According to positive effects of S. hortensis and negative effects of CsA we will evaluate ameliorative effects of Satureja hortensis on histological changes of testicular tissue induced by CsA.
**Materials and Methods:** Twenty four adult male rats weighing 200±20 gr were divided into three groups (n=8): control group which received no treatment; CsA group which received 40/mg/kg/dayCsAand CsA-S. hortensis group received 40/mg/kg/day CsA and 100 mg/kg/day aqueous extract of S. hortensis with oral gavage. Half of each group was sacrificed on 21st day of treatment and the other half on 45th day, and the testicular tissues were collected and fixed and stained with Haematoxylin and Eosin (H&E). The prepared slides were evaluated with light microscope.
**Results:** In CsA group diameter of seminiferous tubules and the percentage of tubules with spermatozoa were decreased. The results announced a decline in uniformity and density of testis interstitial tissue. Negative effects in above mentioned parameters were stronger in 45th day in comparison with 21th day. S. hortensis in CsA-S. hortensis group caused partially amelioration in testicular tissue.
**Conclusion:** CsA can cause testicular toxicity and S. hortensis can decrease its detrimental effects on testis.
***Keywords:*** Cyclosporine-A, Satureja Hortensis, Testis, Histology, Rat

## P-46: Effect of Formaldehyde Toxicity on The Gonadotropin Hormones and Epithelial Cells of Testicular Tubules in Male Rat

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**Background:** Formaldehyde is a chemical that used in fixed tissues and bodies. Formaldehyde during dissection of cadaver, evaporate and be released. Studies have shown that the gas increase endogenous opioids, nitric oxide and cytokines in plasma. These impacts can affect on gonadotropin hormones. The aim of this study was to investigate possible changes in gonadotropin and epithelial cells of testicular tubules in male rat by formaldehyde exposed.
**Materials and Methods:** In this study, 24 adult rats were randomly divided into three groups: 1. control, 2. experimental group: formaldehyde exposure 2 hours per day, 3. in the experimental group formaldehyde exposed 4 hours per day. 18 weeks after the test, serum concentrations of FSH, LH and testosterone by Radioimmunoassay, and changes of epithelial cells of testicular tubules was studied.
**Results:** The results indicated that a decrease in FSH and testosterone in experimental groups compared to the control group. Levels of LH plasma in experimental groups compared to the other groups decreased significantly ($p<0.05$) shows the evaluation of tissue sections for different groups showed that tubule of seminiferous and interstitial tissue that appears normal, epithelial cells of The tubules were uniformly distributed without gaps and without any cell loss.
**Conclusion:** Formaldehyde vapor decreased gonadotropin but has no effect on testicular seminiferous epithelial cells. It is conjectured that the seminiferous epithelial cells of adult rat testis to gonadotropin hormones is not only dependent on many other factors may be involved.
***Keywords:*** Formaldehyde, Gonadotropins, Testicular Epithelial Cells

## P-47: Comparison of Sperm Acrosin Activity and DNA Fragmentation between Varicocele and Infertile Individuals

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**Background:** Introduction assessment of sperm functional tests along with routine semen analysis may provide further information in the etiology of varicocele in different subpopulations. Therefore, in this study, in addition to assessment of sperm chromatin integrity, we aimed to assess the total acrosin activity which has proven to have major role during *in vitro* and natural fertilization in varicocele compared to fertile individuals.

**Materials and Methods:** Sperm parameters were assessed in individuals with a clinical diagnosed varicocele of grade II or III (n= 70) and fertile men as control group (n= 30). In addition, total acrosin activity and DNA fragmentation were evaluated by spectrophotometery and TUNEL assay respectively.
**Results:** Sperm concentration, percentage of normal morphology and total acrosin activity were significantly higher in fertile compared to varicocele individuals. A significant correlation was observed between total acrosin activity and DNA fragmentation in fertile individuals.
**Conclusion:** The results of this study reveal that unlike DNA fragmentations as the negative parameter for fertility, total acrosin activity as the positive parameters in fertility is higher in the varicocele individuals.
***Keywords:*** Varicocele, DNA Fragmentation, Acrosin Activity

## P-48: Effects of Dietary Fat Sources on Reproductive Performance of Mature Rams

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**Background:** The present study was conducted to investigate the effects of dietary fat sources on reproductive performance of mature rams.
**Materials and Methods:** Twelve adult rams, with 32 months of age and a mean weight value of 67 kg, were divided into four dietary treatments using a completely randomized design. Dietary treatments included with the study were: 1. ration with 4% soybean oil (A), 2. ration with 8% full fat soybean (B), 3. ration with 4% calcium salts of soybean oil fatty acids (C), and 4. ration with 4% tallow fatty acids (D). All diets were kept isoenergetic and isonitrogenous. Diets were formulated using the Cornell Net Carbohydrate and Protein System and were fed to the rams for four months (from early July to late November).
**Results:** Diets supplemented with protected soybean oil and tallow improved both the sperm concentration and the scoring appearance of the semen ($p<0.05$). Semen volume was also affected by dietary treatments, with the highest values of 1.07 ml and 0.83 ml in each ejaculation which were belong to treatments C and D respectively ($p<0.05$).
**Conclusion:** As an overall conclusion, the results of this study indicated the benefits of adding fats, specially protected, to the diets of adult rams on their reproductive performance.
***Keywords:*** Fatty Acids, Ram, Spermatozoid, Fertility

## P-49: Comparative Appraisal of Acarbose, Pioglitazone and Repaglinide on Histopathology of Testis in Streptozotocin Induced Diabetic Rats

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**Background:** Experimentally induced diabetes in male rats is associated with alteration in functions of reproductive system. Acarbose, Pioglitazone and Repaglinide via decrease in blood glucose level can prevent the damage of testes.
**Materials and Methods:** A total of 96 adult male albino rats whit a body weighing between 230- 250 g were injected by a single intraperitoneal injection of streptozotocin (65 mg/kg Body weight.) and were randomly assigned to 4 groups and 4 replicates for each group. Group 1 served as diabetic Control, group 2 received Pioglitazone (10 mg/kg/day, orally), group 3 received Repaglinide (1 mg/kg/day, orally) and group 4 received Acarbose (25 mg/kg/day, orally) for a period of 7 days. At the end of the study period, testis samples were taken for histopathological investigations. spermatogonia, spermatocyte, spermatid and sertoli cells in all groups were calculated.
**Results:** Reduction in number of espermatogonial cells, number of spermatocyte cells and sertoli cells in group 4 was less than other groups. Number of spermatid cells in animals treated with Repaglinide was more than other treatment groups.
**Conclusion:** Results indicated that Acarbosehas is more effective for testis of adult diabetic rats by reducing histological Changes of testicular tissue and its negative effects on sperm production.
***Keywords:*** Diabetes, Testis, Acarbose, Pioglitazone, Repaglinide

## P-50: Evaluation of Sperm Parameters and Chromatin Abnormalities in Infertility with CASA and Chromatin Dispersion Test

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**Background:** Although sperm selection according to morphologic and motility characteristic, made to some what the fertility successful, but sperm's normal morphology cannot demonstrate sperm DNA competence. Since the health of sperm DNA is affecting the results of assisted reproductive technology; the aim of this study is to evaluate semen parameters measured by CASA with sperm chromatin abnormalities in Taleghani Medical Center in 1390 and 1391.
**Materials and Methods:** Case-control study was conducted. Subjects who had a diagnosis of infertility and a control group who had previous fertility. Semen samples were taken and sperm parameters such as motility and morphology evaluated with CASA and DNA fragmentation was assessed by chromatin dispersion test. Protamine deficiency was evaluated by chromomycin A3 staining. Parameters in both groups were analyzed statistically.
**Results:** The results showed a significant correlation

between protamine deficiency, DNA fragmentation and sperm parameters. Head and neck anomalies showed the maximum significant correlation with percentage of sperm DNA fragmentation; but tail anomalies even occur with percentage of DNA competence.

**Conclusion:** Protamine deficiency and damage in sperm DNA in infertile cases was more than fertile cases. In cases with high DNA damage ratio, sperm parameters were poorer and the fertility was less, so CASA method cans analysis sperm parameters with high precision.

***Keywords:*** Sperm Chromatin, Infertility, CASA, Chromatin Dispersion Test

## P-51: The Effects of Anethum Graveolens L. Seeds on The Male Reproductive Functions and CREM Gene Expression in The Testis of Rat

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**Background:** It is undeniable that the increasing of the population in the developing countries is an anxiety, therefore finding safe and effective contraceptive Materialss can be very useful. The aim of this study was to investigate the effects of Anethum graveolens L on the reproductive system of male rat and CREM gene expression in testis of rat.

**Materials and Methods:** Animals were randomly divided into two groups of control (treated with normal salin for the period of 56 days, n=8) and experimental (treated with 150 mg/kg/day of Anethum graveolens extract for 56 days, n=8). RT-PCR and histological studies, hormonal assay, and sperm analysis were carried out for evaluating of extract on reproductive function of rat.

**Results:** Results indicated no significant differences between body weights of control and experimental groups. Sperm counts and motility were reduced however there was an increase sperm abnormality by applying of extract. Indeed existence of disorganized germinal epitheliums, degeneration, necrotic cells in some of seminiferous tubules, and lower concentration of sperms in the center of seminiferous tubules of experimental group was noticeable. Results also showed reduction in the diameter of seminiferous tubules of experimental groups comparing with control groups. Administration of extract caused a significant decline in the plasma level of testosterone. Our RT-PCR data revealed that CREM mRNA levels decreased significantly in testes from the experimental group in compared to control group.

**Conclusion:** Anethum graveolens has strong anti-spermatogenic activity by decreasing sperm parameters. This study strongly proposes that this plant can be a good candidate for manufacturing antifertility drugs.

***Keywords:*** Anethum Graveolens, Spermatogenesis, CREM Gene Expression

## P-52: Protective Effects of Korean Red Ginseng on The Testis of Epididymo-Orchitis in The Rat Model

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**Background:** Bacterial infections are mostly the consequence of an ascending infection of the genito-urinary tract which can ultimately lead to epididymo-orchitis. Ginseng has proven its worth with respect to reproductive function in several reports.Therefore, Escherichia coli M39 was employed to investigate whether Ginseng have protective effects on the testis of epididymo-orchitis in the rat model.

**Materials and Methods:** Thirty male Wistar rats were divided into five equal groups as follows:The control group underwent left and Right orchiectomy. PBS (50µl) was injected into the Right and left vasodeferan in the Vehicle group. Escherichia coli M39 ($4\times10^6$cfu/50µl) was injected into the Right and left vasodeferan in the infection group(M39). The fourth group received Ginseng treatment (15 mg/kg/daily; IP) after E. coli M39 inoculation for 7 days (M39-G15). Fifth group received ginseng (15 mg/kg/day) for 7days. In all groups, bilateral orchiectomy was performed 7 days after the challenge.

**Results:** Ginseng caused recognizable histological recovery associated with a decrease in germ cell apoptosis ($p<0.0001$) and decrease Immotile Sperm Percent ($p<0.001$) and decrease abnormal sperm percent ($p<0.0001$) in the experimental groups. The concentration of TNF-α wasn't significantly changed in the experimental groups. Infection caused increase sex organ weight but ginseng was not able to change organ weight except prostate that wasn't significant. Ginseng caused increase in the rat body weight that wasn't significant, as well. Ginseng significantly increased sperm count ($p<0.05$).Ginseng significantly increased Johnson's score in infected group treated with it ($p<0.007$). However, there was not seen significantly different in miller's score.

**Conclusion:** Red ginseng could probably prevent the cytotoxic effects of epididymo-orchitis on the sperm quality and apoptosis of germ cell.

***Keywords:*** Epididymo-Orchitis, Ginseng, Sperm, Rat, Testis

## P-53: Evaluation of The Effects of ElectroMagnetic Field (2.4 GHz) of Laptop Computers on The Sperm Quality, Caspase 3 Activity, Spermatogenic Cells Apoptosis and The Histomorphometry of Testes in The Adult Rat

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**Background:** The qualitative computers (laptop) are as one of the sources of electromagnetic fields (EMF) and these waves are emitted from laptop when you are working with it or connecting to the internet (wireless).The aim of this study is assaying the effects of 2.4GHz waves on the spermatogenesis cycle at the time of connecting to the internet.

**Materials and Methods:** In this study, 36 adult Wistar male rats were divided randomly into three groups. For producing the waves of laptop which connected to the internet (2.4GHz), a laboratory system as generator waves was used. 24 rats as experimental groups were in two groups for one and seven hours irradiated and also 12 rats as a control group were kept in similar laboratory condition. After eight weeks of radiation exposure sperm quality, testes wight, apoptosis of spermatogenic cells and Caspase 3 activity were assayed in the lab.

**Results:** These waves don't have any significant effects on the weight of testes and other sex organs except Seminal vesicle .There has been a significant reduction in the sperm count in two groups of radiation exposure than those of the control group. In terms of motility, the Total Motility of sperm in the experimental groups significantly decreased ($p<0.001$). The number of apoptotic cells in Seminiferous tubes significantly increased in seven hours group in comparision to those of the two other groups ($p<0.004$).Finally the result of Caspase3 activity showed that the activity of this enzyme remarkably increased in 7 hours group which exposure to radiation (2.4GHz- EMF)than those of the two other groups($p<0.0001$).

**Conclusion:** 2.4GHz waves emitting from laptop (wireless) spacially in long exposure significantly induced the Caspase3 activity and apoptosis of spermatogenic cells. Therefore these waves can impair their reproductive capabilities via effect on the sperm count and motilityand increase in cell death (apoptosis).

***Keywords:*** Spermatogenesis, Apoptosis, Sperm, Caspase3, Rat

## P-54: Melatonin Treatment Along Latent and Chronic Phases of Temporal Lobe Epilepsy, Protects Testes Against Destroying Effect of Lithium-Pilocarpine Induced Epileptic Rats

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**Background:** Because of the observed protective effect of melatonin against TLE and the putative role of melatonin to influence neuroendocrine-gonadal axis, we tried to find out whether initiating the melatonin treatment after induction of Status Epilepticus (SE) and all along the different phases of epileptogenesis process, might be able to protect the testes and sperm against devastating effect of chronic temporal lobe epilepsy (TLE).

**Materials and Methods:** Animals were divided into seven groups: group A (control): intact animals (n=5); group B (Epilepsy): animals that received pilocarpine and exhibited SE (n=6); group C (sham): animals that exhibited SE and received the solvent of melatonin; group D (5 mg/60d): animals that received melatonin (5 mg/kg/daily) two hours after onset of SE for 60 days; group E (5 mg/14d): animals that received melatonin (5 mg/kg/daily) two hours after onset of SE for 14 days; group F (20 mg/60d): animals that received melatonin (20 mg/kg/daily) two hours after onset of SE for 60 days; group G (20 mg/14 d): animals that received melatonin (20 mg/kg/daily) two hours after onset of SE for 14 days; group H (Reverse): intact animals which received first pilocarpine (25 mg/kg) and then lithium chloride (3 meq/kg) after passing 20 hours for evaluating the pure effect of lithium and pilocarpine without any seizure induction becase of reverse injection. finally asdsayed Sperm parameters, antioxidant enzymes level, germ cell apoptosis, body and relative sex organ weights.

**Results:** Chronic TLE caused a significant reduction in sperm parameters and the level of antioxidant enzymes. Weight gain and an expanded apoptosis occurred in the testes of epileptic animals. Melatonin administration in three experimental groups were able to improve sperm motility grades. Moreover, spermatogenic cell line apoptosis and the extra weight gain were reduced in some of the treated groups. The increased motility of sperm showed a positive correlation with the level of total antioxidant and a negative correlation with extra weight gain and apoptotic indexes in the treated epileptic animals.

**Conclusion:** Melatonin with reducing the oxidative stress in the testes during epileptogenesis process might be able to improve sperm quality in chronic epileptic animals which probably could be an acceptable cotreatment in epileptic patients.

***Keywords:*** Temporal Lobe Epilepsy, Melatonin, Sperm Parameters, Testis Antioxidant Enzymes, Apoptosis

## P-55: Evaluation of Effects of Gibberellic Acid on Testicular Tissue of Adult Rats

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**Background:** Gibberellic acids are naturally occurring plant hormones that are used in agriculture as plant regulators and enter animals and human cells through diet. This study was undertaken to investigate effects of gibberellic acid on testicular tissue of adult rats.

**Materials and Methods:** Twenty seven adult male

Wistar rats after a one week acclimatization period were randomly divided into three groups of 9 rats each. Control group was untreated, control-sham group received 0.5cc 1% methanol and gibberellic acid group was administered 10 mg/kg body weight gibberellic acid with oral gavage, every other day. The following factors were evaluated on 15th, 30th and 45th day of treatment: Body weight, testis weight, thickness of testicular capsule, seminiferous tubules diameter, thickness of seminiferous tubules epithelium (TSTE), meiotic index (MI), tubular differentiation index (TDI), repopulation index (RI) of seminiferous tubules, spermiogenesis index (SI), Leydig cell count (LeCC) and lymphatic cell count (LyCC).

**Results:** There was no significant difference in first four factors between different groups and days. MI, TDI and SI of gibberellic acid only on 45th day decreased significantly in comparison with the other two groups. TSTE, RI and LeCC on days 30 and 45 of gibberellic acid treated group decreased and LyCC of this group in the same days increased significantly.

**Conclusion:** It can be concluded that Gibberellic acid oral administration in adult rat with its deleterious effects on testicular tissue and spermatogenesis can lead to subfertility or even infertility.

***Keywords:*** Giberellic Acid, Testicular Tissue, Rat, Oral Gavage

## P-56: Effects of Intraperitoneal Injections of Gibberellic Acid on Testicular Tissue in Adult Rats

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**Background:** This study was conducted to evaluate effects of intraperitoneal administration of Giberellic Acid (GBA), a hormone of plants, on testicular tissue of adult rats.

**Materials and Methods:** Thirty six adult male Wistar rats were divided into four groups (n=9). Control group was untreated, control-sham group injected 0.5cc 1% methanol, low dose of GBA group was administered 2 mg/kg and high dose group was injected 20 mg/kg body weightGBA with intraperitoneal injections, every other day. The following factors were evaluated on 15th, 30th and 45th day of treatment: Body weight, testes weight, thickness of testicular capsule, seminiferous tubules diameter, thickness of seminiferous tubules epithelium (TSTE), meiotic index (MI), tubular differentiation index (TDI), repopulation index (RI) of seminiferous tubules, spermiogenesis index (SI), Leydig cell count (LeCC) and lymphatic cell count (LyCC).

**Results:** There was no significant ($p<0.05$) difference in above mentioned first four factors between different groups and days. MI and TSTE of high dose on 30th and 45th day and SI of this group only on 45th day declined significantly ($p<0.05$) in comparison with the control and control-sham groups. 45 days of treatment with both low and high dose of GBA caused a significant decrease in TDI and RI of these groups and an increase in LyCC of high dose group. Finally LeCC on days 30 and 45 of high dose and 45th day of low dose increased significantly ($p<0.05$).

**Conclusion:** According to the results of this study it can be concluded that GBA has a detrimental effect on testicular tissue and spermatogenesis.

***Keywords:*** Giberellic Acid, Testicular Tissue, Rat, Intraperitoneal Injection

## P-57: Effects of Oral Administration of Gibberellic Acid Adult Male Rat on Serum LH, FSH, Testosterone and Testicular MDA

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**Background:** Giberellic acid is a plant growth regulator,apentacyclicditerpene acid promoting growth and elongation of cells, and also a type of pesticide. In this study its oral administration effect on male rat hormones including gonadotropins (LH and FSH) and testosterone, and also lipid peroxidation product (Malondialdehyde) in testicular tissue was investigated.

**Materials and Methods:** Twenty seven mature male Wistar rats were divided into 3 groups (each group n=9) control (no treatment), control- sham (1% methanol) and treatment group (gibberellic acid). Administrations were given with oral gavages every other day. The rats were sacrificed on 15th, 30th and 45th days of the experiment (3 rats for each day of each group), serum samples were taken and LH, FSH and Testosterone levels were measured with radioimmunoassay. Also testicular tissue was used for MDA measurement.

**Results:** Serum FSH had no significant difference between the groups but serum LH had increased and Testosterone had decreased significantly ($p<0.05$) in the treatment group days 30 and 45. MDA in treatment group was higher than control and control- sham groups significantly.

**Conclusion:** Giberellic acid can increase lipid peroxidation in testicular tissue and decrease testosterone, so it may interfere with normal fertility.

***Keywords:*** Giberellic Acid, Gonadotropin, Testosterone, MDA, Rat

## P-58: Which Novel Sperm Selection is Suitable for ICSI? MACS-DGC or DGC-MACS

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**Background:** Super-paramagnetic annexin-V conjugated micro-beads are able to eliminate sperm with phosphatidylserine externalization (PSE). PSE is considered as apoptotic sperm. PES positive sperm can be removed from sperm preparations sample to enhance sperm quality for assisted medical procreation. Therefore, the efficiency of this technique over normal sperm processing procedure such as density gradient centrifugation (DGC) needs further verification. In addition, considering the fact that DGC induces EPS due to capacitation and acrosome reaction, therefore, the role of MACS before DGC (MACS-DGC) and MACS after DGC (DGC-MACS) were assessed.

**Materials and Methods:** Semen samples (n = 15) were collected from infertile individuals and were washed with Ham's buffer and divided into three separate portions. A portion of the sample was kept as controls and a second portion was used for DGC-MACS (MACS procedure was carried out after DGC). The third portion was used for MACS-DGC (DGC procedure was carried out after MACS). Percentages of sperm with normal morphology, DNA fragmentation, protamine deficiency, EPS and caspase-3 activity were determined in each group.

**Results:** Combination of DGC and MACS improved the sperm quality compared to when DGC and MACS were implemented independently. However, comparison of the combined procedures showed only higher efficiency to separate active caspase in the MACS-DGC group.

**Conclusion:** In spite of recent clinical trends in the literature, which are mainly implement DGC-MACS procedure, we strongly recommend MACS-DGC procedure.

***Keywords:*** ICSI, Sperm Selection, MACS-DGC, DGC-MACS

## P-59: Investigation of Simultaneous Exposure to Noise and Formaldehyde Vapor on Mouse Reproductive Function

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**Background:** Formaldehyde (FA) is a member of aldehyde family with a simplest organic molecule, used in various industries. Since there is simultaneous exposure of formaldehyde and noise for workers in most of the workplaces and that noise can reinforce the harmful effects of some chemical pollutants. This study aimed to investigate the effects of simultaneous formaldehyde and noise exposure on the reproductive parameters in male mice.

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

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

**Conclusion:** Exposure to formaldehyde vapor can decrease percentages of sperm count and progressive motility. The present study indicated that reduction of sperm progressive motility may be potentiated by noise in the simultaneous exposure to formaldehyde and noise. Thus, it can be anticipated that simultaneous exposure to formaldehyde and noise in workplaces can increase the possibility of damage to the pituitary-Testes Axis and reproductive functions in male mice.

***Keywords:*** Formaldehyde, Noise, Sperm Parameters, Sex Hormones, Mouse

## P-60: The Comparison of Semen Parameters in Smokers and Non Smokers Who Refer to Infertility Clinic

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**Background:** Facing infertility put too much emotional stress and physical burden on infertile couples that make it difficult to deal with. According to this fact that infertility in some men appears with specified disorder but in most cases there is no clear cause for male infertility which determine the special attention to lifestyle and environmental factors. In this study the effects of smoking on semen parameters in infertile couples are studied.

**Materials and Methods:** This retrospective descriptive study on 1263 men, 948 non smokers and 315 smokers (279 light smokers and 36 heavy smokers) were done. All women who don't have the factors affecting sperm except cigarette were entered into the study.

**Results:** The result showed that the prevalence of smoking in the population studied was 24.9% which was only2.85% of them heavy smokers. Greatest impairment observed in the study population was impaired sperm motility which was 32.4% of the total population. Abundance count of abnormal sperm among smokers was 16.19% and among non-smokers was 10.4% ($p<0.0001$) abundance of abnormal sperm motility among smokers was 42% and among non-smokers was 29.2% ($p<0.0001$), morphology abundance of abnor-

mal sperm among smokers was 6.9% and among non-smokers was 0.73% (p<0.0001). The age distribution in sperm count changes has shown a significant association (p<0.0001).

**Conclusion:** In this study, smoking had an effect on every three factors, sperm number, motility and morphology. Greatest impact was on the sperm morphology and sperm motility disorders are the most common disorder in this study that could be a factor in semen quality reduction. Only light smoker didn't show significant correlation with sperm count but sperm motility and morphology was dependent to light smoker as well and age changes only affected the sperm count.

***Keywords:*** Infertility, Semen Analysis, Smoking, Astheno Spermia

### P-61: Comparing The Protective Effects of Hydro-Alcoholic Extract of Cornus Mas Versus Vitamin E on Methotrexate-Induced Damages on Sperm Parameters

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**Background:** Methotrexate (MTX), as antimetabolite and antifolate compound, is used to treat the certain types of tissue over activity and cancers. The MTX interferes with cell division through inhibiting the purine and pyrimidine synthesis. Moreover, the toxic impact of the MTX on gonadal tissues has been reported. Therefore, current study was designed to evaluate the protective effects of cornus mas hydro-alcoholic extract (CME) and vitamin E, as potent antioxidants, on MTX-induced damages on sperm DNA integrity and chromatin condensation.

**Materials and Methods:** Forty eight mature male mice were divided into six control, test and treatment (N=8 mice for each group) groups. The control group received normal saline (0.25 ml, orally). The test and treated groups were subdivided into five groups including; MTX alone-administrated (20 mg/kg, ip., once a week, for 35 days), three group of MTX+CME at dose levels of 250, 500 and 1000 mg/kg (orally for 35 days) and the MTX+vitamin E (100 IU/kg, orally for 35 days). The sperm count and motility, DNA integrity and chromatin condensation were evaluated.

**Results:** MTX administration remarkably (p<0.05) reduced the semen quality, while the animals in CME and vitamin E-dosed groups revealed with considerably (p<0.05) better sperm parameters. Qua, the high dose CME-administrated animals showed significantly (p<0.05) higher sperm count and motility versus the vitamin E-received groups. Significantly higher percentage of sperms with normal DNA and condensed chromatin was observed in high dose CME-received group versus vitamin E-administrated animals.

**Conclusion:** Our data suggest that high dose CME could fairly protect the sperms against MTX-induced damages even better than vitamin E.

***Keywords:*** Methotrexate, Cornus Mas, Sperm DNA Integrity, Chromatin Condensation, Vitamin E

### P-62: Damage to Sperm DNA and Protamine Deficiency Induced by Risperidone in NMRI Mice

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**Background:** Antipsychotic medication use is a common cause of hyperprolactinemia that shown to have adverse effects on male fertility. Risperidone is a combined serotonin/dopamine receptor antagonist that can cause elevations in prolactine level. Its mechanism of cytotoxic effects on testicular germ cells is not fully understood. In the present study we sought to elucidate the impact of risperidone on sperm DNA integrity and chromatin quality in male mice.

**Materials and Methods:** 8-week old male NMRI mice were randomly assigned to two groups as control-sham and test groups. Risperidone was administered by gavage once daily for 45 consecutive days in dose of 3.2 mg/kg. The control group just received vehicle. At the end of the study period the animals were euthanized by decapitation. Sperms were removed from cauda epididymis and analyzed for chromatin integrity and DNA damage.

**Results:** The level of abnormal single-stranded sperm DNA in Risperidone- treated mice was significantly higher than that of control group. The percentage of sperms with protamine deficiency in treated group was higher than those of control group.

**Conclusion:** Current findings illustrated that risperidone induced its detrimental effects by protamine impairment which was able to increase DNA susceptibly for damages.

***Keywords:*** Protamine, Risperidone, Mice

## Animal Biotechnology

### P-63: Comparison of The Efficacy of Two Culture Media (Handmade vs. Commercial) on *In Vitro* Development of Vitrified and Non-Vitrified Mouse Embryos

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**Background:** While assisted reproductive techniques (ARTs) are considered to be safe, many concerns exist about the safety of these techniques. Two important and indispensable parts of these techniques are *in vitro* culture and vitrification. While much progress has been made in developing culture conditions, it remains suboptimal. In order to investigate the effect of culture medium on *in vitro* development of vitrified and non-vitrified mouse embryos we conducted this study.
**Materials and Methods:** In this regard female mice were superovulated and mated. At ~36 h post hCG injection, the oviducts were flushed for collection of 2-cell stage embryo. These embryos were randomly assigned to vitrified and non-vitrified groups. After vitrification/warming procedure, warmed (not-fresh) and fresh embryos were cultured in mSOF or G1/G2 media up to the blastocyst stage and the effect of the different culture media on the blastocyst formation and the hatching process of blastocysts was evaluated.
**Results:** The survival rate was not significantly different between the treatment groups. The percentage of 2-cell stage embryos that developed to the blastocyst stage was significantly lower in the mSOF/warmed group (76 ± 1.97%) compare to other treatment groups. There were no significant difference between the blastocyst formation of fresh/G1/G2, warmed/G1/G2, and fresh/mSOF groups (89.33 ± 1.2%, 83.67 ± 1.8% and 87 ± 1.5%, respectively). The hatching process of blastocysts was also evaluated. The hatching rate was significantly lower in the mSOF/warmed group (51.67 ± 1.82%) in compare to fresh/G1/G2 and fresh/mSOF (68 ± 1.42%, 64 ± 1.64%, respectively).
**Conclusion:** These results revealed that commercially G1/G2 medium is superior to handmade mSOF medium for culturing and *in vitro* development of 2-cell stage mouse embryos.
***Keywords:*** Mouse, *In Vitro* Culture, Vitrification, *In Vitro* Development, Blastocyst

## P-64: The Effect of Ovine Oocyte Diameter on Nuclear Maturation

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**Background:** The study was carried out to investigate the relationship between the development potential and the diameter of ovine oocytes.
**Materials and Methods:** Ovaries collected from a local abattoir were transported to the laboratory within 1-3 h of slaughter. The oocytes from follicles (2-6 mm in diameter) were recovered by aspiration and stored in a preincubated (at 38.6 C, 5% $CO_2$ and maximum humidity) hepes-modified TCM 199 solution. Good quality oocytes (evenly granulated cytoplasm with at least two layers of surrounding cumulus cells) were selected and subjected to culture in TCM 199 supplemented with 0.05 IU/ml rhFSH, 1 IU/ml hCG and 1 µg/ml E2 (OCM; oocyte culture medium). Before culturing, the selected oocytes (n= 1495) were divided into three categories based on diameter: i. <110 µm, ii. 110-150 µm and iii. >150 µm. In each category half of the oocytes were denuded of the cumulus cells and the rest remained intact (cumulus enclosed oocytes). All of the oocytes, denuded and cumulus enclosed oocytes in each category were cultured in OCM for a period of 26-27 hours. After the incubation period, the nuclear status of the oocytes in each experimental group was assessed using a 2% orcein staining technique.
**Results:** The percentage of oocytes that reached the MII stage was 81, 82, and 84% for the cumulus enclosed oocytes with diameters of <110, 110-150, >150 µm, respectively. The corresponding values for the denuded oocytes were 4, 5, and 6%, respectively. The oocytes displayed no size-related ability to undergo meiotic maturation in both cumulus enclosed and denuded oocytes. However, as expected the rate of nuclear maturation in cumulus enclosed oocytes was higher than that of denuded oocytes in all the size categories.
**Conclusion:** The results suggest that in sheep the antral follicles ranging from 2 to 6 mm contain fully grown oocytes which, despite their variability in diameter, show good competence for *in vitro* nuclear maturation.
***Keywords:*** Sheep, Oocyte,Diameter, *In Vitro* Maturation

## P-65: Maternal Effect Genes in Mammalian Reproduction

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**Background:** Regulation of gene expression in mammalian embryos is not completely known. Pre-implantation embryos need maternal RNA and proteins synthesized during oogenesis, to regulate development before mater-embryo transition, as the grown oocyte and the 1-cell zygote are transcriptionally silent. There are some oocyte-specific genes called maternal effect genes which may account for this early cleavage regulation. Mater-embryo transition is done through gradual degradation and reduction of maternal RNA and proteins, and the occurrence of zygote genome activation. Identification of maternal factors like Mater, Npm2, Hsf1, Floped, Stella, Brg1, Zar1, Dnmt1 and Filia in mice, showed the importance of these genes in mammals.
**Materials and Methods:** In this review, we will focus on

some maternal effect genes causing reproductive dysfunction in different mammalian species.
**Results:** These factors are preferentially expressed in growing oocyte, and their protein products remain until the late pre-implantation developmental stages. They have a highly impressive effect on developing embryos, as the embryos are blocked or growth-delayed in null type. So, maternal effect genes will alter reproduction rate in abnormal inheritance. Loss of maternal effect genes does not make change in female normal ovulation and fertilization, but causes infertility or subfertility due to failure in pre-implantation development.
**Conclusion:** Hence, understanding the biology of such maternal factors and their genomic variations will help to find about the basis of some reproductive abnormalities and troubleshooting.
***Keywords:*** Maternal Effect, Fertility, Pre-Implantation

# Embryology

## P-66: The Effect of Vitrification on Distribution of α9, αv, α1, α3 Integrins Mouse Oocytes at GV, IVM-MII and OV- MII Stages

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**Background:** Integrins are known receptors that facilitate sperm-oocyte interactions and play important roles in fertilization, proliferation and implantation process. The aim of this study was to investigate the incidence of α9 , αv , β1 , β3 integrin in mice oocytes after vitrification by Cryotop.
**Materials and Methods:** A total of 200 germinal vesicle (GV) and 200 metaphase II (OV-MII) oocytes obtained from ovaries and fallopian tubes of NMRI mice respectively and divided into two control and experimental (vitrified) groups. Oocytes in experimental group were vitrified by Cryotop using 1,2-Propanediol / Ethylene glycol vitrification medium (Origio) and were kept in liquid nitrogen for one month. Oocytes in both group after insemination were assessed to hatching stage. The data was compared statistically using SPSS software and chi-square test. Immunocytochemical study were performed in mouse oocytes to demonstrate the distribution of α9 , αv , β1 , β3 integrin.
**Results:** The incidence of fertilization rates in vitrified group showed a significantly decrease compared with the control group ($p<0.05$). The distribution of Integrins was reduced in vitrified group compared to control oocytes. Immunocytochemical analysis revealed higher expression of α9, β1 in GV control oocytes. in other hand β1 expression was the lowest in OV-MII oocytes of vitrified group.
**Conclusion:** Vitrification may play an important role in GV and MII oocyte injury. Therefore reduction of mouse oocyte surface integrin could be one reason for this unsuccessfulness.

***Keywords:*** Vitrification, Maturation , Fertilization, Integrin, Cryotop, Oocyte

## P-67: Reactive Oxygen Species, MDA and NO Concentration of Testicular Extract and Their Effect on Sperm Parameters and IVF Outcome in Hyperprolactinemic Adult Male Mice

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**Background:** ROS are free radicals that have a significant role in many of the sperm physiological processes. In the presence of polyunsaturated fatty acids such as sperm plasma membrane,ROS triggers a chain of chemical reactions called lipid peroxidation.The aim of this study was to determine and compare the concentration of MDA and NO in testicular extract of hyperprolactinemic adult male mice to stablish their effect on sperm quality and assess the fertilization potential of spermatozoa and IVF outcome.
**Materials and Methods:** Eighteen mature male mice were divided in 3 groups as control,sham and test. For hyperprolactinemia induction, test group received 40mg/kg/day sulpiride atypical antipsychotic drug solution , IP for 45 days. Control sham received placebo.45days later spermatozoal suspention was analyzed for sperm parameters and it's concentration in couda epididymis. For each male mouse, 3 female mice were superovulated, killed and ovums collected. 50000-100000 of taken sperms of each male mouse were injected in macro droplets as each oocyte. After 24 hours the rate of fertilization and 2-cell zygots and after 120 hours rate of necrotic cells, cytoplasmic vesicles in each embryoes and arrested emberyos were investigated and were compered with 2 other group,s data. Additionally, the potential impact of hyperprolactinemia on MDA and NO ratio of testicular tissue were checked and compared with control and sham groups.
**Results:** In hyperprolactinemic group negative correlation was found between testicular extract's MDA and NO concentration and sperm morphology,vitality and IVF rate.Also,an inverse correlation was found between sprm DNA damage and moyility.Additionaly,2-cell embryos and blastocyst numbers reduced in this group in compare with two other groups.Data showed no significant differences between sham and control's results ($p<0.05$).
**Conclusion:** High ROS concentration in testicular extract in hyperprolactinemic mice affects the quality of spermatozoa that consequently dose affect fertilization rate and embryonic quality in IVF cycle.
***Keywords:*** Hyperprolactinemia, MDA, Sperm Quality, IVF, ROS

## P-68: Intercourse Recommendations According to Classic and Traditional Medicine

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**Background:** Health is a very essential issue in our world. From the classic and traditional medicine point of view, our surroundings and habitat including geographic location, climatic conditions , eating and drinking habits , training and emotions, contribute to a healthy life style. With an overview in traditional medicine, it is clear that there is another important aspect in preventive medicine and it is sexual life and intercourse.
**Materials and Methods:** We reviewed reference textbooks in traditional Iranian medicine and classic medicine about sexual life and intercourse and their role in a healthy life style. We looked for sexual life and its relationship with eating, types of drinking, bathing, sleeping, special timing in the course of a day and its intervals in those references. Also we reviewed Al-Ghanoon by Avicina, Zakhireh Kharazmshahi and Kamel alsanaeh.
**Results:** In Iranian traditional medicine, having healthy sexual life and intercourse not only serve as a means for pleasure, but they are also important aspects of a healthy life style.
**Conclusion:** Therefore, they need to be performed in certain times and periods, there should be a prologue and foreplay and special limitations should be observed with regard to drinking and eating habits. The role of sexual life and intercourse in health and preventive medicine are essential. Also it is absolutely necessary to educate public about those essential issues in health clinics and primary care settings.
***Keywords:*** Intercourse, Traditional Medicine, Preventive Medicine, Classic Medicine

## P-69: Effect of Fennel (Foeniculum Volgare) on The Postnatal Ovary Development in The Rat

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**Background:** Histological and histometrical effects of Fennel Essential Oil (FEO) on ovary structure were studied during postnatal development in female wistar rats.
**Materials and Methods:** For this purpose, the female rats, after parturition divided in control, sham and experimental groups with equal number of female pups for each mother at random. Sham 1 and experimental 1 groups consisted of mothers received respectively normal salin or FEO during the first 10 days of lactation; sham 2 and experimental 2 groups consisted of mothers were treated same as sham 1 and experimental 1 but during the second 10 days of lactation. In all groups, FEO and salin were administered by gavage in dose 500 mg/kg/day. At age 22, 53 or 84 day old, tissue samples from ovary were removed in different groups and fixed in Bouin's solution. For microscopic studies, following tissue processing, 5-6 µm sections were stained with haematoxylin-eosin.
**Results:** The results showed that FEO increased antral follicles number in 22 and increase corpus luteum number in 84 day old of experimental groups in comparison with control and sham groups but this increase in experimental 2 was more. Also, FEO increased primordial follicles number and decreased developing follicles number in all ages of experimental 1. FEO decreased primordial numbers and increased primary follicles number in experimental 2. Moreover, atretic follicles number in these groups decreased in comparison with control and sham groups. But this decrease in experimental 2 was more.
**Conclusion:** So, present study indicates that consumption of FEO during lactation period may affect rat offspring ovary development by exerting estrogenic effects. Exposure neonatally, causes an abnormal phenotype, postpones primordial to primary follicle transition and decreases number of growing follicles. Exposure prepubertaly promotes follicular development of rat offspring ovary.
***Keywords:*** Rat, Ovary, Ovarian Follicles, Fennel Essential Oil, Postnatal Development

## P-70: Effect of Xylose on Epididymal Sperm Motility in Water Buffalo (Bubalus bubalis)

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**Background:** In mammalian spermatozoa, motility is one of the most important parameters for evaluating the quality of the semen. From a functional point of view, motility is strongly related to the spermatozoa's ability to manage its energy status, Thus, modulation of energy status could be translated into significant changes in motility patterns. The aim of the present study was to evaluate xylose sugar different levels, on buffalo epididymal sperm motility patterns with computer-assisted sperm analyzers (CASA).
**Materials and Methods:** For this study buffalo bull testicles (15 pairs) were picked up in the march till September 2012 in Urmia local slaughterhouse and transported to the laboratory in a cool container (filled with 5oc ice pack).After adding 5 levels of xylose (1-3-5-10-15 mM), in TCM199 media with 10 percent BSA and ,24 hours incubation in 37ºC was performed. Sperm motility was examined at 1, 3, 5, 7, 10, 12 and 24 hours after incubation with CASA. After collection of data from CASA analysis, Statistical analyses were performed with procedures available in ANOVA of SPSS version 20.
**Results:** The results showed that CASA parameters; rapid progressive motility (Class A, %), progressive motility (Class B, %), motile sperms (Class A+B+C, %), straight line velocity (VSL, µm/s), average path velocity (VAP, µm/s), curvilinear velocity (VCL, µm/s), amplitude of lateral head displacement (ALH, µm), angular

displacement (MAD, D),beat cross frequency (BCF, Hz) were significantly higher in control than xylose levels specially in 24 hours after incubation ($p<0.05$).

**Conclusion:** In conclusion, our results indicate that xylose, at concentrations from 1 to 15 mM, decrease CASA parameters, maybe xylose don't activate the glycolytic pathway and ATP production at this concentrations in TCM 199 media in 37 ºC.

***Keywords:*** Xylose, CASA, Buffalo, Epididymal Sperm

## P-71: The Effect of Leuprolide on Prepubertal Mice Sperm Parameters

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**Background:** One of the side effects of chemotherapy agents is gonadal suppression leading to infertility. The present study examined the feasibility of using a gonadotropin releasing hormone agonist (GnRH-A) in the prevention of busulfan induced azoospermia in prepubertal mice.

**Materials and Methods:** Leuprolide(3.8 mg/kg) was given to prepubertal male mice 2 weeks after or prior to busulfan (40 mg/kg) administration and continued every 4 weeks. Two groups received one dose of leuprolide after busulfan administration. Six weeks after the last leuprolide treatment, the sperm parameters were analyzed icluded sperm count, motility, and morphology according to the World Health Organization criteria.

**Results:** At the time of scarifies, testicular weight were increased in animals receiving leuprolide. In animals receiving leuprolide pretreatment, the percentage of progressive and immotile sperm was significantly ($p\leq0.05$) increased and decreased respectively. But, the sperm motility in busulfan treated animals with post treatment didn't change. The sperm count also increased in pretreatment animals but it was not significant ($p\geq0.05$).

**Conclusion:** The present study demonstrates that GnRH-A pretreatment resulted in improvement in sperm motility in prepubertal male mice.

***Keywords:*** Leuprolide, Busulfan, Sperm Parameters, Chemothera

## P-72: Comparative Histopathologic Evaluation of Portulaca Oleracea, Omega-3 Fatty Acids and Combination of Sodium Selenite and Vitamin E on Testis of Experimental Diabetic Rats

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**Background:** Portulaca oleracea (PO) plant, omega-3 fatty acids and combination of sodium selenite and vitamin E can limit negative impacts on testicular tissue and function which may be caused by compensating for defective insulin release, enhancing antioxidant status and decreasing in blood glucose levels.

**Materials and Methods:** A total of 96 adult male Sprague Dawley (SD) rats (weighing approximately 220 ± 10 g) were injected by a single intraperitonial injection of streptozotocin (70 mg/kg Body weight.) and were randomly assigned to 4 groups and 4 replicates for each group. Group 1 served as diabetic control, group 2 received PO juice (1.5 mg/kg/day, orally), group 3 received omega-3 fatty acids (500 mg/kg/day, orally) and group 4 received vitamin E in forms of alpha-tocopheryl acetate (400 Iu/kg/day, orally) and sodium selenite (0.5 mg/kg/day, orally) for a period of 7 days. At the end of the study period, testis samples were taken for histopathological investigations. spermatogonia, spermatocyte, spermatid and sertoli cells in all groups were calculated.

**Results:** Reduction in number of espermatogonial cells in animals treated with combination of sodium selenite and vitamin E was less than other groups. Spermatocytes and sertoli cells in group 4 was more than other treatment groups. Number of spermatid cells in groups treated with omega-3 and combination of sodium selenite and vitamin E was more than control and PO groups.

**Conclusion:** Treatment with combination of sodium selenite and vitamin E in male diabetic rats revealed more positive effects compared to other treatment groups.

***Keywords:*** Portulaca Oleracea, Omega-3, Vitamin E, Testis, Diabetic Rat

## P-73: Effect of Sodium Dodecyl Sulfate and Different Levels of Clove Bud Extract on Motility, Viability and Plasma Membrane Integrity of Ram Spermatozoa during Cooling and Cryopreservation

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**Background:** Hen egg yolk is a common component of most semen cryopreservation extenders. However, diluents containing the egg yolk may have adverse effects on the quality of the semen. Egg yolk could be solubilized by the addition of sodium dodecyl sulphate (SDS) in semen extenders. Many studies have shown beneficial effects of antioxidants addition to semen freezing extenders. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health. The clove bud oil has been powerful antioxidant properties. However, few studies deal with scavenging effect of clove buds extract

in sperm extenders.

**Materials and Methods:** The semen samples were collected from 3 Iranian ram (3-4 years old and 105 kg BW) using artificial vagina, twice a week. Antioxidant property of the ethanol extract of clove bus was determined by DPPH test and then it was added to sperm diluents with concentrations of 0, 35, 75, and 115 mg/ml. The SDS was added at levels of 0 and 0.5 mg per ml of egg yolk. The sperm motility, viability and plasma membrane integrity were evaluated after semen collection, before sperm freezing and after thawing.

**Results:** This study revealed that extenders with SDS was more effective in preservation of motility, viability and integrity of the plasma membrane of spermatozoa ($p<0.05$). Different levels of clove bud extract affect the sperm characteristics before freezing and after thawing ($p<0.05$). The best level for clove bud extract was 75 mg/ml in semen diluents.

**Conclusion:** Therefore we can conclude that adding SDS and plant extract may benefit effects on sperm cryopreservation.

***Keywords:*** Detergent, Plant Antioxidant, Semen Cryopreservation, Ram

## P-74: Crocin Repopulate The Leydig and Sertoli Cells in Testis of Cyclophosphamide Treated Adult Mice

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**Background:** Increases in the survival rate of men treated with chemotherapeutic drugs and their desire to have children precipitate concerns about the effects of these drugs on germ cells. This study performed to evaluation of protective effects of crocin in oxidative stress induced by cyclophosphamide (CP) on testis.

**Materials and Methods:** Three groups (6 mice in each) of adult mice were used. Control group treated with normal saline via ip, and group 2 treated with CP 15 mg/kg/week,ip, and group 3 treated with CP along with crocin 200 mg/kg/day,ip. After 35 days samples were took and fixed in 10% formal saline and paraffin sections were prepared and stained by H&E method. Mean distribution of Laydig and Sertoli cells were counted in 1 mm2 field in 5 regions of each slides by latticed objective device. All obtained data were analyzed by SPSS software in ANOVA and Duncan test.

**Results:** Results showed that the mean distribution of Leydig and Sertoli cells in group that treated with CP (9.27 ± 0.34 and 21.86 ± 0.81), significantly was lower than control group (18.99 ± 0.64 and 61.72 ± 3.17), and mean distribution of Leydig and Sertoli cells (14.41 ± 0.37 and 26.19 ± 0.75) significantly were more than CP treated group ($p<0.05$).

**Conclusion:** This study showed that crocin ameliorate the oxidative stress effects of CP on reproductive organ.

***Keywords:*** Crocin, Leydig, Sertolicells, Cyclophosphamide, Mice

## P-75: The Effect of Hydrostatic Pressure in The Presence of Different Concentrations of Extracellular Calcium, Ethanol and Cytochalasin B on Parthenogenetic Activation of Mouse oocytes

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**Background:** The parthenogenetic of oocytes is important in cloning research, as artificial activation of oocytes is an essential component of nucleus transfer protocols. Hydrostatic pressure can act as a mechanical stimulator that rearranges egg contents. Ethanol promotes a single intracellular $Ca^{2+}$ increase of greater and longer amplitude than the initial increase observed at fertilization. In this study, we investigated the effect of hydrostatic pressure on parthenogenetic activation of mouse oocytes in presence of different concentration of calcium and ethanol and cytochalasin B.

**Materials and Methods:** 6 to 8-week-old female NMRI mouse were superovulated by an injection of 10 IU of PMSG, followed by 10 IU HCG 48 hours later. Metaphase II oocytes were collected from oviduct 14 hr after HCG injection. Oocytes transferred to T6 medium containing different concentrations of calcium (0, 1.7, 3.4 mM) and 7% ethanol and for 5 minutes, treatments I, II,III ; respectively. They were divided to experiment and control groups. In experiment group, oocytes subjected to 20 mmHg pressure for 20 minutes. Oocytes without exposure to pressure were considered as control. Oocytes from two groups were transferred to T6 medium supplemented with different concentration of calcium and 5 µg/ml cytochalasin B for 4 hours. Oocytes were cultured for 72 hours and embryo development was assessed.

**Results:** After 72 hours, in experiment and control groups treatments I , II, III , the percentage of cleavage was 18.51, 69.63, 45.36 and 9.8, 41.17, 48.03%; respectively. Cleavage rate in experiment group was higher than control group ($p<0.05$). The highest cleavage rate associated with treatment II which were significantly different from others in experiment and control ($p<0.05$).

**Conclusion:** The results suggest that exposure to hydrostatic pressure, in the presence of ethanol and cytochalasin B can be considered as an artificial parthenogenic activation protocol in mouse oocyte.

***Keywords:*** Parthenogenetic Activation, Hydrostatic Pressure, Extracellular Calcium, Ethanol, Cytochalasin B, Mouse

## P-76: Cytogenetic Investigation of Parthenogenetic Mouse Embryos Generated from *In Vitro* Activated Oocytes by Hydrostatic Pressure in The Presence of Calcium Ionophore and Ethanol

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**Background:** The advances in cytogenetic techniques during the last few years have permitted not only the study of large populations of wild and domestic animals, but also the detection of chromosome anomalies in embryos. Chromosomal abnormalities are the most common cause of embryonic and fetal mortality in mammals. Most reports of chromosome anomalies in parthenogenetic embryos describe numerical aberrations comprising aneuploidies, haploidy, polyploidy and mixoploidy. Hydrostatic pressure specifically affects centriole microtubules and that proximal microtubuletriplets are more resistant than distal microtubule doublets. The present study has been carried out to investigate the effects of hydrostatic pressure in the absence or presence of calcium ionophore and ethanol on improve of chromosomal complements in 2-cell parthenogenetic embryos.
**Materials and Methods:** 6 to 8-week-old female NMRI mouse were superovulated by an injection of 10 IU of PMSG, followed by 10 IU HCG 48 hours later. Metaphase II oocytes were collected from oviduct 14 hours after HCG injection. Oocytes transferred to T6 medium and randomly assigned to following groups: non- treatment (control), hydrostatic pressure exposure (treatment I), 5μM calcium ionophore exposure (treatment II), ethanol exposure (treatment III), 5μM calcium ionophore with hydrostatic pressure exposure (treatment IV) and ethanol 7% with hydrostatic pressure exposure (treatment V). Groups of activated oocytes were further treated with 5 mg/ml cytochalasin B for 4 hours. About 24-hour post oocytes activation, slides were prepared according to an 'air drying' technique and the chromosomal complement of 2-cell embryos was studied by giemsa staining.
**Results:** Results indicated that in embryos treated by calcium ionophore and hydrostatic pressure observed the most incidence of normal chromosome (Diploidy and Tetraploidy) (84%) and fewest incidence of anormal chromosome (haploidy, triploidy and mixopliody) (16%) observed compared with control group and other treatments ($p<0.05$).
**Conclusion:** It suggests that the exposure to hydrostatic pressure in the presence of calcium ionophore and ethanol can be improve chromosomal complements in 2-cell parthenogenetic embryos.
***Keywords:*** Cytogenetic Investigation, Parthenogenetic Activation, Hydrostatic Pressure, Calcium Ionophore, Ethanol, Mouse

## P-77: HOST Patterns and Sperm Quality

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**Background:** Following the expansion of human infertility, different procedures have been utilized for separation of normal sperm during ICSI (Intra cytoplasmic sperm injection). Hypo-osmotic swelling test (HOST) is one of the tests which have been introduced for assessment of integrity of sperm membrane when confronted with hypo osmotic condition. This test may also reflect intracellular organelle integrity such as chromatin. When sperm expose to hypo-osmotic condition, tail of sperm is curved and form different patterns from "a" to "g". Recently, HOST is suggested to selection sperm during ICSI. Therefore, in this study, we aim to evaluate the correlation of different HOST patterns with sperm parameters, protamine deficiency as sperm maturation marker, and DNA damage.
**Materials and Methods:** Sixteen semen samples were randomly collected from men attending the Andrology Unit of the Isfahan Fertility and Infertility Center. Semen samples were divided to two portions, one portion was assessed for sperm parameters accordingly WHO-2010. Other portion, after HOST procedure was used to assess sperm morphology, DNA fragmentation and protamine deficiency by using papanicoulau, TUNEL assay, and CMA3 staining.
**Results:** Our results show that, the lowest OR (Odds Ratio) of abnormal sperm head morphology and abnormal acrosome were in d-sperm. A significant correlation was observed between percentage of sperm motility and DNA fragmentation. In addition, significant correlations were observed between sperm concentration with percentage of apoptotic sperm and protamine deficiency.
**Conclusion:** This result clearly indicated with different HOST patterns should not be used for ICSI. The best pattern for ICSI is d-sperm and insemination of g-sperm should be avoided. The results of these correlations show that, semen samples with low sperm concentration have low percentage of d-sperm which are mature and intact sperm and therefore, in these cases in cooperation of HOST into ICSI technique are highly recommended.
***Keywords:*** ICSI, HOST, DNA Fragmentation, Protamine Deficiency

## P-78: Supplementation of Sperm Cryopreservation Media with Cell Permeable Superoxide Dismutase Mimetic Agent (MnTE) Improves Goat Blastocyst Formation

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**Background:** ROS production is a well-established phenomenon during sperm cryopreservation. Therefore, supplementation of freezing media with antioxidant is an inevitable event. The aim of this study was to assess whether a cell permeable superoxide dismutase agent such as MnTE, can further improve the quality of frozen/thawed semen sample using a commercially optimized

sperm cryopreservation media (Bioxcell).

**Materials and Methods:** Bioxcell was supplemented with different concentration of MnTE. Sperm membrane integrity, motility, viability and acrosomal status were assessed after freezing. Optimized concentration of MnTE was defined and used to assess fertilization and developmental potential.

**Results:** 0.1μM MnTE significantly improved membrane integrity while 0.01μM MnTE significantly improved acrosmal integrity post thawing. Addition of 0.01μM MnTE also improved blastocyst formation rate.

**Conclusion:** Supplementation of commercially optimized cryopreservation media with MnTE further improves the quality of goat frozen semen sample and may have important consequence of future embryo development. This effect may be attributed to cell permeable behavior of this antioxidant which may protect sperm genome from ROS, DNA-induced damage.

***Keywords:*** Cell Permeable Antioxidant, Goat, MnTE, Semen Cryopreservation

## P-79: The Effect of Alkaline Environments Made with Calcium Carbonate in Order to Migration Spermatozoa for Sex Selection

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**Background:** Due to different such as some sexrelated illnesses, spouses have been encourage to designate special treatments for assessing sexuality. Moreover, Utilizing from identifying sexulityb has been an important aim in industry in order to product humans needs and develop cattle. In this research, the effect of alkaline environments were examined migration spermatozoa for sex selection.

**Materials and Methods:** A number of 40 mice (NMRI) were divided in to a control group and three experimental group. Ovulation of mice was stimulated by using PMSG and HCG hormones. Female mice were killed by cervical dislocation and dissect. Oocytes were collected stril condition from the the oviduct. Spermatozoa from the epididymis were getting and entered on media by pH (7.5, 7.6, 7.7). The faster sperms used for IVF. Embryo of control groups and exprrimental group were transferred into foster mother and after birth, male and female infants were counted. Analysis of data was done by spss, Anova,softwere.

**Results:** The proportion of male and female in control group(pH=7.4), experimental group 1 (pH=7.5) and experimental group2 (pH=7.6) and experimental group3 (pH=7.7) was (48.61), (34.95), (34.35), (33.66) (Mean ± SD) respectively. The comparison of proportion of male to female infant between control group and experimental groups showed significant differences (p <0.05). But there was not significant difference among experiment groups 2, 3 with conrol group.

**Conclusion:** Searches showed that activity of the sperms with X chromosome proportion to Y chromosome in alkaline environments with sodium carbonate increased and chance of female infant birth will increase.

## P-80: The Effect of Alkaline Environments Made with Sodium BiCarbonate in order to Separate Spermatozoa for Sex Selection

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**Background:** There are different factors Tahat effect in sex selection such as the best medium treatment.These techniques can be used in live stock industry for product in ordre to humans need.

**Materials and Methods:** Forty mice (NMRI) were divided in to a control group and three experimental group. Ovulation of mice was stimulated by using PMSG and HCG hormones. Female mice were killed by cervical dislocation and dissect. Oocytes were collected from the oviduct. Spermatozoa from the cauda epididymis were getting and entered on media by pH (7.5, 7.6, 7.7). The faster sperms used for IVF. Embryos of control groups and exprrimental group were transferred into foster mother and after birth, male and female infants were counted. Analysis of data was done by Spsss, Anova,softwere.

**Results:** The proportion of male and female in control group(pH=7.4), experimental group 1 (pH=7.5) and experimental group2 (pH=7.6) and experimental group3 (pH=7.7) was (48.61), (65.99), (67.33), (68.49) (Mean±SD) respectively. The comparison of proportion of male to female infant between control group and experimental groups showed significant differences (p <0.05). But there was not significant difference among experiment groups 2,3 with conrol group.

**Conclusion:This research showd that sperm activity in Y chromosome increased with alkaline invironments and Sodium Bicarbonate.**

## P-81: Distinct Epigenetic Status of Fully Grown and Growing Oocytes Selected by BCB Staining

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**Background:** Since the sheep 'Dolly' was successfully cloned by somatic cell nuclear transfer (SCNT), the inefficiency of SCNT cloning and abnormality of cloned animals have been a focus of considerable controversy. Oocyte quality has been shown to contribute to poor somatic cloning and cell reprogramming efficiency with many factors affecting oocyte quality and developmental potential. It has been found that immature oocytes with reduced reproductive performance or slaughtered at the end of their use are heterogeneous in quality. Then selecting immature oocyte is necessary and important for embryos laboratory. It is generally believed that glucose-6-phosphate dehydrogenase (G6PDH) protein is active in the growing oocyte, but its activity is decreased in oocytes that have finished their growth phase, then likely to have achieved developmental competence. The enzyme G6PDH can degrade brilliant cresyl blue (BCB). Thus, oocytes yielding decreased G6PDH (finished growth phase) show a blue cytoplasm (BCB+) after BCB staining, while growing oocytes (unfinished growth phase) have abundant G6PDH and a colorless cytoplasm (BCB-).
**Materials and Methods:** The oocytes were exposed to 26μM BCB and classified according to their cytoplasm coloration. However, to address the effects of oocyte selection by BCB staining on the nuclear reprogramming capacity of oocytes, embryo quality, we analyze developmental competence, status of epigenetic MII oocyte modifications, embryo quality assessment.
**Results:** Levels of DNA Methylation, H3K9ac and H3K4me3 in BCB + group was significantly higher (89.19±1.49), (90.08±2.18), (98.53±2.62) in compare to the BCB- group (81.95±1.27), (78.73±2.16), (80.32±3.82). The percentage of Parthenogenetic blastocyst development was higher in BCB+ and was significantly different between BCB+ (39.25±9.03) and BCB- (23±7.48) oocytes. Total cell number, TE cell number, ICM cell number: TE ratio of blastocysts were significantly higher in the BCB+ group compared with the BCB- group.
**Conclusion:** Therefore, the BCB test is a useful method for selecting more competent immature sheep oocytes and it made improve Scnt method.
***Keywords:*** Brilliant Crysel Blue, Quality Oocyte, Epigenetics, Parthenogenetic

## P-82: Effect of SCNT Steps on Relative mRNA Abundances of Sheep Oocytes

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**Background:** The oocyte is a unique cell committed to reprogram fertilizing sperm and to support early stages of embryonic development until the species-specific stage of zygote genome activation that occurs around the second to third cell cycle in sheep embryos. In this sense, considering the huge list of oocyte transcripts, we selected some candidates genes based on their roles of regulating different cell functions.
**Materials and Methods:** The effect of zona removal and two main steps of SCNT (enucleation and nuclear transfer) on relative abundances of eight developmentally important genes (CCNB1, POU5F1, PAP, CX43, GMNN, NPM2, ATP1A1, and HSP90) and two housekeeping genes (GAPDH and ACTB) were studied and compared with the transcript profile of zona-intact oocytes. For this purpose, 30 minutes after zona removal, enucleation, and nuclear transfer, oocytes were used for quantitative realtime PCR.
**Results:** For all genes assessed, neither zona removal nor enucleation significantly affected the relative abundances of transcripts, except for RNA abundance of CCNB1, which was significantly reduced following enucleation compared to zona-intact oocytes. However,transcript abundances of CCNB1, POU5F1, NPM2, GMMN, and CX43 were all significantly reduced after electrofusion of enucleated oocytes with the nuclei donor cells. Unlike other genes, the reduction in abundances of PAP, ATP1A1, and HSP90 was not affected by the SCNT steps.
**Conclusion:** The RNA contents that were just affected by the reconstruction procedure were NPM2, POU5F1, CX43, and GMNN, which are involved in pluripotency, gap junction communication and regulation mechanisms, DNA replication, and reprogramming during early embryogenesis, respectively. These results suggested that SCNT steps have determining effects on oocyte transcripts of the reconstituted oocytes compared to intact counterparts.
***Keywords:*** SCNT, Sheep Oocyte, Relative mRNA

## P-83: Cloned Sheep Blastocysts Derived from Oocytes Enucleated Manually Using A Pulled Pasteur Pipette

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**Background:** The potential applications of a simplified method of somatic cell nuclear transfer (SCNT) that is improved in both efficiency and throughput is considerable. Technically, a major step of SCNT is to produce large pools of enucleated oocytes (cytoplasts) efficiently, a process that requires considerable micromanipulation skill and expensive equipment. Here, we have devel-

oped an efficient and high-throughput method of manual oocyte enucleation using a simple device, a pulled Pasteur pipette, that can be connected to standard zona-free method of embryo reconstitution.

**Materials and Methods:** Common Pasteur pipettes were pulled on a flame to produce finely drawn pipettes with inner diameters approximately less than half the oocyte diameter (*50-60 lm), and slightly larger than cytoplasmic protrusion (*20-30 lm) that was induced after demecolcine treatment of MII-stage oocytes. Oocyte manipulation was performed under a stereomicroscope by either bisecting the oocyte into two approximately equal demioocytes (blind manual enucleation), or by positioning the oocytes so that the cytoplasmic extrusion that contains the MII chromosome mass is removed with the minimum amount of cytoplasm (oriented manual enucleation).

**Results:** The survival rate of the manually enucleated oocytes was 81.4-91.5%, comparable to standard zona-free method of oocyte enucleation (>95%). A total of 80-120 oocytes could be enucleated in 10 minutes, which was considerably higher than standard zona-free enucleation method. *In vitro* development rates of cloned embryos derived from manually enucleated cytoplasts with varying cytoplasmic volumes (50, 95, and 100%) was comparable, and embryonic developmental rates of the two latter groups were at least as good as standard zona-free method.

**Conclusion:** The manual method of oocyte enucleation described here can be learned and mastered for simple, fast, and cheap production of cloned embryos with comparable efficiency to other available methods

***Keywords:*** Sheep, Enucleated Manually, Pulled Pasteur Pipette

## P-84: Protective Effect of Black Grapes Seed Hydroalcholic Extract Against Fluoxetine-Induced Embryo Toxicity in Male Mice

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**Background:** Fluoxetine (FLX) is a selective serotonin reuptake inhibitors antidepressant. FLX affects chemicals in the brain that may become unbalanced and cause depression, panic, anxiety, or obsessive-compulsive symptoms. Black Grape seed extract (BGSE) antioxidants neutralize free radicals and oxidative stress. Therefore this study was designed to investigate Protective effect of RGSE hydroalcholic extract against fluoxetine -induced damage on embryo development.

**Materials and Methods:** To follow up present study 24 mature male mice were used. The animals divided into four groups as, control, FLX (20 mg/kg), FLX (20 mg/kg) + BGSE (100 mg/kg) and BGSE (100 mg/kg) orally for 42 days. The caudae epididymides spermatozoa were obtained on day 42 in all groups. Superovulation was induced in 7-8 weeks-old female mice by intraperitoneal injection of 10 IU PMSG followed by intraperitoneal injection of 10 IU HCG 48 hours later. $1\times10^6$ sperm /ml HTF+ 4mg/kg BSA medium add to fertilization medium containing 10 to 15 oocytes. Fertilized oocytes or zygotes were evaluated. 24 hours after the zygotes culture, the two cell embryos rate was assessed and *in vitro* embryonic development was evaluated.

**Results:** The results showed that in FLX group percentage of zygote, Blactocyst as well as hatching embryos significantly ($p<0.05$) reduced in comparison with the control, BGSE and FLX+ BGSE groups. There was no significant value between groups in two cell embryo percentage.

**Conclusion:** BGSE extract caused partially improvement in all above mentioned embryos parameters. Therefore, grape seed extract can have positive effects on male fertility.

***Keywords:*** Fluoxetine, BGSE, Mice, Blactocyst

## P-85: Detrimental Effects of High Concentration Taurine on Sperm Motility and Viability

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**Background:** Taurine regulates an unusual number of biological phenomena,including heart rhythm, contractile function, blood pressure, platelet aggregation, neuronal excitability and body temperature. It appears to be a sperm motility factor (SMF), although the mechanism that maintains motility has apparently not been elucidated. It may act by alterations in either ion transport (osmoregulation) or protein phosphorylation or it may be decrease lipid peroxidation. In this syudy, we investigated the effects of various concentrations of taurine on *in vitro* sperm motility over time.

**Materials and Methods:** This study was performed on 6 lamb testis which gathered from abattoir. Semen samples were collected from epididymis and mixed with S-TALP medium + 5 mg/ml V fraction of Bovine Serum Albumin. Before and after 120 minutes incubation at 37 ºC and 5 % $CO_2$, sperm motility parameters were measured by CASA (Hooshmand Fanavar, Iran) in the presence of 0.05, 0.2 and 0.4 M taurine.

**Results:** Our result showed that 0.2 M concentration of Taurine cause increase in sperm motility, although this effect was not significant ($p>0.05$). In 0.4 M concentration of taurine, class A of sperm motility significantly decreased ($4.44 \pm 0.72$ vs $35.68 \pm 7.4$ for Taurine and control group respectively $p<0.01$) and class C motility significantly increased ($11.24 \pm 1.25$ vs. $4.58 \pm 1.49$ for

Taurine and control group respectiely p<0.01).

**Conclusion:** Present study demonstrated that although taurine could improve sperm motility parameters, higher concentration of this compound (0.4 M) had detrimental effects on sperm motility and viability.

***Keywords:*** Sperm, Motility, Taurine

## P-86: Determining The Most Oocyte Challenging Step during Vitrification-Warming in Sheep

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**Background:** Oocyte cryopreservation is of utmost importance in the current repertories of assisted reproductive technology (ART). Despite this, oocyte vitrification is yet a problematic issue with limited successful pregnancies has been arisen after transfer of embryos derived from vitrified-warmed oocytes. In this sense, great attention has been paid to investigate possible mechanisms underlying low developmental competence of vitrified-warmed oocytes. In this study, we provide a sequential and detailed observation on early and delayed responses of *in vitro* matured sheep oocytes to different steps involved in a routine oocyte vitrification process to define the most critical stage of vitrification-warming process and to clarify the essentiality of post-warming interval.

**Materials and Methods:** This study was carried out to investigate how different steps of a common vitrification-warming process affect the early (immediately after warming) and delayed (2hours after warming) aspects of the treated oocytes including; volume changes, microtubule and chromosome organization, distribution of cortical granules (CGs), mRNA abundances, ultrastructure, and *in vitro* embryo development.

**Results:** Cellular, molecular and ultrastructural results indicated that the first moments of oocyte exposure to cryoprotectants (CPAs) are the most challenging stage with comparable features to oocytes endured a complete process of oocyte vitrification-warming. However, developmental competence of oocytes exposed only to CPAs in response to parthenogenetic activation (PA), *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI) were quite comparable to control oocytes. Whereas, developmental competence of vitrified-warmed oocytes dramatically decreased compared to control oocytes, and only immediately activated (PA) oocytes had reasonable competency for development compared to control oocytes. Time-dependent assessment of CPAs and cryo -shocks indicated that although some of the injuries are reversible, the overall advantages and disadvantages of this time rest are not in favor of the oocyte developmental competence.

**Conclusion:** The first set of cellular, molecular and ultrastructural evidences obtained in this study provided some indications to suggest that the most challenging step of current routine process of vitrification-warming maybe the first step in which oocyte is equilibrated in a mix solution of DMSO and EG cryoprotectants (ES step).

***Keywords:*** Oocyte, Vitrification, Cryoprotectants, Cryo-shocks, Embryo Development

## P-87: High Fat Diet Affects *In Vitro* Maturation and *In Vitro* Fertilization of Mouse Oocytes

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**Background:** Physiological effects of obesity are increasingly common in women of reproductive age and are associated with infertility. Obese women have dysfunction in hypothalamic-pituitary-ovarian axis, menstrual cycle and are up to three times more likely to have decreased oligo-/anovulation. The purpose of present study was to evaluate the high fat diet on IVM and IVF outcomes in mouse.

**Materials and Methods:** Balb-c mice (21 days of age, n=40) were divided into control and high fat diet groups. Experimental group fed with high fat diet (60% fat) during 12 weeks. After 12 weeks the oocytes obtained and *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) were done according to routine protocols.

**Results:** The results showed significantly decrease in number of oocytes obtained at GV stage in high fat diet group and had weaker expansion as well as fewer oocytes reached to metaphase II stage. IVF results showed that number of MII oocytes in HFD group decreased significantly compared to control group and 30% of them reached to two-cell embryo.

**Conclusion:** Our Findings show that high fat diet causes a significant reduction in differentiated oocytes from GV stage to methaphase II and also reduce the number of two-cell embryos in balb -c mices. It is suggested that in obese women with high body mass index maturation of oocyte and pregnancy rate is lower than normal ones.

***Keywords:*** IVM, IVF, HFD, Infertillity

## P-88: Assessing Expression Changes of Some Wnt Pathway Genes During Goat Early Embryonic Development

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**Background:** The developmental competency of embryos is affected by several factors, including the developmental pathways and their elements. In mammalian species including goat, fertilized oocyte undergoes several divisions to form a structure called blastocyst. These events depend on the successful control of temporal and spatial expression of genes involved in genome activation. One of the critical mediator signaling events during embryogenesis is Wnt signaling pathway. This pathway possesses large number of proteins that can control and regulate the production of Wnt signals. A growing body of evidence suggests that, there is a correlation between the appropriate regulation of Wnt signaling and proper embryonic development.

**Materials and Methods:** In order to assess the mRNA expression of Frizzled, β-catenin and C-myc gene in caprine embryos, *in vitro* matured caprine oocytes were chemically activated and Day 3, 7 and 14 embryos and also MII stage oocyte were used for real time PCR.

**Results:** Frizzled and c-myc expression in D3 increased dramatically compared to other stage, however, after this stage is accompanied by a sharp decline in expression. But for β-catenin highest expression levels can be seen in the oocyte stage and after that until D14 is facing a decline.

**Conclusion:** Base on literature review, it was expected that pattern of changes for β-catenin gene was correlate with the two other genes but it was not, then we suggested, wnt pathway in Capra species maybe non-canonical pathway.

***Keywords:*** Wnt Pathway, Gene Expression, β-catenin

## P-89: Effect of Ruta Graveolens Aqueous Extract on Embryo Development in Mouse

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**Background:** The purpose of this study was to evaluate the effect of Ruta graveolens (RG) Aqueous Extract on *in vitro* fertilization (IVF) rate and embryo development. RG, commonly called sudab or rue, has been known as a medical plant since ancient times. Recently some medicinal properties were reported for RG such as: antioxidant, anti-inflammatory, anti-tumor, anti-androgenic, anti- conceptive and anti-fertility activity. Flavonoids, rutin, quercetin, furocoumarin and lemonins are major active constituents in RG.

**Materials and Methods:** 20 adult female mice were divided into four groups as control, control-sham and tests. Control-sham group received saline normal 0.2 ml per day. The test group was subdivided into two groups of high dose (300 mg/kg) and low dose (150 mg/kg) and received the aqueous extract of RG daily, orally for 14 days. 48 hours after PMSG injection, Germinal vesicle (GV) stage oocytes were collected from mentioned groups. The GV oocytes were dissected out of the ovary and put into a plastic dish containing α-MEM medium. After 12 to 24 hours of incubation, matured oocytes collected and put into *in vitro* fertilization medium. And then capacitated sperm were added to fertilization drop, containing mature oocytes. Fertilized oocytes and embryo development were evaluated in next 5 days.

**Results:** The results for *in vitro* fertilization of the RG group animals were remarkably lower than the controls group. The considerable point was that most of the 2-cell embryos had stopped division and did not continue to four and/or more cells embryos in test groups.

**Conclusion:** RG was able to induce embryo toxicity and can induce infertility.

***Keywords:*** *In Vitro* Fertilization, Mice, Ruta Graveolens

## P-90: Ascorbic Acid Effects on *In Vitro* Fertilization of Ovine Oocyte with or without Cumulus Cells

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**Background:** *In vitro* maturation and fertilization are techniques to increase efficiency of mammals reproduction. However, *in vitro* culture conditions exert an imbalance between oxidants and antioxidants which may reduce live birth and pregnancy rates when compare to *in vivo* conditions.

**Materials and Methods:** The present study was conducted as a part of the ovine IVF program organized by University of Tehran, Department of Animal Science between the 16th April and the 24th of June (Spring), 2012. About 250 ovaries were obtained at a local abattoir in Karaj city, Iran, from the slaughtered animals. Ovarian follicles, 2-6 mm in diameter were aspirated and collected ina pre-incubated Hepes-modified TCM199 medium. The oocytes were allocated to 2 groups, cumulus-oocyte complexes (COCs) and denuded oocytes (DOs). Each 20 oocytes were cultured in a drop of IVM medium (200 _mL) and different levels of ascorbicacid (0, 150, 250 and 300 μM), covered with mineral oil for 24 hours, at 37.5°C in 5% $CO_2$ in air and 85% humidity. mature oocytes were washed three times in IVF medium. Thereafter, 20 matured oocytes were transferred to 200 ml of the already incubated IVF medium droplets. Then, sperm suspension was finally added to the each fertilization drop to obtain a final concentration of ≈$1\times10^6$ sperm/ml. The gametes were cultured under mineral oil for 18 hours at 38.5°C in 5% $CO_2$ humidified air.

**Results:** Interestingly, the fertilization rate of denuded oocytes that 250 and 300 μM ascorbic acid concentrations supplemented to their maturation culture medium was significantly lower than cumulus-oocyte complex group ($p<0.05$).

**Conclusion:** This may express the pro-oxidant effects of high concentrations of ascorbic acid on ovine oocytes fertilization.

***Keywords:*** *In Vitro* Maturation, *In Vitro* Fertilization, Asorbic Acid, Cumulus Cells, Ovine Oocyte

## P-91: Phenyl Hydrazine-Induced Anemia-Related Hypoxia Effect on Mice Testes Parameters: Implication for Protective Effect of Crocin

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**Background:** There are reports indicating that among the others, hypoxia is one of the testes threatening factors, which may occur intentionally or accidentally. Hypoxia could be created secondarily after some pathological conditions such as anemia. In this study we aimed to investigate the crocin protective effect on the testes parameters in the animals that were exposed against phenyl hydrazine to induce anemia-dependent hypoxia.
**Materials and Methods:** Forty nine male and clinically healthy mice (20-25 g and 6-8 weeks old) were divided to 7 groups including control and test groups. The anemia-dependent hypoxia was induced by using Phenyl hydrazine. The control (C) animals were received only saline normal during the experiment and the test groups were treated as follow: Phenyl hydrazine (PHN, 2 mg/100 g, 4mg/100g and 6 mg/100g) via IP.Three other groups revived crocin 40 mg/kg/day via IP. PHN was administered each 48 hours for 35 days. At the end of experiment testes were fixed in 10% formal saline and after tissue processing and staining with H&E method, samples were studied histomrphometricaly including capsule thickness, tubular diameter, thickness of the germinal epithelium, Sertoli and leydig cells number, tubular differentiation index (TDI), republication index(RI) and spermiogenesis index (SI) were investigated.
**Results:** The results of this study showed that anemia-related hypoxia resulted in a drastic reduction in germinal epithelium, Sertoli and leydig cells number, TDI, RI and SI, and Capsule thickness and tubular diameter did not change. While those groups which were treated with CRN showed a remarkable improvement in testes parameters.
**Conclusion:** These results indicate a protective effect of CRN against the anemia-related hypoxia on testes. The protective capacity of CRN might relate to its known antioxidant properties.
*Keywords:* Hemolytic Anemia, Hypoxia, Phenyl Hydrazine, Crocin, Testes Morphometry

## P-92: An Experiment on Osmotic Behavior of The Goat Epididymal Sperm Following Exposure to Different Cryoprotectants above Zero Temaperature

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**Background:** Sperm is known as an osmo-sensitive cell in biological studies. The studies showed the good response of human sperm to cryobiologic assays. However, in farm animals because of different composition of the cell membrane and presence of more unsaturated fatty acid make sperm more sensitive to oxidative stress that is one the most important factors that can affect the sperm viability during cryopreservation. The aim of the present study was to find the effects of different cryoprotectants on the goat epididymal sperm total motility.
**Materials and Methods:** The goat testes-epididymides were collected (6 pairs) and epididymal sperm retrieved in warmed TALP (5 mL) for 15 minutes. The sperm suspension was homogenized and 200 µL was overlaid with TALP within 1.5 mL Eppendorph micro-tube and left in room temperature (25-33 C) for 30-45 minutes. The swimmed-up sperm were collected from upper layer of micro-tube. Suspension of sperm adjusted with concentration of $20\times10^6$ sperm /mL with different cryoprotectant. The cryoprotectant solutions were provided in double distilled water (DW) or TALP. The solution composed of Ethylene glycol (EG 1 and 5 %), Glycerol (Gly 1%) and different molarities of sucrose (0, 0.2, 0.4 and 0.6 M). The combinations of EG2.5 and Gly2.5 with 0.2 M sucrose also were considered. A combination of EG5 with 0.4 M sucrose was assessed, too. After 7-10 minutes of exposure, total motility of sperm was evaluated on a warmed slide under cover-slip. The proportion of total estimated motility after exposure to before exposure was calculated and analyzed using GLM procedure in SAS.
**Results:** The results showed that basic medium for cryoprotectant can be DW (54.7 ± 4.52) or TALP (49.7 ± 6.52 %) with no significant difference (p=0.53). A linear negative tendency between sucrose osmolaity and sperm motility was detected; after 10 min exposure in sucrose alone no motile sperm was seen. After 7 min exposure, the rates of estimated motile sperm were the highest in solutions composed of EG2.5+S0.2, Gly2.5+S0.2 and S0.2 alone. The highest concentration of EG, 5%, with (43.3 ± 11.2) or without (30 ± 11.2) S (0.4 M) significantly decreased rate of total sperm motility. The highest concentration of sucrose stopped motile sperm during assay (0%).
**Conclusion:** The results of the present study showed a linear toxic effect of sucrose on goat epididymal sperm and also showed that incorporation of the permeable cryoprotectants with low levels of sucrose can be an option for diluting epididymal sperm for above zero temperature.
*Keywords:* Osmotic Behavior, Goat, Epididymal Sperm

## P-93: Comparison of TRIS and TALP in Reversibility of The Goat Epididymal Sperm Motility Following Exposure to Different Sucrose Concentrations

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**Background:** Nowadays, cryoprotectant-free vitrifica-

tion of human sperm in large or small volumes facilitated storage and use of human sperm for long time. However, this procedure was not successful in farm animals. The main finding in the studies is higher sensitivity of these kinds of sperm to sucrose osmolarities. Preliminary studies showed a reversible effect of sucrose on goat epididymal sperm motility. However, higher concentrartions of sucrose are toxic for goat epididymal sperm. The aim of the present study is to compare recovery rate after of sucrose exposed-goat epididymal sperm in two different media (TALP or TRIS).

**Materials and Methods:** The goat testes-epididymides were collected (4 pairs) and epididymal sperm retrieved in warmed (5 mL) basic media (TALP or TRIS (TRIS 24 mg/mL; citric acid 10 mg/mL; Glocose 8 mg/mL; genetamycine 1mg/mL and 1% (w/v) BSA; pH=6.8-7.2)) for 15 min. The sperm suspension was homogenized and an aliquot of 200 µL was overlaid with the basic media within 1.5 mL Eppendorph micro-tube and left in room temperature (25-33 C) for 30-45 minutes. The swimmed-up sperm were collected from upper layer of micro-tube. Suspension of sperm adjusted with concentration of $20\times10^6$ sperm /mL with different concentrations of sucrose (0, 0.1, 0.2 and 0.4 M in distilled water). After 30 minutes of incubation the equal volumes of the basic media added to the tubes, to reduce sucrose concentrations, and followed 60 min for sperm total motility. The proportion of total estimated motility after dilution of sperm suspension to before exposure was calculated and analyzed using GLM procedure in SAS.

**Results:** The results showed that sucrose exposed sperm recovered its motility (p=0.024) after withdrawing sucrose in TRIS (0.9 ± 0.03) than TALP (1.03 ± 0.03). The rate of sperm motility that recovered was highest in Sperm that exposed to 0.4 M sucrose; however, the value of sperm motility in 0.4 M sucrose (60 ± 1.73 %) after recovery was not elevated compared to other groups (p<0.0001). The proportion of sperm that exposed to 0.4 M sucrose more recovered their motility (p<0.0001) in TRIS (1.3 ± 0.06) than TALP (0.83±0.06).

**Conclusion:** The results of the present study showed impact of sucrose high concentration on the goat epididymal sperm motility that can be recovered in TRIS better than TALP.

***Keywords:*** TRIS, TALP, Epididymal Sperm Motility, Sucrose

## P-94: The Effect of Superovulation on Oviduct and Ovary Structure of NMRI Mice

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**Background:** To determine the effect of ovarian stimulation on the height of the epithelial cells of fallopian tubes and development of ovarian follicles and corpus luteum in NMRI mice.

**Materials and Methods:** Forty female NMRI mice of 6 to 8 weeks were selected and divided into 4 groups: control group, 10 mice that were injected with saline. The second group of 10 mice that were injected with 7 uI PMSG. The third group 10 mice that were injected with 10 UI PMSG. Fourth group of 10 mice that were injected with 12 UI PMSG. 48 hours after injection of PMSG, HCG was injected intraperitoneally. 24 hours after the injection of HCG, the mice were killed by cervical dislocation and their ovaries removed and studied by light microscopy, were stained with H & E. And the height of the epithelial cells of the fallopian tube, ovary size, number and size of follicles and corpus luteum in the four groups were compared.

**Results:** In group 2 (injected with 7 UI) there was no significant change in the number of follicles, size and number of the corpus luteum and ovary size compare with control group. Also there was no significant change in size multilayer primary (preantral) and secondary (antral) follicles compared with control group. But the size of the graafian follicle increased than the control group. The size of unilayer primary follicles was significantly lower than the control group. In group 3 (injected with 10 uI) number of multilayer primary follicles (preantral) was increased compared to control. But number of other follicles, ovarian size and graafian follicle size was no significant change. Increased number and decreased size of corpus luteum compared with group 2 and control. Decreased size of unilayer follicle compared with group 2. Increased size of secondry follicles compared with group 2 and control. In group 4 (injected with 12 uI) ovarian size significantly increased as compared to groups 1 and 3. Increased number of the corpus luteum compared with group 2 and control.Increased multilayer primary (preantral) and graaphian follicles compared to other groups. The size of the unilayer primary follicle was significantly decreased compared to control. Secondary follicle size was significantly increased compared with group 2. Graafian follicle size was significantly decreased compared to group 2.

**Conclusion:** The findings of this study indicate that PMSG and hCG hormone even with increased dose has no effect on the number of unilayer primary follicles, but their size is reduced. But the basic multi-layered follicles (preantral), increasing the gonadotropin dose, increase their number, but they have no influence on their size. Increased gonadotropin dose increased Secondary or antral follicles size but has no effect on their numbers. Increase the number of Graaphian follicles, occurs only in high doses but reduces the size of the Graaphian follicle while low dose increasing the size of the Graaphian follicle. High doses may cause negative effects on the follicle size, that needs to be more investigated. The study also showed that increasing the dose of hormones, reduces the size and increasing the number of the corpus luteum . Ovarian size is increased only in the high dose and low dose did not affect the size of the ovary. Another result of this study is reducing the height of the oviductal epithelium with PMSG and hCG hormone stimulates ovulation and if the hormone level increase, the height of the epithelium reduced.

***Keywords:*** Superovulation, Oviduct, Ovarian Follicle, Corpus Luteum

## P-95: Effect of Nano Silver on Nitric Oxide Production in Chicken's Sertoli Cells

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**Background:** Chicken sertoli cells are a model to evaluate the effects of toxic Materialss. These cells can actively proliferate in the *in vitro* conditions and they are able to passage several times. Because of the widespread use of nanosilver in life and also few studies on the effects of these Materialss on different cell lines, evaluation of its toxic effects is felt. Mechanism of nanosilver toxicity is not completely obvious; there are only some reports of its harmful effects. In the present study, an attempt has been done to study the effect of nanosilver on the chicken Sertoli cells in nitric oxide production, lipid per oxidation and antioxidant power. Nitric oxide has a dual role; in low concentrations is considered as a scavenger agent while in high concentrations acts as a free radical.
**Materials and Methods:** Rooster sertoli cells were isolated and cultured, then were exposed to different doses of nanosilver (25, 75 and125 µg/ml). After 48 hours, cells were counted and levels of nitric oxide, TBARS and FRAP were examined.
**Results:** The results indicated that with increasing of nanosilver concentrations, the percentage of living cells was reduced. This reduction of living cells in concentrations of 125 and 75 µg/ml of nanosilver, was significant ($p < 0.05$). In nitric oxide measurement, there was a significant reduction in concentrations of 125 µg/ml of nanosilver with compared to control. It is likely that nitric oxide acted as a scavenger agent. TBARS level was significantly ($p<0.05$) increased with compared to control.
**Conclusion:** Studies indicated that the antioxidant power was increased but no significant difference was shown. Taken together, it can be noted that large amount of nanosilver induce its toxic effects by producing oxidant compounds in sertoli cells. Nanosilver had no increasing effect on nitric oxide production or possibly oxidants compounds neutralize high concentrations of nitric oxide.
***Keywords:*** Nanosilver, Chicken Sertoli Cell, Nitric Oxide

## P-96: Survey of *In Vitro* Effect of Noscapine on Nitric Oxide Secretion of Human Endometrial Stromal Cell

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**Background:** Noscapine is cough suppressant drug with cell inhibitory effect. It is introduced for anti-cancer treatment. The aim of present study was to determine *in vitro* noscapine effect on nitric oxide (NO) secretion by human endometrial stromal cells.
**Materials and Methods:** Human endometrial biopsies (n=8) were obtained in sterile condition and were chopped mechanically and digested by enzymatic (collagenase, 2 mg/ml) methods. Stromal cells were harvested after using cell strainer (70 and 100 µm) and centrifugation. The cells were divided into four groups: control, 10, 25, 50 and 100 µM noscapine concentration for three culture periods (24, 48, 72 hours), Nitric oxide secretion were assessed by Geriss assay. Data was analyzed by one way ANOVA and $p< 0.05$ was considered significant.
**Results:** Difference between NO amount were significant in control, 10, 25, 50 and 100 µM noscapine concentration were 35.14, 33.79, 20.7, 19.69 and 18.75 µM at 24-hour culture period ($p=.009$), 56.22, 53.54,26.84. 25.03 and 22.35 µM at 48-hour($p=0.01$), and 73.99, 63.42, 33.33, 32.77 and 30.6 µM at 72-hour ($p=0.037$) respectively.
**Conclusion:** Noscapine decreased endometrial stromal cells NO secretion in dose dependant manner.
***Keywords:*** Noscapine, Stromal Cells, Nitric Oxide, Cell Culture

## P-97: Optimized Combined Electrical-Chemical Parthenogenetic Activation for Zona Free Sheep Oocyte

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**Background:** Artificial oocyte activation (AOA) has important implications in assisted reproductive technique (ART) with particular importance in somatic cell nuclear transfer (SCNT). Parthenogenesis activation using zona-free oocytes offers an alternative model that could be applied to develop protocols for the activation of reconstructed embryos for cloning.
**Materials and Methods:** To establish the reliable combined electrical-chemical activation for zona-free oocytes,oocytes were first exposed to an electrical pulse and then treated with 18 chemical activation regimens designed through modifying duration (5, 2.5, 1 minutes) and concentration of ionomycin (5, 2.5, 1 µM) and 6-dimethyl aminopurine (6-DMAP) (2 mM for 2 or 4 hours), which is routinely used for SCNT.
**Results:** Maximum parthenogenetic blastocyst development was obtained when oocytes were submitted to electric pulse and then to (1) 5 µM ionomycin for 5 or 2.5 minutes, both followed by 2 hours of incubation with 6-DMAP (41.7 - 1.1, and 42.4 - 1.4%, respectively), (2) 5 µM ionomycin for 1min + 6-DMAP for 4 hours (43.1 - 1.4%), and (3) 2.5 µM ionomycin for 1min + 6-DMAP for 2 hours (42.4 - 1.4%), with significant differences compared to all the other groups.
**Conclusion:** Statistical assessment of interactions between duration and concentration of ionomycin and duration of 6-DMAP exposure revealed that (1) concentration of ionomycin may be a more important factor than

its duration, (2) both a long exposure period and a low concentration of ionomycin had marked decreasing effects on parthenogenetic development of zona-free oocytes, and (3) high duration of exposure to 6-DMAP can reduce parthenogenetic development.

***Keywords:*** Zona Free Oocyte, Sheep, Parthenogenetic Activation

## P-98: Ultrastructural Study of Basement Membrane of Seminiferous Tubules in Adult Diabetic Rats with Spermatogenic Alterations

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**Background:** The complications of diabetes are the major problems that occur in diabetic patients. It has been reported that, insulin insufficiency and the impairment of regulatory action of this hormone on Leydig and Sertoli cells have an important role in testicular alterations. Testicular disorders and change in normal spermatogenesis is one of the consequences of diabetes. This study was designed to find the relationship between structural alterations of basement membrane of seminiferous tubules and impairment of normal spermatogenesis.

**Materials and Methods:** Induction of experimental diabetes was done by intraperitoneal administration of Streptozotocin (45 mg/kg BW). In control group, sodium citrate was administrated with the same volume of Streptozotocin. At the end of 70 days, the animals were scarified; the testicles were separated and processed for electron microscopic studies.

**Results:** Ultrastructural studies showed that the thickness of basement membrane of seminiferous tubules was increased in diabetic group in comparison to control rats. Moreover, the amount of collagen fibers increased between basement membrane and myocytes in diabetic group. These morphologic changes, was observed more in basal compartment of Sertoli cells compared to other areas of seminiferous tubules.

**Conclusion:** The results study showed that, the structural changes of basement membrane of testicular seminiferous tubules in chronic diabetes, lead to alteration in normal spermatogenesis by affecting the activity of Sertoli cells and transport of Materialss.

***Keywords:*** Basement Membrane, Diabetes, Rat, Seminiferous Tubules, Ultrastructure

## P-99: Survey of Noscapine Effect on Apoptosis of Human Endometrial Stromal Cells: An *In Vitro* Study

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**Background:** Noscapine is a water soluble alkaloid with antitussive property and very less toxicity. It is obtained from the Papaveraceae family. Recently, new pharmacological properties such as anticancer, anti stroke and anti-angiogenic effects are introduced. There is no scientific report on noscapine effect on reproductive system. The aim of present study was to determine *in vitro* effect of noscapine on apoptosis of endometrial stromal cells in *in vitro* culture.

**Materials and Methods:** In this experimental study, normal endometrial biopsy (n=8) were collected in sterile condition and chopped by sterile blade. Stromal cells were isolated after collagenase digestion (2mg/ml); cell filtration through filter mesh and ficoll layering. The cells were divided into 5 groups; control, 10, 25, 50 and 100 µM noscapine concentrations, and were cultured for 48 hours in DMEM/F12 containing FBS (5%) and antibiotics. Cell apoptosis was determined by TUNEL Staining . Data was analyzed by one way-ANOVA test and $p< 0.05$ was significant.

**Results:** Endometrial stromal cell apoptosis were 2.32, 3.19, 3.7, 6.15, 8.49%, in control, 10, 25, 50 and 100 µM of noscapine concentration respectively. The difference between groups was significant ($p= 0.004$).

**Conclusion:** Stromal cell apoptosis were increased by noscapine in a dose dependent manner.

***Keywords:*** Endometrial Stromal Cell, Noscapine, Apoptosis, Cell Culture

## P-100: Determination of The Proper Pregnancy Age for Collection and Culture of Guinea Pig Fetal Fibroblast

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**Background:** Fibroblast cell lines have different application in biological research including nuclear transfer in cloning, feeder layer of embryonic stem cells, wound healing research and tissue engineering. The objective of the present study was to determine the proper pregnancy age for collecting and culturing guinea pig fetal fibroblast.

**Materials and Methods:** Guinea pig fetuses were collected after determination of their age (day 20, 30 and 45 of pregnancy) by measuring the fetal biparietal diameter using a real-time B-mode ultrasound scanner (SIUI V900, China) equipped with a 7.5 MHz, linear-array transducer. Skin of fetuses was cut into small pieces (1 $mm^2$) and cultured in 88% Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), 1% penicillin and streptomycin, 1% L-glutamine in 25 $cm^2$ flasks. When the cell cultures reached 80-90% confluence, they were passaged. The time intervals between the first and second passages in three different ages were determined.

**Results:** The time interval between the first and second passages for guinea pig fetal fibroblasts were 30 days for day 20 pregnancy, 10 days for day 30 pregnancy, and 15 days for day 45 pregnancy.
**Conclusion:** The proper pregnancy age for collection and culture of guinea pig fetal fibroblast was at the age of day 30 of pregnancy which made the shortest interval for reaching the maximum confluence in the fibroblast cell culture.
*Keywords:* Fibroblast, Fetus, Ultrasound Scanner, Guinea Pig

## P-101: Study of The Effect of Alkaline Medium on Separation of Spermatozoa Containing X and Y Chromosomes in order to Sex Selection

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**Background:** Sex Selection in livestock happens in order to increase productivity and it also occurs in human society to avoid genetic disease related to gender and culture, social and religious trends. By separating spermatozoa carrying X and Y chromosomes from each other, we could achieve a desire gender by using assisted productive techniques. In this study the effect of alkaline environment were examined on the speed of spermatozoa carrying X and Y chromosomes
**Materials and Methods:** A number of 60 mice (NMRI) were divided in to a control group and three experimental groups. Female mice stimulated by injecting PMSG matter to supper ovulation. Oocyte retrieved under sterile condition from oviduct. Spermatozoa provided from cauda epididymis and vas deferan and entered to media by pH(7.5, 7.6, 7.7). The faster spermatozoa were used for IVF. The embryo in control and experimental groups were transferred to foster mother(Pseudo pregnant) and the female and male infants were counted after birth. Analysis of data has been done with the help of spss, Anova software.
**Results:** The proportion of male and female in control group(pH=7.3), experimental group 1 (pH=7.5) and experimental group2 (pH=7.6) and experimental group3 (pH=7.7) was (47.98),(64.75), (65.41), (67.31) (Mean±SD) respectively. The comparison of proportion of male to female infant between control group and experimental groups showed significant differences ($p < 0.05$). But there was not significant difference among experimental groups.
**Conclusion:** With respect of results of this study, it is appear that by increasing the pH of media up to 7.7, the speed of spermatozoa carrying Y chromosom will increase. Therfore, by increase of pH of spermatozoa media, the chance of male infant birth will increase.
*Keywords:* Sex Selection, IVF, PMSG, PH

## P-102: Effect of Vitrification in Open Pulled Straw Vitrification of Live Birth Rates, Fertility and The Development of Mouse Oocytes at Metaphase II

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**Background:** The cryopreservation of human oocyte would make significant contribution to fertility treatments. It could also provide on alternative to embryo preservation for avoidance ethical problems. Embryo cryopreservation is now a successful procedure, but oocyte cryopreservation has poorer results.
**Materials and Methods:** For retrieval of oocyte NMRI mouse were used. after administration of HMG and HCG, mature mouse oocyte were delivered from fallopian tube.800 mature oocyte were allocated in tree groups, 250 for verification in conventional straw(Cs),280 for verification in open pulled straw (ops), 270 as control group.
**Results:** Survival rate of oocyte in Cs was significantly lower than ops ($p=0.01$). After insemination, fertilization rate in oocyte was in Cs was significantly smaller than ops ($p=0.001$). There is significant difference in fertilization rate between ops and control group ($p=0.031$). After culturing, developmental capability in oocyte that were treated in ops significantly over than of oocyte who were treated in Cs ($p=0.001$)
**Conclusion:** Ops technology has provide better results from point of view of survival rate, fertilization and developmental capability than that's of Cs but use of this method as usual in IVF clinics remains collusive.
*Keywords:* Open Pulled Straw, Survival Rate, Oocyte, Verification

## P-103: Enhancement of Colonization on Mouse Spermatogonial Stem Cell by Low Intensity Ultrasound Stimulation

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**Background:** Spermatogonial stem cells (SSCs) are the foundation of spermatogenesis. New procedure such as sound wave especially low intensity ultrasound (LIUS) can be effective on increasing the number of cells. In this study we investigated the effect of LIUS stimulation on mouse SSCs.
**Materials and Methods:** Isolated SSCs from neonate mice cultured in DMEM culture medium with 10% fetal bovine serum (FBS). In the first phase of study, temperature controlled by LIUS stimulation of plate containing culture medium and in the second phase, SSCs stimulated by LIUS with 2 different Intensity dose (100 and 200 $mW/cm^2$) for one week and then SSCs colonization

assessed at 7th day.
**Results:** The LIUS treatment of mouse SSCs increased the number and diameter colonies of SSCs in experimental groups compared to the control group. Average number of colonies in 100, 200 mW/cm$^2$ and control group were 20 ± 1.1, 29 ± 1.7 and 10 ± 0.5, respectively. Average diameters of colonies in 100, 200 mW/cm$^2$ and control group were (209 ± 20) × (165 ± 10), (254 ± 33) × (146 ± 5) and (217 ± 48) × (148 ± 16), respectively. Our results showed that there was significant increase in number of colonies in experimental groups compared to control group (p≤0.05), whereas there were not significant differences between groups regarding to diameter of colonies.
**Conclusion:** These results suggested that LIUS treatment is an efficient and cost-effective method to improve proliferation and colonization of SSCs during *in vitro* culture.
***Keywords:*** Colonization, Mouse, Stem Cell, Ultrasound

## P-104: Embryo Sex Selection by Migration of Spermatozoa in Different Acidic pH Environment

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**Background:** Today factors including cultural, social, religious, economic and medical such as X Linked diseases encouraged couples to choose traditional methods or new treatment for selecting the gender of child. It seems that Spermatozoa containing chromosomes X and Y have different speed in various pH. In this study the velocity of spermatozoa in different acidic pH was evaluated.
**Materials and Methods:** A number of 60 mice (NMRI) were divided in a control group and three experimental groups. Female mice stimulated by injecting PMSG matter to super ovulation. Oocyte retrieved under sterile condition from oviduct. Spermatozoa provided from cauda epididymis and deferane and entered on media by pH 6.7، 6.8 and 6.9. The fastest spermatozoa were use for IVF. Produced embryo were transplanted to pseudo pregnant mother. The female and male infants were counted after birth. Analysis of data done with the help of SPSS, Anova Software.
**Results:** The proportion of female to male in control group; experimental group1(pH=6.7); experimental group 2 (pH=6.8); and experimental group 3 (pH=6.9) were (Mean ± SD) 49.54 ± 2.83, 66.20 ± 3.37, 65.76 ± 3.76, 63.31 ± 4.12 respectively. The comparison of mean of proportion of female to male infant in control group and experimental groups showed significant difference(p≤0.05). There was not significant difference between mean of proportion of female to male infant in experimental groups(p≥0.05). The Duncan and Tuky test were the same.
**Conclusion:** The results showed that the speed of spermatozoa X in acidic media is higher and this technique is cheap and accessible.
***Keywords:*** IVF-Sex Selection, PMSG, PH

## P-105: Gibberellic Acid Can Decrease *In Vitro* Fertilization Success Rate in Adult Male Rats

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**Background:** Enzymatic and histological change in the testicular cells of rats treated orally, intraperitoneally and intradermally for 45 days with Gibberellic acid (GBA) in independent studies is reported. This study was conducted to evaluate *in vitro* fertilization (IVF) rate and preimplantation embryonic development in GBA treated adult male rats.
**Materials and Methods:** Thirty six adult male Wistar rat was divided into four groups as following: 1. control group; 2. control-sham group; 3. Low-dose of GBA and 4. High dose of GBA. All injections were intraperitoneally (IP) and every other day. 0.5 CC 1% methanol, 2 mg/kg and 20 mg/kg body weight GBA was injected to groups 2, 3 and 4, respectively. To perform IVF, oocytes from healthy untreated female rats and sperm from mentioned groupswere collected on days 15, 30 and 45 of treatment (three rats for each day of each group) and mR1ECM medium was used.
**Results:** GBA had significantly (p<0.05) decreased oocyte fertilization rate and blastocyst stage embryos rate in day 45 of low dose and days 30 and 45 of high dose in comparison with groups 1 and 2. Also there was a significant (p<0.05) decrease in two-cell stage embryos rate in groups 3 and 4 on days 30 and 45compared to control and control-sham groups.
**Conclusion:** Intraperitoneal administration of GBA acid may have a deleterious effect on IVF success and preimplantation embryo development which may be dose-dependent and time-dependent.
***Keywords:*** Giberellic Acid, *In Vitro* Fertilization, Male Rat, Intraperitoneal

## P-106: Evaluatiny the Effectiveness of Vitamin C Protection on Testicular Germ Cell Apoptosis in Male Rat Treated with Formaldehyde

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**Background:** Formaldehyde is associated with overproduction of endogenous opioids (EOP), nitric oxide (NO) and cytokins in the blood streams. These consequences can affect sex hormones. Since proper fertility will result physiological balance of sex hormones so that we investigated the relationship between Formaldehyde and sex hormones and apoptotic germ cells in adult male rats.

**Materials and Methods:** To study, we used five groups of animals: (1) control; (2) FA treatment group 2-hour per day(FA2hours); (3) FA + Vc group 2-hour per day (FA2hours)+Vc. (4) FA 4-hour per day (FA4hours); and (5) FA+ Vc group 4-hour per day (FA2hours)+Vc. Treatment groups were exposed to FA by inhalation at a concentration of 10 mg/m3 for 18 weeks. In addition, FA+ Vc group were orally administered Vc during the 18-week FA treatment. After the treatment, all animal were killed by cervical dislocation, serum concentrations of FSH, LH and testosterone were determined by Radioimmunoassay respectively. Apoptosis was evaluated by DNA fragmentation detected by in situ terminal deoxynucloetidyl Transfrase-mediated dUTP nike end labeling (TUNEL).

**Results:** A significant reduced LH and testosterone in FA4hours group compared with those in the control group ($p<0.05$). There was not significant reduced FSH in FA group compared with control group. and not significant reduced FSH, LH and testosterone in FA+ Vc groups compared with control group. But Formaldehyde had no significant effect on apoptotic index germ cell testis ($p>0.05$).

**Conclusion:** These findings have shown Formaldehyde decreased the levels of serum gonadotropins but it has no significant effector on testicular germinal cells apoptosis. We suggest that testicular germinal cells apoptosis were not only dependent on gonadotropin hormones but also other factors may be involved.

***Keywords:*** Formaldehyde, Gonadotropins, Apoptosis

## P-107: Vitrification and *In Vitro* Culture of Mouse Preantral Follicles in Presence of Growth Factors

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**Background:** Cryopreservation of oocytes is an effective technology in assisted reproductive technology. Although successful vitrification of gametes has been reported in several mammalian species, the survival is generally low. The aim of this study was to investigate the effects of fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) on preantral follicle development after vitrification *in vitro*.

**Materials and Methods:** Preantral follicles with diameter of 150-180 µm were mechanically isolated from 18-21 day old NMRI mouse ovaries. Follicles were vitrified and warmed and then cultured in α-minimal essenstial medium supplemented with 0,20 ng/ml FGF,20 ng/ml HGF, 20 ng/ml from each of FGF and EGF. After 12 days hCG/EGF was added to culture medium and after 18-20 h later the presence of cumulus oocyte complexes (COC) and oocyte maturation state assessed.

**Results:** The results of this study showed that the rate of MII oocytes in FGF group increased in comparison with control and other treatment groups ($p<0.05$) There was significant increase in survival rate of follicles in FGF-HGF group in comparison with control and other treatment groups ($p<0.05$ ). After *in vitro* ovulation induction the follicles in HGF group showed a lower ovulation rate ($p<0.05$) than those cultured in other groups.

**Conclusion:** FGF in mouse vitrified follicle culture system increases oocyte maturation rate and HGF decreases COCs number. Addition of HGF and FGF simultaneously to the culture medium increase follicle survival.

***Keywords:*** Vitrification, Preantral Follicle, Epidermal Growth Factor, Fibroblast Growth Factor

## P-108: Effect of Phosphodiesterase Type 3 Inhibitor on Nuclear Maturation and *In Vitro* Development of Ovine Oocytes

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**Background:** Present study aims to investigate if PMC of ovine oocytes in the presence of cilostamide a phosphodiesterase type 3 (PDE3) inhibitor can improve the shortcomings of conventional IVM system.

**Materials and Methods:** Immature ovine oocytes were retrieved from pre antral follicles of abattoir ovaries by aspiration and after washing in Htcm + %10 FBS were immediately classified in four groups: three groups were cultured in PMC medium in the presence of 1, 10 and 20 µm cilostamide for 22 hours to arrest meiotic maturation and then they were washed and transferred to MM to resume meiotic maturation. In control group, oocytes were cultured in MM in the absence of cilostamide. The effect of cilostamide on gap junction communications and nuclear status was studied. The variables assessed were chromosome organization, spindle pattern, polar body extrusion, and embryonic development.

**Results:** According to the results, inhibition of PDE3 could not permanently block nuclear maturation in ovine oocytes but it delayed the process of nuclear maturation. Elevation of intra-oocyte cAMP concentration could inhibit cumulus cells expansion and maintain gap junction communications between oocyte and cumulus cells. Deletion of cilostamide and refreshing maturation medium after 22 hours culture revealed that cumulus cells were completely expanded. The inhibitory effect induced by 1 µM cilostamide was reversible, and it increased the number of mature oocytes with aligned chromosomes and normal spindle. However, the inhibitory effects of 10 and 20 µM cilostamide was not fully reversible and was associated with deleterious effects on chromo-

some organization and spindle pattern. Investigation of embryonic development via parthenogenetic activation and *in vitro* fertilization revealed that the blastocyst rate of oocytes that were prematured with 1 µM cilostamide was not significantly different from oocytes that underwent conventional IVM but it was significantly reduced in oocytes that were prematured with 10 and 20 µM cilostamide.

**Conclusion:** Our results provide the evidence that reduced cAMP via PDE3 is not the only mechanism that controls the progress of nuclear maturation in sheep oocytes, and that alternative or additional mechanisms may also exist.

***Keywords:*** Two-Step Culture System, Conventional IVM System, PMC, Embryonic Development, Gap Junction Communication

## P-109: Using SMAD2/3 Inhibitor to Investigate the Importance of GDF9 Signaling on Ovine Cumulus Expansion and Subsequent Embryonic Development

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**Background:** Oocyte *in vitro* maturation success rate is still low in compareision to oocyte *in vivo* maturation. Thus prediction and improvement of oocyte competency are two critical issues in assisted reproductive technology. New insights into oocyte-cumulus cell regulatory loop may assist our optimization of *in vitro* maturation technique. Maturation and developmental competency of cumulus oocyte complexes (COCs) are the result of cross talk between two pathways including endocrine hormones and paracrine growth factors (also known as oocyte secreted factors). The contribution of these factors in oocyte maturation and development is an active area of research. The objective of this research was to investigate whether growth differentiation factor 9 (GDF9) secreted by oocyte affects cumulus expansion and oocyte development in sheep.

**Materials and Methods:** Immature ovine COCs were cultured in presence of recombinant GDF9, denuded oocytes, SB-431542, a specific inhibitor of activin like kinase 4/5/7; or a combination of these factors. Routine *in vitro* maturation of COCs and denuded oocytes were considered as external controls. Cultured COCs were used for assessment of i. cumulus expansion and ii. yield and quality of embryo development.

**Results:** It was observed that cumulus expansion was not affected by any of these treatments. In the presence of exogenous GDF9, cleavage rate was reduced; blastocyst rate did not differ from other groups while trophectoderm cell number significantly increased.

**Conclusion:** This suggests that exogenous GDF9 could improve embryo quality. This study provides evidence that GDF9 signaling has a minor influence on ovine cumulus expansion and oocyte development; it appears that other signaling pathway have dominant role.

***Keywords:*** *In Vitro* Maturation, Oocyte Secreted Factor, Developmental Competency, Ovine

## P-110: *In Vitro* Developmental Competence of ICSI-Derived Activated Ovine Embryos

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**Background:** This study was designed to respond to the controversy regarding the necessity of oocyte activation after the ICSI procedure for normal embryo development in sheep.

**Materials and Methods:** The effect of chemical stimulation with either 5mM ionomycin (I) for 5 minutes or ionomycin + 2 mM 6-dimethylaminopurine (6-DMAP) for 3 hours on the efficiency of ICSI, was compared in six experimental groups: (1) ICSI, (2) ICSI + I, (3) ICSI + I + 6DMAP, (4) sham, (5) sham + I, and (6) parthenogenetics (sham and parthenogenetic groups were used as controls). In the present study, ovine oocytes needed additional chemical stimulation, after conventional ICSI, to activate (female pronucleous formation) and to form zygotes with male and female pronuclei (2PN).

**Results:** The percentage of cleaved embryos obtained and developed to blastocyst stage was higher ($p<0.001$) for ICSI-derived zygotes, followed by activation (I and I + 6DMAP; 18.2 and 22.5%, respectively) than ICSI and Sham injection without activation (3.0 and 0.0%, respectively). There was, however, no significant difference between activation protocols I or I + 6DMAP. Furthermore, there was no significant difference among chemically activated, ICSI-derived zygotes in term of hatchability rate; however, the percentage was significantly higher in parthenogenetic and IVF groups than ICSI and Sham injection.

**Conclusion:** Neither sperm alone nor mechanical activation was sufficient for ovine oocyte activation and pronuclei formation. Therefore, in our study conditions for *in vitro* embryo development, chemical activation of oocytes must be considered an essential part of the ICSI procedure in sheep.

***Keywords:*** ICSI, Activation, Parthenogenetic, Embryo, Ovine

## P-111: Inhibitory Effect of Noscapine on Human Endometriotic Epithelial Cells

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**Background:** Endometriosis is the presence of a functional endometrial tissue outside the uterine cavity and defines as cancer like model. Noscapine is a safe cough suppressant which has been introduced as cancer sup-

pressor. The aim of this study was to investigate the *in vitro* effect of noscapine on human endometriotic epithelial cells.

**Materials and Methods:** Human endometriotic biopsies (n=7) were digested by enzymatic method (collagenase, 2 mg/ml). Epithelial glands were collected by sequential filtration through nylon meshes (70 and 40 µm). The cells were divided to five groups: control and 10, 25, 50 and 100 µM concentration of noscapine and cultured for 72 hours. Viability of cells was assessed by trypan blue staining; also Nitric oxide concentration was measured by standard Griess reagent. Data was analyzed by one way ANOVA.

**Results:** Viability of endometrial epithelial cells were 88.2, 82.0, 73.6, 72.1, and 60.7% in control, 10, 25, 50 and 100 µM noscapine concentration respectively. Nitric oxide concentrations were 57.7, 33.8, 31.4, 29.2 and 20.4 µM in control, 10, 25, 50 and 100 µM groups respectively ($p<0.05$).

**Conclusion:** Noscapine decreased endometriotic epithelial cells viability in dose dependant manner. It can suggest for endometriosis treatment.

***Keywords:*** Epithelial Cell, Endometriosis, Nitric Oxide, Noscapine

## P-112: Kinetic Evaluation of Buffalo Epididymal Spermatozoa Preserved in Different Culture Media

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**Background:** Epididymal sperm has been recovered with the aim of conserving potentially valuable genetics Materialss from post mortem males for subsequent usage in assisted reproduction technologies. Among the quality parameters of spermatozoa, motility and velocity patterns are sensitive for chemical and physical components of medium which they were diluted. Also close correlation between sperm motion characteristics and sperm fertility has been found. So the aim of this study was to evaluate, motion characteristics of buffalo epididymal spermatozoa preserved in different culture media during 6 hour incubation.

**Materials and Methods:** Thirty testes from 15 mature buffalo bulls (3-5 year) were collected immediately after animal's death from Urmia slaughterhouse and transported to laboratory in cool condition. Spermatozoa were collected from caudal epididymis and pooled together. The samples were diluted in Ham's F10, human tubal fluid (HTF), Tissue culture medium (TCM) with 7.2 PH and concentration of $20\times10^6$ sperm/ml. sperm motion characteristics were evaluated 1, 3 and 6 hours of incubation at 37 °C with computer assisted sperm analyzer(CASA HFT version 6.0).

**Results:** Result showed that, all of the kinetics parameters reduced dramatically in time depend manner. Preservation of epididymal sperm in HTF and TCM media yielded higher motility and progressive motility as compared with Ham's F10 at 1, 3 and 6 hours of preservation ($p<0.05$). Velocity patterns (straight line, curvilinear and average path velocity) were highest in HTF medium and lowest in Ham'sF10 medium in all of the incubation time ($p<0.05$).

**Conclusion:** It can conclude that preservation of buffalo epididymal spermatozoa in HTF medium at 37°C seems to be suitable for sperm motility and velocity patterns.

***Keywords:*** Buffalo Epididymal Spermatozoa, Ham F×10, HTF,TCM, Kinetics Parameters

## P-113: Quality Improvement of Buffalo Frozen -Thawed Spermatozoa by Supplementation of Cysteine and Glutamine in Cryopreservation Extender

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**Background:** Sperm cryopreservation has been associated with over production of reactive oxygen species (ROS). Buffalo spermatozoa are susceptible to ROS inducing damages due to insufficient level of cytoplsmic antioxidant along with high amount of poly unsaturated fatty acids composition in membrane structure. Therefore, appropriate antioxidant in buffalo cryopreservation extender would reduce this oxidative stress, leading to achieve optimal fertility with frozen-thawed buffalo spermatozoa. Cysteine and glutamine are the main component of glutathione which act as a major antioxidant in sperm cell. Therefore this study aimed to assess functional and structural characteristics of buffalo spermatozoa in order to detect the optimum concentrations of cysteine and glutamine for buffalo semen cryopreservation

**Materials and Methods:** Twenty ejaculates of four buffalo bulls were diluted in tris-egg yolk extender and divided in to seven equal groups consisting of cysteine (5, 7.5 and 10 mM), glutamine (10. 15 and 20 mM) and no additive. Diluted sample equilibrated, filled in 0.5 straws and frozen in liquid nitrogen. The post thawed sperm motion characteristics, membrane integrity were assisted with computer assisted sperm analyzer (SCA, Spain) and hypo-osmotic swelling test. Also flowcytometric evaluation of intracellular ROS and mitochondrial membrane

potential were analyzed with Dihydroethidium and JC-1 dye respectively

**Results:** Addition of cysteine at 5 and 7.5 mM and glutamine at 15 mM in cryopreservation extender significantly increased post thawed motility and plasma membrane integrity with significant reduction of intracellular ROS compared to control groups ($p<0.05$). Also 7.5 mM cysteine elevated both progressive motility and mitochondrial membrane potential as compared with the control group ($p<0.05$). Supplementation of both amino acids did not present any significant effect on sperm motion characteristics following cryopreservation

**Conclusion:** It is concluded that addition of 7.5 mM cysteine and 15 mM glutamine could be suitable for cryopreservation of buffalo spermatozoa

**Keywords:** Buffalo, Cryopreservation, Cysteine, Glutamine, Sperm Parameter

## P-114: Gene's Expression Assessment of GDF9 Signaling Pathway in Ovine Cumulus Cells

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**Background:** Revisiting molecular dialog between the oocyte and cumulus cells may improve *in vitro* maturation (IVM) process. In this regard, background literature reveals that oocytes regulate many of the distinctive functions of cumulus cells. These regulatory effects are mainly attributed to the growth differentiation factor 9 (GDF9). Consequently, this study was carried out to investigate the effects of exogenous recombinant human GDF9 on ovine cumulus gene expression by using a specific inhibitor of ALK 4/5/7 (SB-431542) as a SMAD2/3 pathway inhibitor.

**Materials and Methods:** COCs were cultured with denuded oocytes (as a possible natural source of GDF9), 175 ng/ml recombinant human GDF9, 4 μM SB-431542 or a combination of these factors. After 22h IVM, quantification of mRNA expression for Pentraxin 3 (PTX3), Hyaluronon Synthase 2 (HAS2), Tumor necrosis factor alpha-induced protein 6 (TNFAIP6), Prostaglandin synthase2 (PTGS2), B-cell lymphoma 2 (BCL2) and Bcl-2-associated X (BAX) were determined using real time RT-PCR.

**Results:** Evaluation of relative expression of HAS2 revealed no significant difference between the experimental groups. The maximum production of PTGS2 mRNA was shown in COC+DOs group. The lowest expression of TNFAIP6 was observed in COCs +DOs. There was no significant difference in BCL2 mRNA expression among experimental groups. COCs Co-cultured with DOs, reduced pro-apoptotic BAX mRNA level.

**Conclusion:** In the present study, the majority of the differentially expressed genes were not up or down-regulated. The cumulus expansion index confirmed this observation. Thus, CC gene expression is suggested as a non-invasive method to predict COC competency.

***Keywords:*** *In Vitro* Maturation, Ovine, Growth Differentiation Factor 9, Cumulus Cell, Gene Expression

## P-115: Melatonin Increases Developmental Rate of *In Vitro* Mouse Somatic Cell Nuclear Transfer Embryo

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**Background:** The beneficial effect of supplementing culture medium with melatonin has been reported during *in vitro* embryo development of species such as mouse, bovine and porcine. However, the effect of melatonin on the mouse somatic cell nuclear transfer remained unknown.

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

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

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

***Keywords:*** Melatonin, Mouse Somatic Cell Nuclear Transfer, Reactive Oxygen Species

## P-116: Antioxidant Effects of MnTE and Catalase on Goat Sperm Motility Following Freezing and Thawing

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**Background:** This study was carried out to evaluate the protective effects of a cell permeable antioxidant, Manganese (III) meso-tetrakis (N-ethylpyridinium-2-yl) porphyrin chloride (MnTE), alone or in combination with different concentration of Catalase on motility of frozen-thawed goat semen.

**Materials and Methods:** Fresh semen ejaculates were collected with artificial vagina from 3 goats, mixed and divided into four equal fractions, and diluted (1:20 v/v) with commercial extender, Bioxell®, containing no antioxidant (control), 0.1μM of MnTE, 0.1μM MnTE + 200 IU Catalase and 0.1μM MnTE + 400 IU Catalase. All diluted sperm suspensions were cooled to 5 °C for 2 hours followed by transfer into 0.5 ml French straws before being

stored in liquid nitrogen. The straws were removed from the liquid nitrojen and thawed individually by immersion in a water bath at 37◦C for 30 second. Motility of spermatozoa in each sample was individually evaluated by Camputer Assisted Sperm Analyzer.

**Results:** The results showed that combination of two antioxidants in freezing media for goat semen cryopreservation, improved sperm motility following thawing. Accordingly, total motility was higher in 0.1µM MnTE + 200 IU Catalase (41.11%) and 0.1µM MnTE + 400 IU Catalase (44.92%) groups which was significantly higher than those for control (31.57%) and 0.1µM MnTE (32.56%) groups.

**Conclusion:** This study demonstrated that semen extender supplemented with MnTE in combination with Catalase can improve sperm motility due to reducing the oxidative stress provoked by freeze/thaw processes.

***Keywords:*** MnTE, Catalase, Goat Semen, Cryopreservation

## P-117: Gene Expression and Developmental State of Mouse Cloned Embryos after Treatment with Histone Deacetylase Inhibitor, Suberoylanilide Hydroxamic Acid (SAHA)

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**Background:** It is known that acetylation level of the nuclear histones in cloned embryos is lower compare to normally developed embryos. Histone deacetylas inhibitors (HDACi) with improvement of acetylation level in these embryos can affect embryo quality in pre-implantation stage and expression level of different genes especially developmental genes.

**Materials and Methods:** In this research, SAHA as an HDACi is used for cloned embryo treatment. These embryos treated with 5µM SAHA after beginning activation during 10 hours. Then development to blastocyst stage, total cells count(Tukey HSD test) and rate of cells undergo apoptosis (with TUNEL test) were measured(Dunnett T3 test) and expression level of Oct4, Cdx2, Hspc117, Nanog and Igf2 genes were studied with Real Time PCR method(Mann-Whitney test).

**Results:** The average of total cells number of the treated cloned embryos to the blastocyst stage compared with untreated group were increased ($p \leq 0.05$), and also cells that had undergone apoptosis were reduced statistically ($p<0.05$). Expression level of specified genes in cloned untreated embryos showed significant reduction compared with natural produced embryos. However SAHA treatment resulted increasing of genes expression ($p<0.05$). Pre-implantation development to blastocyst stage in treated group showed non-significantly enhancement compared with untreated group.

**Conclusion:** Although, the SAHA did not cause the enhancement of pre-implantation development to the blastocyst stage, the numbers of cells were increased and apoptosis were decreased. So the quality of blastocyst obtained from cloning procedure improved. Increasing expression of the mentioned genes especially Nanog and Igf2 to normal level of natural produced embryo maybe improves developmental potential and survival rate of embryos in post-implantation stages.

***Keywords:*** Cloning, Histon Deacetylas Inhibitors (HDACi), Pre-implantation Embryo, Suberoylanilide Hydroxamic Acid (SAHA)

## P-118: Developmental Competence of Ovine Embryos Derived from Frozen Oocytes Co-Cultured with Embryos Derived from Fresh Oocytes

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**Background:** Currently, a limited number of studies have been done on the vitrification of small ruminant oocytes, especially in sheep, where poor developmental rates are obtained following immature and mature oocyte vitrification. Poor ovine oocyte cryopreservation could attribute to the damage to enzymes which has role in metabolic pathways. Therefore, production of cytokines and growth factors such as epidermal growth factor, platelet-derived growth factor, and interleukin 6, which are critical for subsequent embryo development, could negatively be affected in zygotes derive from vitrified oocytes. This study was aimed to evaluate the probable improvement of developmental competence of embryos derived from vitrified oocytes co-cultured with embryos derived from fresh oocytes.

**Materials and Methods:** The *in vitro* matured oocytes were either vitrified or as fresh oocytes were *in vitro* fertilized and the presumptive zygotes were subjected to *in vitro* culture as 3 different groups; I. zygotes derived from fresh oocytes (fresh), II. co-culture of zygotes derived from fresh and vitrified oocytes (fresh-vitrified), III. zygotes derived from vitrified oocytes. The cleavage rate was then evaluated on Day 3 (Fertilization=Day 0).

**Results:** The cleavage rate in embryos derived from vitrified oocytes in fresh-vitrified group (58.3 ± 5.3) was slightly higher than vitrified group (50.8 ± 3.5, $p<0.05$). Although, the corresponding rate in fresh group (80.9 ± 3.0, $p<0.05$) was significantly higher than other groups.

**Conclusion:** Co-culturing of presumptive zygotes derived from vitrified oocytes with those derived from fresh oocytes could relatively improve the developmental competence of the former.

***Keywords:*** Coculture, Vitrified, Ovine Oocytes, Developmental Competence

## P-119: Comparison of Weymouth and TCM 199 for Ovine Oocyte Maturation

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**Background:** Different culture media are used for *in vitro* maturation of ovine oocyte. The Weymouth medium are used for *in vitro* maturation of rat and mice oocytes with very low levels of supplementation (Just FBS, while to acquire the good results in TCM hormone, and serum supplementation and in some cases growth factor are necessary to gain the optimized results. The aim of the present study was to compare the Weymouth and TCM199 media for maturation of ovine oocytes *in vitro*.
**Materials and Methods:** The ovine ovaries were collected from the local abattoir and transported to the laboratory in saline supplemented with required antibiotics. The oocytes were aspirated, qualified and subjected to Weymouth with 10% FBS (n= 110) or TCM FSH (2.5 IU/mL; IBSA, Switzerland,) and estradiol (30 µg/mL) and gentamycin (50 µg/mL). (n=95) in an atmosphere of 5 $CO_2$, 95 % humidity and 38.6°C for 17-19 hours. The cultured oocytes were denuded and examined for nuclear maturation using a phase contrast microscopy.
**Results:** The results showed a comparable efficacy of Weymouth (67.3%: 74/110) and TCM (68.4%: 65/95) in maturation of ovine oocytes.
**Conclusion:** The Weymouth medium was efficient in maturation of ovine oocytes.
***Keywords:*** Weymouth, TCM199, Ovine, Maturation

## P-120: The Impact of Progesterone and Sildenafil on Endometrial Ultrastructure in Superovulated Mice

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**Background:** Implantation is proceeded by a process referred as adhesion. Adhesion requires maturation of endometrium and blastocyst to confer adherence ability to them. During IVF cycles, endometrial maturation is facilitated by progesterone administrating. Considering vasodilatory effect of Sildenafild, in genital organs, it appears that Sildenafil may have similar effect as progestrone. The aim of the present study is to compare ultrastructural characteristics of mice uterine endometrium in natural cycle with those at superovulated cycle received progesterone or Sildenafild.
**Materials and Methods:** For this purpose, 60 adult female and 30 adult male mice were used. The female mice were divided into 4 groups as: controls, gonadotropin, gonadotropin + Sildenafil and gonadotropin + progesterone. In experimental groups the mice received 7.5 IU HMG as intra peritoneal injection and 48 hours later 7.5 IU HCG. Then in all groups, 2 female and one male mouse were placed in a cage for mating. In the gonadotropin + progesterone and gonadotropin + Sildenafil groups, the mice respectively received 1mg progesterone and 3mg sildenafil citrate at 24 , 48 and 72 hours after HMG injection. 96 hours after HMG injection, the mice were sacrificed and their uterine specimens were fixed in 10% formalin, embedded in paraffin and 5 µm thick sections were stained with H & E and studied with light microscope. For electron microscopic studies the specimens were fixed in 2% glutaraldehyde, embedded in araldite and thin sections studied with LEO 906 transmission electron microscope. Statistical analysis was performed using ANOVA.
**Results:** Microscopy revealed that in control group the cells had numerous apical microvilli and some granules were found in basal portion. In gonadotropin + progesterone group, the granules were found in basal and apical portions and their microvilli appeared shorter than the control and gonadotropin groups. In this group, the apical membrane also contained some pinopodes projecting toward the lumen. In gonadotropin + Sildenafil group the granules were found in both apical and basal portions and the microvilli appeared shorter than the control and gonadotropin groups. In this group, pinopodes appeared slightly numerous than the other groups.
**Conclusion:** Superovulatory drugs in mice stimulate endometrial maturation but injection of Sildenafil and progesterone has a negligible effects on it.
***Keywords:*** Implantation, Progesterone, Viagra, Mice, Uterine

## P-121: Follicle Development in Vitrified Sheep Ovarian Tissue Transplanted to Immunodeficiencent Rat

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**Background:** Ovarian cortex xenotransplantation into immunodeficient rats is a follicles protection method in rare animals and investigation on folliclogenesis. The aim of this study was to evaluate the effects of gonadotropin and erythropoietin (EPO) treatment on follicular development in the transplanted ovaries.
**Materials and Methods:** Prepared sheep ovarian cortical strips were vitrified by cryopin method using combination of etilenglycol (EG) and dimetylsulfoxide (DMSO) as cryoprotectans.Vitrified-warmed strips were grafted in the neck muscle of castrated male rats that treated with or without EPO for 5 days. 14 days later, rats were treated with HMG for 9 weeks. Ovarian fragments from each animal were divided into the control (fresh ovarian

tissue) and experimental groups (XI: vitrified xenograft+ HMG), (XII: vitrified xenograft+ HMG+ EPO). XI and XII groups were carried out for 11weeks and were repeated 7 times. E2 serum was measured before and after gonadectomy and at the end of xenograft. Histological analysis was done before and after grafting.

**Results:** In all grafted tissues, follicular growth was decreased. The number of primordial and preantral follicles increased after transplantation in XII group as compared to the control group (44.19 ± 11.40, 10.20 ± 3.79 vs. 41.33 ± 2.86, 3.18 ± 0.57). In XI group the primordial follicles numbers was decreased after transplantation as compared to the control group whereas the preantral follicles were increased (38.13±12.12, 19.94 ± 11.82 vs. 41.33 ± 2.86 3.18 ± 0.57, respectively). E2 secretion were also increased in xenograft+ HMG+ EPO group and it showed a significant differences with male castrated rat ($p<0.01$) and xenograft+ HMG group, $p<0.05$).

**Conclusion:** Co administration of human menopausal gonadotropin (HMG) and EPO seems to be more effective in surviving the primordial follicles in xenotransplanted vitrified ovaries.

*Keywords:* Follicle, Ovarian Tissue, Rat, Sheep, Xenotransplantation

## P-122: Morphine Effects on Placental Layers in Wistar Rats

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**Background:** Experiments have shown that opioid drugs abuse during pregnancy can cause a delay in the development of the placenta. In mammals, the placenta is the most important channel for the exchange of Materialss between the maternal and fetal bloods. The present study investigates the time-dependent effect of the addiction duration of maternal morphine consumption during pregnancy.

**Materials and Methods:** Female Wistar rats with an average weight of 170 to 200 grams were used in this experimental research. Experimental groups received morphine (0.05 mg/ml of drinking water) after one night coupling with male rats for mating. On 9th, 10th, 14th days of pregnancy, pregnant animals were killed, placentas removed and fixed. The cells of the placentas layers were calculated by light microscope, MOTIC and SPSS software.

**Results:** The thickness of the maternal portion of the placenta increases with morphine abuse in all three experimental groups compared to the control groups with a time-dependent manner. On the other hand, the findings of the present study, aligned with the body of research concerning the time-dependent effect of morphine consumption, revealed a significant reduction in the thickness of the fetal portion of the placenta in the experimental groups.

**Conclusion:** Overall, these results show that most of the negative effects of oral morphine consumption by pregnant women are time-dependent, and the severity of the delay in the development of the placental layers, depending on the duration of morphine consumption is more evident. In a general conclusion, it can be stated that the time-dependent effects of oral morphine consumption, can cause a delay in the natural development of cytotrophoblast and syncytotrophoblast cells of the placental layers in rats.

*Keywords:* Development, Cytotrophoblast, Syncytiotrophoblast, Morphine

## P-123: The Effect of Salvia Officinalis Extract on Serum Levels of Testosterone and Testes Tissue Differentiation in Male Rats

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**Background:** Studies show that Salvia officinalis administration can influence reproductive functions in animals. The purpose of this study was to determine the effects of Salvia officinalis extract on serum levels of testosterone and testes tissue differentiation in male rats.

**Materials and Methods:** Male Wistar rats were randomly divided into control, normal saline, and Salvia officinalis extract receiving rats. Salvia officinalis extract (200 mg/Kg/day) was injected intraperitoneally for 30 days. Blood samples were collected using cardiac puncture method. Serum testosterone level was measured by radioimmunoassay method. Testes tissue were examine microscopically. Data were statistically analyzed and compared between groups using "one way- ANOVA".

**Results:** The results indicated that serum testosterone level was significantly increased in Salvia officinalis receiving animals compared with control groups ($p<0.001$). Seminiferous wall diameter was significantly increased in Salvia extracts receiving animals compared with control rats ($p<0.01$). There was also higher number of spermatozoon in seminiferous tubules in Salvia extract receiving animals than control animals ($p<0.001$).

**Conclusion:** Our findings show that Salvia officinalis extract has a potential to improve testicular spermatogenesis and increasing of testosterone level.

*Keywords:* Salvia officinalis, Testosterone, Testes, Rat

## P-124: Association between Fertilization Rate and Externilization of Phosphatidyl Serine

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**Background:** Externalization of PS (EPS) is considered

as a sign of early apoptosis. In addition, EPS also occurs during capacitation and the acrosome reaction. During this study we aimed to evaluate the relationship between EPS and fertilization, embryo quality and pregnancy outcomes in couples who were candidates for ICSI.

**Materials and Methods:** Semen samples (n = 43) were collected from infertile individuals ICSI candidates and assessed according to World Health Organization guidelines for semen parameters. EPS was assessed by Annexin V and propidium iodide (PI) staining.

**Results:** There is a significant positive correlation between percentages of fertilization and annexin-positive PI-negative (An+PI-) sperm and percentage of An+ PI- sperm was significantly high in individuals with fertilization rates higher than 50%. In addition, the percentage of annexin-positive PI-positive (An+PI+) sperm in semen of the partners of pregnant women significantly differed from the partners of non-pregnant women. Furthermore, a significant negative correlation between percentages of fertilization and protamine-deficient sperm was observed.

**Conclusion:** The result of this study suggest that An+PI- is a sign of capacitation and An+PI+ is a sign of apoptosis, therefore, semen samples with high percentage of An+PI-, have a higher ability to undergo capacitation and, also have a higher chance to result in successful fertilization post-ICSI.

***Keywords:*** Externalization of Phosphatidyl Serine, Fertilization, Pregnancy, Protamine Deficiency

## P-125: The Effect of Eicosapentaenoic Acid (Omega-3) on The Expression of HAS2 in Cumulus Cells on Mice

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**Background:** Omega-3 fatty acids can modify gene and protein expression through altering the function of transcription factors controlling gene expression. Cumulus expansion, is a critical process for normal oocyte development, ovulation, and fertilization. This event needs to the synthesis of hyaluronic acid and its production is controlled by the enzyme hyaluronic acid synthase 2 (HAS2). The aim of this study was to determine if Omega-3 can affect on the expression of HAS2 in Cumulus cells and how its outcome in the *in vitro* fertilization was.

**Materials and Methods:** For this purpose, 30 female mice were super ovulated with hMG and HCG as IP injection. For the collection of cumulus- oocyte complexes(COCs) mice were killed by cervical dislocation and COCs were collected by flashing method. COCs were cultured in 2cc universal medium supplemented with 20 λ omega-3 and incubated for 4-6 hours. Surrounding cumulus cells were removed mechanically by using 20 λ hyaluronidase. The cumulus cells was stored at- 80°C until real-time PCR. PCR was performed by using a master mix containing CYBR green. The expansion level of mRNA HAS2 was evaluated by real-time PCR. For the IVF sperms from male mice were added to the medium drops containing 50 COCs in each group.

**Results:** The result showed that the level of expansion of HAS2 in experimental group comparing with control was significantly increased.

**Conclusion:** The result indicates that omega-3 could improve Cumulus expansion through increasing the amount of expansion of HAS2.

***Keywords:*** Omega-3, HAS2, Cumulus Cells

## P-126: Duration of Exposure to Polyvinylpyrrolidone (PVP): The Effects on Fertilization Rate and Embryo Development

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**Background:** Recently, it has been found that exposure of sperm to PVP may cause submicroscopic changes in sperm structure and the sperm nucleus. The PVP-induced nuclear damage may have been due to breakdown of sperm membranes. Consequently, it is probable that exposure of sperm to PVP may impair embryo development. There are apparently no detailed reports regarding the specific effects of PVP on fertilization rate and embryo development .

**Materials and Methods:** In the present study, 358 oocytes from 58 cycles were evaluated. We recorded the time interval between exposure of sperm to PVP and ICSI insemination, also we recorded the time of exposure of oocytes to hyaluronidase, and then each oocyte was put it in a single droplet of G1 media. All of the procedures were performed by a single embryologist over 6 month's period of time. Post-ICSI rates of fertilization and blastomere number as well as embryo quality were assessed.

**Results:** No statistically significant differences were found between the time of exposure of oocytes to hyaluronidase with blastomere number, embryo quality and fertilization rate, but there are significant differences between the time of exposure of spermatozoa with PVP and Fertilization rate (p=0.002), embryo quality (p=0.03) and blastomere numbers (p=0.001).

**Conclusion:** It seems different time of exposure of sperm to PVP can affect subsequent cleavage rate and embryo development, so it is suggested to decrease the time of sperm exposure to PVP as possible.

***Keywords:*** Time Interval, PVP, Embryo Development

## P-127: Characterization of Filia, A Maternal Effect Gene, in Bovine Oocytes and Embryos

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**Background:** Genetic analysis in mice has lead to find about maternal effect genes such as Filia. Filia knock out mice have a 50% decrease in fertility. Filia dysfunction causes disorders in pre-implantation development. Mutations in human Filia gene, cause FBHM (Familial Biparental Hydatidiform Mole) in women. Filia protein in mice is homologous to that of rat and human, so this idea has emerged that maybe it is present in the bovine oocytes/embryos.
**Materials and Methods:** To find Filia gene expression, primers were designed with OLIGO5 software and the amplified fragment was sequenced, and then translated to protein. A Real-Time experiment was done to identify the expression pattern in oocytes, and blastocysts. A bioinformatic analysis was done on bovine Filia gene (AC_000166.1) and its 2 kb upstream promoter, in FGENESH online tool, and the consensus transcription start site (TSS) and number of exons were predicted. The partial protein sequence was analysed in PHYRE2 online tool and the consensus domains were predicted. A 3' RACE-PCR analysis was implemented to identify different Filia splice variants.
**Results:** Real-Time Data showed that Filia is expressed in bovine oocytes and pre-implantation embryos with a specific pattern. Three exons and one TSS were predicted in FGENESH and according to PHYRE2 results, the 5' protein sequence consisted of a RNA-binding KH-domain, previously found in mouse Filia protein. The 3' RACE-PCR results showed that just one splice variant was amplified.
**Conclusion:** Filia has a maternal inheritance in bovine, and can be an essential factor in bovine embryo development and female fertility.
***Keywords:*** RACE-PCR, Bioinformatic, Fertilily, Bovine

### P-128: The Effect of DNA Methyl Transferase1 Inhibitor (RG108) on DNA Methylation Pattern of Cloned Mouse Embryos

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**Background:** In somatic cell nuclear transfer (SCNT) method of cloning, transferred nucleus should be dedifferentiated from differentiated state to embryonic state. Molecular analysis showed that the reprogramming in the transferred nucleus was incomplete and cloned embryos have epigenetic abnormalities such as high DNA methylations levels. Since methylation in pre-implantation embryos has a critical role, hypermethylation or any other methylation disorders can alter the expression of genes related to the embryo development, that this changes in the later stages of fetal growth, appear to block development. Therefore, in this study for correcting epigenetic errors, the effect of RG108 as DNA-methyltransferase1 inhibitor on pre-implantation embryo development and methylation was assessed. And will discuss whether the RG108 can be reduced methylation in embryos obtained from cloning and increased potential of it.
**Materials and Methods:** Two-cell stage embryos obtained from cloning, treatment with 5 and 10 µmol RG108 until morula stage and the pattern of DNA methylation was measured and analyzed quantitatively.
**Results:** The percentage of development to the morula/blastocyst stage in the group treated with 10 µmol RG108 (6.9 ± 3.3) were significantly lower in comparison to the non treatment group (35.2 ± 3.9). Also a significant difference was observed in developmental rate to the morula/blastosyst stage in the group treated with 5 µmol RG108 (17.9 ± 2.9) and non treatment group of cloned embryos (34.82 ± 3.99). But the difference between two treatment groups was not significant. Intensity of methylation between cloned embryos treated with 5 µmol RG108 (34.55 ± 3.19) and none treated (42.57 ± 5.99) showed significant differences, also the difference between normal fertilized (*in vivo*) embryos (36.53 ± 9.24) and those derived from cloning was significant while no significant difference showed between treated and *in vivo* embryos.
**Conclusion:** Results of this study showed that treatment of mouse cloned embryos from 2-cell to morula stage with 5 µmol RG108, although it cannot increase to the level of development morula/blastosyst stage, but as one can possibly have a positive effect on correction of hyper methylation in the embryos obtained from SCNT.
***Keywords:*** Cloning, SCNT, DNA Methylation, RG108

## Ethics and Reproductive Health

### P-129: Study of Sexual Behavior and Related Factors in Infertile Couples Referred to Fatemeh Zahra Infertility Center in Babol

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**Background:** Infertility is a major challenge to balance the emotional and sexual life of a couple. Sexual dysfunction is common in infertile couples and women are affected more often than men. This may be related to infertility or infertility diagnosis and treatment process. Infertile couples look sex as part of the process in the treatment so sexy and sensual aspects of human needs will be removed. The Disruption in the couple's sex life will stabilize in the lives of them and eventually lead to feelings of failure, self-concept changes and changes in relationships between couple. The aim of study is assessment the effect of infertility diagnosis and treatment on sexual behavior of infertile couples.
**Materials and Methods:** In this study, 230 infertile

couples attending Fatemeh Zahra infertility clinics in Babol were studied. For all participants, after the filing and history taking, physical examination was performed and laboratory tests needed were requested. The data collection tool was questionnaire, which has separate sections for men and women, each with two parts of demographic and sexual dysfunction information. The questionnaires to assess sexual function were FSFI questionnaire (Female Sexual Function Index) for women and IIEF (International Index of Erectile Function) for men, which were completed by the subjects. For content validity and reliability, Cronbach's alpha was used. Data were analyzed by chi-square test and ANOVA.

**Results:** 41.6% of women and 34.8% of men reported changes in their sexual relationships. Most changes in both men and women were, reducing the number of sexual contacts and sexual anxiety. The most common sexual dysfunction was dyspareunia in women (27.3%) and after that sexual desire (26.1%) and premature ejaculation in men (34.5%). Cycle of female sexual dysfunction and orgasmic dysfunction in men was significantly associated with primary infertility and increase the duration of infertility and idiopathic infertility ($p<0.001$). Also, economic status, physical and mental diseases and the dissatisfaction of sexual and asexual relationships was significantly associated with sexual dysfunction in couple ($p<0.001$) and as well as husbands'job with sexual dysfunction cycle in women ($p<0.01$).

**Conclusion:** Given the importance of sexual health in quality of life and treatment process of infertile couples, consulting services and attendance of a sex therapist is essential in infertility centers.

*Keywords:* Infertility, Sexual Behavior, Sexual Dysfunction

## P-130: Frequency and Associated Causes of Induced Abortions in Northwestern Iran

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**Background:** The extensive education on contraceptive methods, particularly in rural areas of developing countries, play a pivotal role in preventing of induced abortion. The main purpose of this study was to determine the prevalence and major cause of induced abortion in women in northwestern Iran.

**Materials and Methods:** The hospital documentary of 848 women encountering medical examinations due to abortion during recent 2 years, was studied. Subsequently, by using standard questionnaire, the major causes of induced abortions were investigated and statistically analyzed.

**Results:** Our findings indicated that the frequency of induced abortion leading to infectious abortion was very remarkable (480 cases) and most of them (over 60%) were inhabitants of rural areas who had not considerable knowledge about contraceptive methods. The used devices and tools for induced abortion were urethral sound or Foley catheter (56%), hook, clarinet key or feather (27%), plant extractions, Mg sulfate or Permanganate (9%), and fork or spoon (8%). In most cases (over 50%) the mean age range was 24 - 38 years old.

**Conclusion:** Based on our results, the frequency of induced abortion was remarkable among the women population in rural west areas of Iran. Since the major cause of induced abortion was lacking of contraceptive methods and poor knowledge on contraceptive methods, the encouragement and education on contraceptive methods application is necessarily required in developing countries and particularly in rural areas.

*Keywords:* Induced Abortion, Frequency, Causes

## P-131: Prevalence of Cesarean in Women Who Were Pregnant by Using Assisted Reproductive Techniques in Imam Ali Hospital in the Province of AZNA 1391

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**Background:** Maximum infertility as the inability to conceive after one year of marriage without the use of contraceptive means. About 12-8% are couples without children, 35% of male infertility is just as relevant to their In addition, approximately 25% of infertile couples are men and women are involved

**Materials and Methods:** This study was a descriptive - analytical and the study of 50 pregnant women who were referred to Imam Ali hospital in the province of assisted reproductive techniques and questionnaires Bardarshdh a variable delivery, place of delivery, cesarian Median age was Rtrahy.

**Results:** In this study, the rate of cesarean delivery 93/4% normal delivery 6.6% reported an increase in cesarean rates increase as education level and age.

**Conclusion:** Due to the increasing cesarean rate and its complications, education of women enjoying the benefits of natural childbirth and reduce its complications is recommended.

*Keywords:* Delivery, Assisted Reproductive Technology, Casarian

## P-132: Female Adolescents Attitudes toward Sexual and Reproductive Health Education in Iran

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**Background:** Despite clear reasons for necessity of sexual health education for adolescents, it is a contested issue and has faced challenges in most cultures. Adolescents, specially females in Iran have so many unmet needs in sexual health issues, yet appropriateness of sexual health education to them is controversy. This study aimed to examine female adolescents attitudes to sexual health education for female adolescents in Iran.
**Materials and Methods:** In this descriptive study, a questionnaire was developed to measure the adolescents' attitudes regarding sexual health education issues. After measuring the construct validity and reliability of the instrument, it was utilized in a survey in 2011 on 359 adolescent girls in Mashhad and Ahvaz schools who were selected through cluster sampling.
**Results:** The majority of the adolescent girls were agree to the necessity of sexual education, school-based sexual education, low quality of current sexual education, reluctance of reticence and censoring in sexual education, the important supportive role of adults, the gradual nature of sexual education in accordance with developmental stages, and also providing an inclusive sexual health educational program for adolescents.
**Conclusion:** It is the time to take steps towards overcoming the cultural taboos and barriers to sexual health education by advocacy and developing and implementing programs with comprehensive contents, instead of being entrapped in the circle of suspicion and distrust.
***Keywords:*** Female Adolescents, Sex Education, Reproductive Health

## P-133: The Influence of Gender Role and Women Empowerment on Fertility Experiences in Urban Society of Mashhad, Iran

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**Background:** Iran has experienced a great variation in women status in recent years. There is a little information on how and why advancing gender equality and equity and the empowerment of women play a role in recent fertility reduction in the country. This study was conducted to gain an insight into the role of gender beliefs and women empowerment in couples' experiences of fertility in Mashhad, Iran in 2011-12.
**Materials and Methods:** In this exploratory qualitative study in-depth interviews were conducted with 54 purposefully selected eligible male and female participants and some key informants who lived in urban society of Mashhad. Data was gathered until saturation was happened and analyzed adopting conventional content analysis approach through giving analytical codes and identification of categories using MAXqda software. Study rigor verified via prolonged engagement and validation of cods through participants' confirmation.
**Results:** Findings from data analysis demonstrated three major categories including: 1. gaining the power and evolution of gender roles, 2. interacting with repeated/ balanced gender roles, and 3. realization of reproductive preferences. The participants viewed advancement of women's knowledge on their socio-legal right and health literacy and decision making skills preceded by increased women education, as essential elements and cornerstone of women empowering that result in achieving the equity in fertility decisions and also ability for administrating such decisions. "Equal roles in fertility decisions", "choosing and using best fit family planning methods" and "participative child care" influenced the women's fertility behaviors.
**Conclusion:** Managing the fertility behaviors need to understand the roles of spouses in their mutual interaction and their quota in fertility decision making and related behaviors. Reproductive health policy makers and family planning counselors can apply the study findings in order to disseminate proper fertility behaviors.
***Keywords:*** Fertility, Childbirth, Empowerment, Gender Role, Couple's Interaction, Qualitative Study, Conventional Content Analysis

## P-134: Women's Perception of Infertility Counseling in A Religious and Spiritual Context

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**Background:** To provide infertility counseling for diverse religious groups it is important to have a good understanding of how patients' religious background affects their understanding of infertility. Considering the dearth of research on how religious/spiritual viewpoints impact choice of counseling, this study explored how infertile women perceived counseling using their religious/ spiritual frame of reference.
**Materials and Methods:** The design was a grounded theory study on 30 infertile women affiliated to different denominations of Christianity and Islam who were interviewed in one Iranian and two UK fertility clinics. Data were collected through semi structured in-depth interviews and analyzed using straussian mode of analysis.
**Results:** Religious participants using a religious/spiritual meaning-making framework had a divine understanding of infertility. This worldview resulted in optimism which gave them empowerment to accept God's plan with enthusiasm. They gradually tried to contribute actively to take responsibility and control over all aspects of their lives through adopting religious coping strategies. As a

result, they relied less on formal support resources like counseling services. Additionally, they criticized health professionals for not addressing religious/ spiritual issues in practice and wished their religious concerns to be discussed in counseling.

**Conclusion:** We argue that providing infertility counseling in a religiously sensitive context taking religious/ spiritual values into account encourages infertile women to use counseling services more and will help them to cope better with their stressful situation. It might enhance the therapeutic outcome for infertile women and therefore can contribute to build a more holistic approach to infertility counseling.

***Keywords:*** Infertility, Religion, Spirituality, Counseling, Grounded Theory

## P-135: Iranian Women's Decisions to Seek Help for Self-Discovered Breast Symptoms: A Qualitative Study

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**Background:** Breast cancer (BC) is the most common cancer in women worldwide. Approximately 25-42.5% of Iranian women seek help within three months or more after discovery of a breast cancer symptom.The aim of this study was to explore influencing factors on decision making to detecting potentially malignant breast symptoms to evolve new insight about help seeking process in Iranian women.

**Materials and Methods:** A qualitative method was conducted involving in-depth semi-structured interviews with 23 Iranian women with self-discovered breast cancer symptoms. Participants were purposefully selected form women who attended Cancer Institute of Tehran University of Medical Sciences. Interviews were transcribed and analyzed using conventional content analysis with MAXqda software.

**Results:** The main influencing factors on decision making to detect potentially malignant breast symptoms of breast cancer emerged from data were categorized in four categories including: 1. individuals' symptom appraisal, 2. symptom disclosure, 3. emotional reactions nd 4. individual characteristics and its relation with health services utilization. Decision about how to deal with symptoms mainly was made based on individual and interactive understanding of seriousness of symptoms. Palpable lumps with pain often perceived as a serious condition and lumps without pain were ignored in most cases. Disclosing the symptoms to others yielded more positive than negative results and led to prompt help seeking in most cases. Women experienced varying degrees and types of emotional reactions after discovering symptoms with conflicting effect in help seeking behavior.Women who did not perceive the seriousness of the symptoms dismissed them without emotional reactions. Perceived competing priorities such as familial and social responsibilities, inaccessibility and also unacceptability of care had undeniable effect on decision making about health care services utilization.

**Conclusion:** This study suggests considering improving women's knowledge and self-awareness beside correcting their social beliefs about breast cancer to inform the development of effective programs to shorten patients delay.

***Keywords:*** Breast Cancer, Breast Symptoms, Help Seeking Behaviors, Qualitative Research

## P-136: The Protective Effect of Vitamin C on Adverse Effect of Bisphenol A on The Ovary in Adult Rat

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**Background:** Bisphenol A (BPA), an estrogenic environmental contaminant is also known for oxidative stress-inducing effect. The aim of this study was to investigate the effect of vitamin C as an antioxidant on the ovary tissue in adult rats treated with BPA.

**Materials and Methods:** Adult female rats (200 ±20 g) were divided into 4 groups (n=6):control, bisphenol A (60µg/Kg/day) , vitamin C (150 mg/Kg/day) and bisphenol A + Vitamin C. Oral treatments were performed for 20 days. After treatment, the right ovaries removed, fixed and tissue processing was carried out. Using stereological technique, the total volume of ovary, cortex, medulla, corpus luteum, the mean number of follicle, the volume of oocyte and its nuclei and thickness of zona pellucida were estimated. Data were analysed with SPSS using one-way ANOVA, and the mean difference was considered significant at the ($p<0.05$).

**Results:** Body and ovary weight showed no significant change in 4 groups ($p>0.05$). A significant decrease in the total volume of ovary, cortex, medulla and volume of corpous luteum in BPA group was found in comparison with the control group ($p<0.05$). The mean number of antral follicle in group treated with BPA significantly decreased compared to control group ($p<0.05$), while atretic follicle increased in this group. The volume of oocyte and its nuclei in antral follicles and also the thickness of zona pellucida in secondary and antral follicles indicated a significant decrease in BPA group compared to control group. Co-administration of vitamin C and BPA compensated for the adverse effects of BPA on the above parameters.

**Conclusion:** This study showed that co-administration of vitamin C with BPA could prevent the adverse effects of BPA on the ovarian tissue in adult rat.

***Keywords:*** Bisphenol A, Vitamin C, Ovary, Stereology, Rat

## P-137: Sex Hormones Abnormalities and Female Sexual Dysfunction in Multiple Sclerosis Patients

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**Background:** Multiple sclerosis (MS) is the most common cause of progressive neurological disability in young adults who may be sexually active. MS patients experience high levels of sexual dysfunction with hypoactive sexual behavior and abnormalities in hypothalamus-pituitary-gonad (HPG) axis. In the present study, we aimed to evaluate the correlation between serum levels of sex hormones during menstrual cycle and the sexual response of women with MS.
**Materials and Methods:** A total of 30 female MS patients with relapsing-remitting MS (RRMS) aged from 20 to 40 years divided into four groups according to expanded disability status scale (EDSS, mean EDSS score 2.5, range 0-6) and 30 female age-matched healthy control subjects were included into the study. The female sexual function index (FSFI) questionnaire was used to determine sexual dysfunctions. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), β-oestradiol, progesterone, prolactin (PRL), total and free testosterone were checked in follicular and leutal phases of menstrual cycle.
**Results:** FSFI scores in RRMS patients were significantly lower than the healthy group. Domain scores for desire, arousal, orgasm, pain, lubrication and satisfaction measured between RRMS patients and controls showed significant difference. We observed significant hormonal abnormalities consisted of decreased progesterone and testosterone levels and increased oestradiol and prolactin levels during menstrual cycle.
**Conclusion:** Although, we showed the abnormalities in sex hormones level and sexual dysfunction, we could not define a statistically significant negative correlation between EDSS and FSFI scores in the RRMS patients and there was no significant correlation between abnormal hormonal status and the presence of a specific sexual dysfunction, as assessed with the FSFI.
***Keywords:*** FSFI Scores, Sex Hormone Level, EDSS

## P-138: What Should We Know about Ethical Problem in ART? A Study of Systematic Review

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**Background:** Advances in medical technology have had major impacts on human reproduction. The development of effective contraceptive methods has reduced unwanted fertility, and the development of a variety of drugs and procedures has, for the first time, permitted women with fertility problems to reasonably expect that they can fulfill their desires to have children. So, The aim of this review article is to determine some ethical problem in ART that used for treatment of infertility.
**Materials and Methods:** In this review research we used databases Web of Science, Bireme and Scopus. The inclusion criteria are as follows: full papers, published between 2001 and 2011, in English. Thirty-four papers were found, 27 of which were excluded because they did not comply with the inclusion criteria, resulting in a final sample of 7 papers.
**Results:** In this review article, we found that any types of technique used for being parents have problem specially in ethical field, so based on culture and social problem, parents at first attention to ethical problem of that culture, if they can accept that consideration, then try to being parents.
**Conclusion:** Legal rules and ethical guidelines around consent to health research involving children will frustrate the creation of research protocols that investigate health and safety issues related to the use of ARTs. In order for such research to proceed, it is essential that these issues and uncertainties be addressed. Consideration of these consent and risk-related issues is clearly needed in order to create some certainty around law and consent in this specific research context. Some of the key ethical questions that have been raised in this paper for society and cultures, we should know that artificial instrument have their problems and couple should know them.
***Keywords:*** Assisted Reproductive Technique, Infertility, Ethical problem

## P-139: The Relative Frequency of Depression in Infertile Women

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**Background:** Infertile women suffering from more psychological distress than other people. Women with involuntary childlessness have higher depression scores than others. Psychological factors are predictor for successive treatment and fertility. Cognize needs to be taken of these factors for effective treatment. The aim of this study was to evaluate the relative frequency of depressive disorder in infertile women in an infertility clinic in Rasht.
**Materials and Methods:** In a descriptive study, 240 infertile women with simple sampling were evaluated. Variable were included: depressive disorders, age and duration of infertility. Beck questionnaire was used to evaluate the psychological status. Then, infertile women with positive Beck score (16≤) were examined by psychiatrist make definitive diagnosis of depression. Collected data were processed with EPI-2000 (a=0.05).
**Results:** 84(35%) of 240 women with infertility had positive Beck scores (16≤). Of 84 patients with positive Beck score, 8 were excluded for different reasons such as re-

fusal and inability for cooperation. Clinical diagnosis and interview proved 57 (24.56%) of them to be depressed, respectively. The most relative frequency of depression was in 24-30 years old women and women with duration of infertility more than 5 years. Minor depression was the most prevalent depressive disorder.
**Conclusion:** According to this study, the prevalence and severity of psychological disorders of infertile women indicates the appropriateness of screening and referring these patients for psychological evaluation.
***Keywords:*** Depressive Disorder, Infertility, Female

# Female Infertility

## P-140: Effect of Ovarian Stimulation on The Endometrial Integrin α9, αv, β1, β3 and Apoptosis in The Peri-Implantation Period

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**Background:** Integrins are important markers of endometrial receptivity that will be recognized by the embryo and facilitate its growth, differentiation and implantation. In addition, Apoptosis is one of the most important topics that plays an important role during early stage of implantation but the relationship between ovarian stimulation and apoptosis is still a matter of debate.
**Materials and Methods:** Six-week-old NMRI female mice were stimulated using an intrapritoneal injection of 0 (solvent as control), 7, 10 and 12 IU PMSG followed by another injection of 10 IU hCG after 48 hours. then the mice were rendered pseudopregnant. Samples were obtained from 1/3 middle part of uterine horns. Integrins and apoptosis were assessed in four groups at peri-implantation period using immunohistochemistry and Tunel staining imaging analysis.
**Results:** Qualitative analysis indicate that the endometrial integrin α9, αv, β1, β3 distribution in the peri-implantation phase were lower in stimulated cycles compared to controls. TUNEL analysis indicate that the percentage of TUNEL-positive cells was higher in stimulated group compared to control group ($p \leq 0.05$). The ratio of apoptosis was the highest in 12 IU PMSG stimulated group ($p \leq 0.05$).
**Conclusion:** The ovarian stimulation could change the distribution of Endometrial integrin α9, αv, β1, β3 and enhanced the incidence of endometrial apoptosis at peri-implantation period; therefore, it could affect on the endometrial receptivity and implantation rate.
***Keywords:*** Stimulation, Apoptosis , TUNEL, Integrin, Endometrium

## P-141: The Comparsion of Glucose-6-Phosphate Dehydrogenase Level between Preeclamptic Postpartum Mothers and The Healthy Post-Partum Mothers in Alzahra Hospital in Rasht, 2011- 2012

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**Background:** Preeclampsia has high prevalence and important complication. The relationship between Glucose-6-Phosphate Dehydrogenas (G6PD) and preeclampsia are expressed previously but their relationships are controversial yet. Some studies confirm this possibility and some others dismiss it. So we decided to examine the relationship between G6PD activity and preeclampsia although it is not denoted this test as clinically useful predictor for preeclampsia.
**Materials and Methods:** In this case-control study, women with gestational age more than 20 weeks with or without preeclampsia admitted to Al-Zahra Hospital were included. Exclusion criteria were gestational diabetes mellitus, history of diabetes, chronic hypertension, use of any particular drug during pregnancy, thyroid disease, history of high fever in pregnancy, RH-negative blood group, more than 35 years, multiple pregnancies, body mass index more than 35, vasculopathy, antiphospholipid antibody syndrome and all factors affecting on G6PD enzyme levels. Demographic variables and levels of G6PD enzyme, hemoglobin, red blood cells and reticulocytes were assessed. Then statistical analysis was done with SPSS.
**Results:** In this study, 115 women with a mean age of 28.4 ± 5.4 years were studied. They divided in two groups: 58 postpartum women with preeclampsia and 57postpartum women without preeclampsia. Two groups were matched in age, gestational age, gravidity, hemoglobin and red blood cell level. The G6PD enzyme levels ($p=0.0001$) and reticulocyte levels ($p=0.005$) were significantly lower in women with preeclampsia.
**Conclusion:** This study showed that G6PD enzyme and reticulocythe levels were significantly lower in women with preeclampsia. The result of previous studies were inconsistent with others. Therefore, it is suggested to survey further studies with larger sample to find exact relationship between G6PD and preeclampsia
***Keywords:*** Preeclampsia, Glucose-6-Phosphate Dehydrogenas, Pregnancy

## P-142: Adenosine Deaminase Activity during Menses, Follicular and Luteal Phases of Menstrual Cycle

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**Background:** In recent years, numerous regulators of gonadal function have been studied. Adenosine deaminase(ADA) is widely distributed throughout human tissues and may contribute in the regulation of menstrual cycle. The purpose of this study was to determine the plasma activities of total adenosine deaminase (ADAT), and its isoenzymes, ADA1 and ADA2, and ADA1/ADA2 ratio during the menses, follicular and luteal phases of the menstrual cycle.
**Materials and Methods:** Plasma activities of ADAT,

ADA1, ADA2 and ADA1/ADA2 ratio were measured in 20 women aged 19-25 years and with regular menstrual cycle (26-30 days). For the menses, follicular and luteal phases, blood samples were obtained between 1-2, 7-8 and 21-22 days after beginning of menstruation, respectively.

**Results:** Although, there were not significant differences in ADA and its isoenzymes activity during three phases of the menstrual cycle, but we observed regular pattern for alteration of ADA activity during menstrual cycle. The activity of ADAT, ADA1, ADA2 and ADA1/ADA2 ratio were the highest during the menses phase, the lowest during follicular phase and followed by increased during the luteal phase.

**Conclusion:** Our results suggest that the change of ADA activity during menses, follicular and luteal phases might involve in the regulation of menstrual cycle. We speculate that reduction of ADA activity in follicular phase might lead to an increase in adenosine levels which may contribute in the ovulation process.

***Keywords:*** Adenosine Deaminase, Menstrual Cycle, Follicular Phase, Luteal Phase

## P-143: Investigation of Female Related Infertility in Patients of Kermanshah Infertility Treatment Center

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**Background:** Our current knowledge of the epidemiology and the most common cause of infertility are limited and outdated in our geographic area. It is known that, one important cause of infertility is female factor. This study was done to investigate the incidence of female factor and the most common cause of female infertility in patients of Kermanshah infertility treatment center in Mo'tazedi Hospital with primary infertility.

**Materials and Methods:** Data were studied from 313 patients with primary infertility (age between 20 and 48 years) who were referred to Kermanshah Infertility treatment Center (Iran) during March 2011 until April 2012. Analysis of data performed by SPSS.

**Results:** Our results showed that, the female factor in 28.5% of patients with primary infertility was known as the cause of infertility. In the patients with female related primary infertility, 21.5% ovulation disorders, 11.6% tubal factor, 3.7% uterine factor, 3.4% endometriosis, 2.6% recurrent abortion and 12.8% idiopathic causes were reported. However, 44.4% of females had normal fertility.

**Conclusion:** This study showed that in primary infertile patients, disorders in ovulation was the most common cause of infertility in females of our geographic area. On the other hand, recurrent abortion was reported more than normal that, it may be due to recent environmental changes such as influx of haze and dust in the west of Iran.

***Keywords:*** Epidemiology, Primary Infertility, Female Factor

## P-144: Egg and Embryo Donor's Application Forms in Infertility Centers; Medical, Genetic and Psychosocially Forms in Evaluating Potential Donor Candidate

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**Background:** Nowadays, gamete and embryo donation is applied as effective process in treatment infertility problems. In order to participate in the process of donation, donors should complete an extensive questionnaire that details their personal and family medical history. Included in this questionnaire should be a detailed sexual history, substance abuse history, history of family disease, and psychological history. A donor is ineligible if either screening or testing indicates the presence of a risk factor for, or clinical evidence of, a communicable infection or disease.

**Materials and Methods:** In this study, we review books and articles in Internet sites about egg and embryo donor's application forms. Then we collected and summarized this information to present important sections of them.

**Results:** This questionnaire is composed of three parts. The first part contains confidential information the donor. Confidentiality section has been included information donation history, personal health history, sexual, contraceptive and reproductive history. The second part contains information that will be shared and viewed by recipients. The shared section has been included physical characteristics, social history and habits, genetic history and personal and motivational. Finally, the third section included of donor acknowledgement sheet. The laboratory testing of all donors should include screening and testing for medical and genetic disease. All donors undergo a psychological evaluation during screening to limit the possibility of emotional harm. Psychological screening is performed aimed at identifying emotional problems, evaluating donor motivations.

**Conclusion:** The main purpose of donor's application forms is to ensure the safety of donor's egg and embryo.

***Keywords:*** Egg Donor, Embryo Donor, Application Forms, Donor Candidate, Infertility Centers

## P-145: Role of Human Leukocyte Antigen in Miscarriage

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**Background:** During pregnancy, the maternal immune system is in close contact with cells and tissue from the semiallogenic fetus .The human leucocyte antigen (HLA) class Ib molecules, HLA-E, -F and -G, are expressed at the materno-fetal interface. Because of the apparent immunoregulatory functions of these proteins, they may be involved in successful acceptance of the semi-allogenic fetus during pregnancy.in this presentation we have reviewed studies regarding role of hla class Ib in pregnancy success and gathered their results in order to gain a better understanding of the relationship of these molecule and pregnancy success.
**Materials and Methods:** We have conducted a thorough literature search using search engines in the medical databases and collected the data and research results regarding these molecules and pregnancy. We focused on polymorphisms of the three genes, expression patterns of the proteins, and interactions with immune cells and the have been evaluated to elucidate whether HLA-E, -F and -G are involved in the pathogenesis recurrent miscarriages.
**Results:** Non-classical antigens such as Human leukocyte Antigen class Ib may interact with receptors of NK cells ,Macrophage, CD8+ T cell, CD4+ T cell and monocyte such as Killer Inhibitory Receptor, Leukocyte Ig Like Receptor which results into limit activity of these immune cells and downregulation of immune response and helps in the maintenance of pregnancy and any changes in pattern of expression of these HLA molecule because of polymorphisms or other reason may cause miscarriage.
**Conclusion:** The HLA class Ib molecules seem to induce suppression of the maternal immune system. Evidences show changes in expression pattern and function of these molecules are associated with miscarriage . To clarify the functions of HLA-E, -F and -G future studies need to link investigations of the polymorphisms in these genes to measurements of protein levels, and examine the role of these proteins in the complex interplay of immune cells and cytokines at the materno-fetal interface.
***Keywords:*** Miscarriage, HLA, Leukocyte Antigen

## P-146: Toxoplasmosis: Experimental Vaginal Infection in NMRI Mice and Effect on Uterin, Placenta and Fetus Tissues

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**Background:** Toxoplasma gondii is an important zoonotic pathogen. Vertical transmission of the parasite occurs when females were infected primarily during gestation.This parasite transmitted to the fetus through the placenta and may cause miscarriage, permanent neurological damage, premature birth and visual impairment.
**Materials and Methods:** Two 6-8-week NMRI females mice were crossed with one male. The pregnant mice were divided into 2 groups: experimental group that infected by parasite via intra-vaginal (IV) and control group that received the same volume of normal saline via IV. One mouse from each group was killed on the fifth day after infection. The peritoneal fluid, ovary and uterus of mouse samples were taken and divided into two parts. One part used for DNA extraction and other keep in formalin and sent for histological study.
**Results:** PCR using DNA extracted from experimental group showed the parasite was existed in tissues of the uterus and placenta but not in the embryos and peritoneal fluid. PCR using DNA extracted from control group was negative.
**Conclusion:** Tachyzoite of Toxoplasma and DNA of this parasite were observed in sub mucosa and muscles of the uterus and in the villis of placenta but were not observed in histological sections of the fetus. Therefore, histological and molecular results were consistent.
***Keywords:*** Infection, NMRI Mice, Toxoplasma, Vaginal

## P-147: Ovarian Function over Time in Adult Rats after Tubal Sterilization

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**Background:** Tubal sterilizatio (TS) is a widely applied contraceptive method worldwide. Although most studies have described sterilization as a safe method, there are reports of TS complications. The aim of this study was to evaluate the effects of TS on ovarian function.
**Materials and Methods:** The study comprised of 2 protocols A and B. A Protocol: survey ovarian hormone and B protocol: study of estrous cycle. Each of the protocols included control, sham and TS (Pomeroy) groups. In protocol A, rats serum estradiol was measured 15, 45 days 3 and 6 months after intervention. In protocol B, on days 15 and 45 and months 3 and 6 after the intervention, animal's estrous cycle was followed for 15 days.
**Results:** Our results showed that serum estradiol significantly decreased in rats undergone TS ($p<0.05$). Also TS caused irregularity in estrous cycle after 15, 45 days, 3 months, and 6 months.
**Conclusion:** TS decreased estradiol of serum. Complications of TS may be due to decreased ovarian function.
***Keywords:*** Tubal Sterilization, Estrous Cycle, Estradiol

## P-148: Predictive Factors for Pregnancy after Intrauterine Insemination: A Retrospective Study of Factors Affecting Outcome

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**Background:** Controlled ovarian hyper stimulation (COH) in conjunction with intrauterine insemination (IUI) is frequently used to treat various causes of infertility in couples. In this study we evaluate the relation between IUI outcomes and infertility with the intent to detect the most important parameters that pertain to a successful IUI.
**Materials and Methods:** In this cross-sectional study, we included 994 IUI cycles in 803 couples who referred to the Royan Institute between 2010 and 2011. All statistical analyses were performed with the SPSS program, chi-square and t-tests. Logistic regression was performed to build a prediction model of the IUI outcome.
**Results:** Overall pregnancy rate per completed cycle (16.5%), ectopic pregnancies (1.8%), miscarriage rate (5.4%), multiple pregnancy rates (17.5%) and live birth rate per cycle (14.5%). Pregnant women were of a lower mean age than nonpregnant women (p=0.01). There was an association between cause of infertility and clinical outcomes (p<0.001). The mean duration of infertility in pregnant women was significantly lower (p=0.002), however body mass index (BMI), type of infertility, endometrial thickness and sperm parameters were not related to IUI success (p>0.05). Logistic regression identified five significant IUI variables [female age (OR: 0.9 CI: 0.91-1.007 p= 0.09), menstrual history (OR: 0.4; CI: 0.2-0.6; p<0.001), duration of infertility (OR: 0.8; CI: 0.7-0.9;p<0.001), total dose of gonadotropin (OR: 1.00; CI: 1.00-1.001;p= 0.01) and volume (OR: 1.1; CI:1.003-1.2;p=0.04)] which were the most predictive of IUI success.
**Conclusion:** This study defined prognostic factor prognostic factors for pregnancy in COH+IUI. These variables have been integrated into a statistical model to predict the chance of pregnancy in subsequent COH+IUI cycles.
*Keywords:* Intrauterine Insemination, Infertility, Pregnancy Rate, Prognostic Factors

## P-149: GnRH Antagonist versus Agonist in Normoresponders Undergoing ICSI: A Randomized Clinical Trial in Iran

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**Background:** General concern is that the pregnancy rate is higher with GnRH-agonist as a protocol of pituitary suppression. GnRH-antagonist protocol provides a shorter period of administration and an easy flexible protocol. In this study, the outcomes of GnRH agonist and antagonist in ICSI cycles are compared in normo responder patients.
**Materials and Methods:** In this randomized clinical trial, 300 normoresponders undergoing ICSI were randomly divided to GnRh agonist (n=150) and GnRh antagonist (n=150) groups. The main outcome measurements were chemical, clinical and ongoing pregnancy rates (PR).
**Results:** The mean duration of stimulation were 9.6 ± 1.6 and 8.2 ± 1.6 days in agonist and antagonist groups respectively (p=0.001). The mean number of MII oocyte retrieved in agonist and antagonist groups were 7.7 ± 4.0 and 6.9 ± 4.3 respectively (p=0.03). There was no significant difference between two groups regarding mean number of gonadotrophin ampoules, follicles, oocytes, total embryos and good quality embryos, OHSS incidence, and abortion rate. Chemical pregnancy rate was 35.3% in agonist and 39.3% in antagonist group. Clinical pregnancy rate was 35.3% in agonist and 34% in antagonist group. Ongoing pregnancy rate was 45 (31.3%) in agonist and 44 (29.3%) in antagonist group. There was no significant difference between two groups in pregnancy rates.
**Conclusion:** It was shown that antagonist protocol was an easy, safe and friendly protocol in Iranian normoresponder patients, having similar outcomes with standard agonist protocol but shorter period of stimulation.
*Keywords:* IVF, GnRH Agonist, GnRH Antagonist, Normoresponder

## P-150: The Role of Using Antioxidant Supplementation by Men on Recurrent Abortion

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**Background:** Recurrent abortion is defined as 3 or more pregnancy loses. It is estimated that between 0/5 to 3 percent of couples suffer from recurrent abortion. The male gamete contributes 50% of genomic Materials to the embryo and contributes as well as to placental and embryonic development. Oral antioxidant treatment has been reported to reduce the percentage of damaged spermatozoa and improved pregnancy outcomes.
**Materials and Methods:** This article is a review article and the findings has been received by reviewing 5 articles.
**Results:** All the articles demonstrated the role of using antioxidant supplementation by men and improving the pregnancy outcomes in women with history of recurrent abortion.
**Conclusion:** The review showed that, increased intake of antioxidant supplementation by men, could result in improvement in pregnancy outcomes.
*Keywords:* Male Factor, Recurrent Abortion, Antioxidant

## P-151: Assisted Reproductive Technology Outcomes in Too Young and Young Patients: Single Embryo Transfer versus Multiple Embryo Transfer

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**Background:** This retrospective study compared pregnancy outcomes from single embryo transfer (SET) with multiple embryo transfer (MET) in too young (≤25 years) and young (>25 years) infertile patients.
**Materials and Methods:** Data from infertile patients who referred for ART treatment to Yazd infertility center during 1992 to 2012 were studied retrospectively. Records of patients with SET or MET were studied in two groups: ≤ 25 (375 patients), and >25 years (346 patients). Demographic data including age, job, place of residency, clinical information, including cause and duration of infertility, type of infertility and kind and number of treatment, details of ART treatment cycles consisting number of antral follicles, number and grade of oocyte, semen analysis, number and grade of embryos, number of transferred embryos, and pregnancy outcomes were compared.
**Results:** In≤ 25 years group the mean number of follicles >14mm 11.03, the number of oocytes 9.71, the number of MII oocytes 8.38, and the Grade of transferred embryos was 17.19. In >25 years group the mean number of follicles >14mm 9.26, the number of oocytes 8.45, the number of MII oocyte 7.23, and the Grade of transferred embryos was 16.99. The pregnancy rate from SET in ≤25 years group was 15.8% and from MET was 29.4%. In >25 years group, the pregnancy rate from SET was 11.5% and from MET was 39.3%.
**Conclusion:** The pregnancy outcomes with SET or MET didn't differ in very young infertile women. So, it is better to perform SET to avoid multiple pregnancy complications.
***Keywords:*** Assisted Reproductive Technology, Single Embryo Transfer, Multiple Embryo Transfer

## P-152: Expression of Recombinant Human Luteinizing Hormone (hLH) in CHO Cells

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**Background:** Human luteinizing hormone (hLH) stimulates steroid biosynthesis of the ovary, triggers ovulation and prepares androgen production of testicular Leydig cells. LH belongs to the family of glycoprotein hormones that are heterodimers consisting of a common α-subunit and specific β-subunit. hLH is necessary for clinical and infertility treatment. Recombinant DNA technology provides a useful tool for hLH production with high specific activity and purity. The objective of the present study was gene cloning of hLH subunits simultaneously in the innovative multigenic plasmid for gaining high levels of expression.
**Materials and Methods:** In this study, open reading frame (ORF) sequences of subunits were separately amplified by PCR and cloned into pTZ57R/T vector and then they were subcloned simultaneously into pVITRO2-neo-mcs expression vector. All mechanisms were confirmed by PCR, digestion reaction and sequencing. Recombinant pVITRO2-neo-mcs expression vector was linearized by using restriction enzyme, the products were transfered into Chinese hamster ovary (CHO) cells by electroporation and examined by PCR. Expression of recombinant hLH was confirmed by SDS-PAGE and Western blotting techniques.
**Results:** The recombinant vector pVITRO2-neo-mcs was able to insert subunits with hFerH and hFerL composite promoters in CHO cells genome and both subunits were expressed.
**Conclusion:** The carbohydrate moieties of glycoprotein hormones perform main roles in the biopotency, so mammalian cells could prepare appropriate position for accurate glycosylation of hLH. Cloning of both subunits in one expression vector is good idea to insert them at the same time and near the each other. These functions makes more probability to associate subunits after expression processing in mammalian cells.
***Keywords:*** hLH, CHO, pVITRO2-neo-mcs

## P-153: Role of Chemokines in The Pathogenesis of Infertility Related to Enometriosis

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**Background:** Chemokines, proteins that operate within the body's immune system, play numerous roles in menstruation, bacterial infection, implantation of embryos, and the maintenance of early pregnancy. They are also strongly related to the pathogenesis of endometriosis.
**Materials and Methods:** This is review article that were indentifical by computer search of MEDLINE (1984-2013).
**Results:** Several chemokines including interleukin (IL)-8, growth-related oncogene (GRO) alpha, regulated on activation, normal T expressed and secreted (RANTES), and macrophage inflammatory protein (MIP)-1 are reported to be elevated in the peritoneal fluid (PF) of women with endometriosis. Chemokines IL-8 and GRO alpha as well as epithelial cell-derived neutrophil-activating protein (ENA)-78, eotaxin, and interferon-inducible protein (IP)-10 might be involved in macrophage activation, inflammatory reaction, and adhesion of endometriotic tissues in the peritoneal cavity, and enhanced angiogenesis in the progression of endometriosis.
**Conclusion:** Cytokines, which are produced by many cell types in peritoneal fluid, play a diverse role in constructing the peritoneal environment that induces the development and progression of endometriosis and en-

dometriosis-associated infertility. Intense basic research into the specific role of these cells and soluble factors may improve our understanding of endometriosis and result in novel therapies for endometriosis.

*Keywords:* Endometriosis, Chemokines, Pathogenesis

## P-154: Comparison between The Effect of 17-Bestradiol at Preovulatory and Pregnancy Levels for *In Vitro* Generation of Regulatory T Cells

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**Background:** Naturally occurring CD4+CD25+ regulatory T cells (Treg) exert an important role in mediating maternal tolerance to the fetus during pregnancy. During pregnancy the elevated number of regulatory T cells (Tregs) correlates with 17-b oestradiol (E2) level. The role of maternal hormones in successful pregnancy is well known. Maternal hormones through re-engineering of the maternal immune response provide protection for the semi-allogeneic fetus, while any changes in maternal tolerance may lead to abortion, and reproductive abnormality. The aim of this study was to compare the effect of E2 at preovulatory and pregnancy levels for generation of Tregs *in vitro*.
**Materials and Methods:** We treated the magnetic bead separated peripheral blood naïve T cells (n=4) with E2 and anti-CD28 antibody in anti-CD3 coated plates for 96hours at 37°c and 5%$Co_2$. Two different concentrations of E2 (pregnancy and preovulatory) were used. Naïve T cells with no treatment were termed as control cells. The phenotype of induced Tregs was investigated through analysis of CD25 and Foxp3 expression. XTT assay was used to monitor PHA-mediated proliferation of conditioned T cells and their effect on proliferation of autologous/allogeneic PBMC in co-culture experiments. ELISA technique was applied for measurement of cytokines: IL-10,TNF-a and IFN-g in cell supernatants of a secondary culture in the presence or absence of PHA.
**Results:** After 4 days of incubation, CD25 and Foxp3 were detected by flow cytometry. In comparison to E2preg, E2preov-conditioned T cells showed significantly higher frequencies of Foxp3 expression. E2preov-conditioned T cells showed a significantly reduced proliferation capacity in comparison to other groups of conditioned T cells. Decreased TNF-a level and IFN-g/IL-10 ratio were noted especially in cultures with preovulatory concentration of E2. In addition, production of TNF-a by E2 preovulatory treated cells was significantly lower than that by E2 pregnancy in the presence or absence of PHA.
**Conclusion:** Our data suggest estrogen as a good candidate for *in vitro* generation of Tregs. This data might along with the development of new therapeutic methods for treatment of autoimmune diseases and pregnancy complications, reduce abortion incidence in the future.

*Keywords:* Regulatory T Cells, 17-Bestradiol, Preovulatory, Pregnancy

## P-155: Pattern of Serum Adiponectin Concentrations in Normal Estrous Cycles of Postpartum High-Producing Dairy Cows

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**Background:** There are accumulating evidences showing the role of adiponectin, a newly discovered adipocytokine secreted from adipose tissue, in the regulation of the ovarian function in dairy cows. The objective of the present study was to evaluate the pattern of serum adiponectin concentration during a normal estrous cycle in the postpartum dairy cows.
**Materials and Methods:** Coccygeal vein blood samples were collected from 71 high producing dairy cows, with a history of being clinically healthy, twice weekly (three days apart) from the 1st to 7th week postpartum. Serum progesterone (P4) concentrations were determined twice weekly using a validated commercial radioimmunoassay kit (Immunotech kit, France). Based on the progesterone profile, a subset of cows (n=6) with a pattern of normal luteal activity was randomly selected. Serum adiponectin was determined twice weekly using a validated commercial ELISA kit. Changes in mean serum adiponectin concentrations were analyzed using paired sample t test (SPSS 11.5, $p<0.05$).
**Results:** Serum adiponectin concentration gradually decreased ($p<0.05$) while the serum progesterone concentration increased after ovulation by day 17 of cycle. During the periovulatory period, adiponectin concentration were in maximum levels, when the P4 was in minimum concentration; but when the level of P4 reached to the highest level during the estrous cycle, serum adiponectin concentration was in its nadir.
**Conclusion:** During a normal estrous cycle in high-producing dairy cows with normal luteal activity, the fall in serum adiponectin concentrations was accompanied by a marked increase in progesterone concentrations. Inspection of the adiponectin profile suggests that this increase was initiated a few days before next ovulation.

*Keywords:* Adiponectin, Estrous Cycle, Dairy Cow

## P-156: Survey of *In Vitro* Effect of Resveratrol on Apoptosis of Human Endometrial Epithelial Cells

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**Background:** In apoptosis is physiological cell death which eliminates injured and old cells. Any disruption in apoptosis, leads to disease. Resveratrol is a natural polyphenol with estrogen-like, antioxidant and anti-inflammatory properties; also it showed cell prolifratory and inhibitory effect. The aim of present study was to determine resveratrol effect on apoptosis of human endometrial epithelial cells in *in vitro* culture.

**Materials and Methods:** In this experiment study, human endometrial biopsies (n=8) were chopped in sterile conditions. Epithelial cells, were isolated after enzymatic digestion (collagenase 2 mg/ml), cell filtration though filter mesh. Cell viability was defined by trypan blue staining. The epithelial cells were divided into five groups, control, 1, 10, 20 and 50 µM resveratrol concentration for 48 hours in DMEM/F12 medium containing antibiotics. Cell apoptosis was determined with tunnel assay. Data was analyzed by one-way ANOVA and $p<0.05$ was considered significant.

**Results:** Apoptosis were 5.9 ± 0.35, 8.4 ± 1.7, 10.03 ± 2.4, 15.46 ± 0.4 and 18.03 ± 1.9% in control, 1, 10, 20 and 50 µM resveratrol respectively and difference between groups was significant ($p=0.015$).

**Conclusion:** Resveratrol can induce significant apoptosis in human endometrial epithelial cells in higher doses.

***Keywords:*** Human Endometrium, Epithelial Cells, Apoptosis, Resveratrol

## P-157: A Comparison of The Effect of Levonorgestrel IUD with Oral Medroxyprogesterone Acetate on Abnormal Uterine Bleeding with Simple Endometrial Hyperplasia and Fertility Preservation

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**Background:** Endometrial hyperplasia is important clinically, because it can lead to abnormal uterine bleeding (AUB) which itself can precede to endometrial cancer. Endometrial carcinoma is the most common malignancy of the female genital tract, occurring about 75-85% in younger, perimenopousal women as hyperplasic endometrial. The treatment is hysterectomy or hormone therapy with progesterone. The aim of this study was, therefore, to compare the effect of levonorgestrel IUD (Mirena) with medroxyprogesterone acetate on simple endometrial hyperplasia for fertility preservation.

**Materials and Methods:** Forty women in reproductive age(22-47) with AUB that their endometrial biopsies reports were simple hyperplasia were enrolled in this study and then randomly divided into two groups. All patients presented with designed special checklist which was filled with satisfaction. Complete history and physical examination especially BP, BMI, breast examination, bimanual vaginal examination and trans vaginal sonography (to measure the thickness of endometrial and exclude the other pathologic lesions) were performed. In first group, treatment was performed with medroxyprogestron (20mg/daily) for 10 days and in other group Levonorgestrel IUD was prescribed. After 3 months, Trans vaginal sonography and biopsy of endometrium were done. Therefore; status of AUB and side effects of two methods along with the rate of satisfactory were evaluated.

**Results:** Our findings showed the significant differences in the treatment of simple hyperplasia between two groups (levonorgestrel IUD group vs. MPA group) ($p<0.047$). Recovery of AUB in the group of Mirena was enhanced ($p<0.047$). Endometrial thickness was reduced in both groups ($p<0.001$), but reduction of endometrial in Mirena group was even more. Also, Mirena was tolerated more than MPA. Side effects of MPA was more and reached significance ($p<0.003$).The rate of satisfactory with Mirena was higher than medroxyprogesterone acetate and reached significance ($p<0.048$).

**Conclusion:** The results of this study show that levonorgestrel IUD is more effective than MPA in treatment of simple endometrial hyperplasia and can be helpful in young women who want to preserve their fertilities.

***Keywords:*** Mendometrial Hyperplasia, Levonorgestrel IUD, Medroxyprogesterone Acetate (MPA), Abnormal Uterine Bleeding

## P-158: ART and Gynecological Cancer: Report of Our Experiences and Literature Review

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**Background:** Infertility is an important and common problem in couples that needs to assisted reproductive technology (ART) or the other drug therapies. Infertility has been known as a risk factor for ovarian cancer, breast cancer and endometrial cancer but there is a question about the relationship of these cancers to infertility itself or drugs and methods of infertility treatment.

**Materials and Methods:** We evaluated all risk factors in patients with breast cancer, ovarian cancer and endometrial cancers who referred to Gynecological Oncology Clinic in Shahid Sadoughi Hospital within 2002-2012. In a retrograde study, we investigated the history of primary infertility and ART before diagnosis of cancers in patients.

**Results:** We registered 92 patients with endometrial cancer, 84 patients with advanced epithelial ovarian cancer and 113 patients with breast cancer. 39.1% of patients with endometrial cancer who were obese body mass index (BMI>30) and 18.8% of patients with normal (BMI=25-29) had infertility history. ART was found in 7.3% of all patients with endometrial cancer. In 28.4% of patients with epithelial ovarian cancer, female infertility has been also diagnosed and in 14.1% ART was found. Clomiphen therapy with or without HCG and HMG was the most common drugs which was used for patients with ovarian cancer. 16.5% of all patients with breast cancer had infertility problem, and in

7.3% of them ART was diagnosed.

**Conclusion:** Although infertility was diagnosed as an important and fairly common risk factor in endometrial, ovarian and breast cancer, but some other factors are more important. Age, body mass index and cause of infertility are also important. Finding the association of ART to gynecological cancers need some other long cohort studies which follow the infertile women who get the ART or drug therapy for over 15-20 years. The other studies in this field cannot answer to our question about increasing gynecological cancer due to ART. We think BMI and age are co-factors to cancers which should added to infertility or ART. We had better discus this relationship to the partners and have a multidisciplinary management for obese infertile women who had had polycystic ovarian syndrome or age more than 35 years. Breast cancer screening should be investigated in infertile women after 35 years because breast is the most common site of primary cancers which send metastasis to ovaries.

***Keywords:*** Assisted Reproductive Technologies, Infertility, Gynecological Cancers, Ovarian Cancer, Endometrial Cancers, Breast Cancers, Poly Cystic Ovarian Syndrome, Age, Body Mass Index

## P-159: Women's Infertility and Polycystic Ovarian Syndrome, Concepts and Treatment

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**Background:** Polycystic ovarian syndrome (PCOS) is a medical condition that affects women's menstrual cycles, fertility, hormone levels, and physical appearance. PCOS is thought to be one of the leading causes of female infertility, and is the reason of more than 75% of cases of anovulatory infertility.

**Materials and Methods:** In this article, we review the different aspects of PCOS including genetic biasis and treatment.

**Results:** The mechanism of anovulation is uncertain, but there is evidence that arrested antral follicle development is associated with the abnormal endocrine profile, in particular the interaction of insulin and LH on granulosa cell differentiation. Clinical evidence indicates that polycystic ovary syndrome has a heritable basis, at least in part, which could result from a genetic etiology, epigenetic changes, or an admixture of the two causes.

**Conclusion:** Although, there is no cure for PCOS, but controlling it lowers the PCOS risks of infertility, miscarriages, diabetes, heart disease, and uterine cancer. Management of infertility in polycystic ovary syndrome includes lifestyle modification as well as assisted reproductive technology such as ovulation induction, oocyte release triggering and surgery.

***Keywords:*** Polycystic Ovarian Syndrome, Genetics, Infertility

## P-160: Study of Association between Polymorphism in Estrogen Response Element (ERE) in Promoter Region of C3 Gene and Spontaneous Recurrent Abortion

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**Background:** There are different etiological factors in spontaneous recurrent abortion which are one of the complications in pregnancy. Primary embryo development in uterine tube and oviduct are affected by different factors such as embryotrophic elements. ETF3 as a embryotrophic factor that contains a complex of complement 3 protein and its derivatives specially iC3b, causes embryo trophoblast growth and expression of genes involved in embryo development. There are different response elements in promoter region of C3 gene such as two estrogen response elements (ERE).

**Materials and Methods:** In this research, we proposed that polymorphism in two ERE in promoter region of C3 gene can associate with Spontaneous Recurrent Abortion in early months of pregnancy. Peripheral blood samples of 40 women with Spontaneous Recurrent Abortion registered to infertility center and 30 fertile women as control group were collected. After DNA extraction and amplification of regions harboring ERE utilizing PCR with specific primers, SSCP method used for study of probable polymorphisms in this region.

**Results:** Our studies showed no significant correlation between polymorphism in ERE of C3 gene promoter and desired symptoms. However, this study closed our opinion to an individual asymptomatic infertility.

**Conclusion:** Study of effective factors on this promoter region can be lionized. Accurate selection of more cases can be helpful in improvement or rejection of this proposal. Likewise as a new proposal, we emphasize on association between polymorphism in this region with some of heredity infertility of women.

***Keywords:*** Spontaneous Recurrent Abortion, C3, Estrogen Response Elements

## P-161: Effect of Naloxone on Endometrial Growth

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**Background:** There is fine correlation between the infertility and lessening of endometrial thickness. In this study the effects of sedative naloxone, and L-arginine, the precursor of nitric oxide (NO), on endometrial thickness of rat uterus was examined.

**Materials and Methods:** Wistar rats (weighing 200-250 g) in Diestrous phase received intra-peritoneal (i.p.) 1. single L-arginine (50 mg/kg), 2. single naloxone (0.4 mg/kg) and 3. naloxone (0.4 mg/kg) 30 minutes priorly to the L-arginine (50 mg/kg) during 9 days/ once a day. The control group exclusively received saline (1 ml/kg, i.p.)

throughout the treatment period. After completion of the treatments, all rats were coupled with intact males and after observation of vaginal plaques they were isolated and graded 0 of gestation. The females were subsequently passed surgical examination in days 19-20 of gestation.

**Results:** Based on the findings the group that only administered L-arginine, nevertheless of endometrial development, showed low pregnancy and fetal count to control. However, naloxone pre-treatment caused significant raise in the rates. The rate, furthermore, showed an increase in single naloxone group.

**Conclusion:** Endometrial wideness may occur due to angiogenesis and expansion of blood flow in the endometrial wall and by growing of the wall glands and matrix. The L-arginine may produce the NO, a putative increasing factor of blood flow. This factor, however, is not as effective as do in the presence of naloxone. The naloxone can develop the endometrial growth in Wistar rat.

***Keywords:*** Naloxone, L-Arginine, Endometrial Thickness

## P-162: Pathophysiology of Endometriosis is Interacted by MIF, its Receptor and COX-2

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**Background:** Endometriosis is a gynecological disease associated with severe pelvic pain and infertility. Immunological changes that occur in patients with endometriosis include reduced natural killer cell and T-lymphocyte cytotoxicity in the peritoneal fluid, and an elevated number of activated macrophages. 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 leads to positive effect on the expression of COX-2. In this study, the expression of MIF, CD74 and COX-2 genes in eutopic, ectopic and the normal endometrium was evaluated in menstrual cycle by Q-PCR. The expression level of MIF protein in peripheral blood samples of patients was another variable factor checked in this study.

**Materials and Methods:** All women taking part in this study, were between 20-45 years old, had no endometrial hyperplasia or neoplasia. Twelve ectopic and 8 eutopic endometriosis samples and 12 normal endometrium during menstrual cycle as control group were tested in this study. Peripheral blood samples were likely obtained from each group. The level of MIF, CD74 and COX-2 genes expression was investigated. Also, protein level of MIF in blood serum were measured by ELISA assay.

**Results:** The expression of MIF, CD74 and COX-2 genes in ectopic, eutopic and normal endometrium during menstrual cycle were varied. Also women with endometriosis had 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 biomarker for endometriosis development and pathophysiology. Variation in the expression of these genes in normal endometrium during menstrual cycle could play an essential role in reproduction, inflammation and endometrium reconstruction. Also, high level of MIF in blood serum in endometriosis could act as a biomarker in diagnosis of endometriosis.

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

## P-163: Evaluation of Ovarian Masses in Shahid Sadoughi and Mother Hospital during 2008-2012

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**Background:** Ovarian cancer is the fifth most common cancer in woman and the fifth most frequent cause of cancer death. Ovarian cancers are usually asymptomatic until metastasized, patients have advanced disease at diagnosis in more than 2/3 of the cases. Totally 75-80 % of ovarian masses are benign. The strongest patient related risk factor for ovarian cancer is increasing age. The most ovarian masses in premenopausal are functional and ovarian cancer is rare (2% malignant, 20% benign, 72% functional) and in postmenopausal report of ovarian masses are: 10% malignant, 10% functional, 80% benign. Benign ovarian cysts characteristically are unilateral mobile, smooth but bilateral, solid and fixed masses should alert the clinician to a possible carcinoma. The size of masses shows the chance of malignancy, which usually smaller than 5 cm are benign.

**Materials and Methods:** In this study, 659 ovarian masses evaluated, using the reports of pathology of patient with ovarian mass since Jan 2008 till Dec 2012 in mother and Shahid Sadoughi Hospitals. Questiannare is containing age, size ,essence, location, benign or malignant and type of pathology. The data was analyzed by spss (ver.15) and the following results were achieved.

**Results:** Totally 95.1% (627 patients) of the masses were benign and 4.9% (32 patients) were malignant. Among the malignant type (32 patients), epithelial masses were the most common malignant tumor (53.1 %). Among the neoplastic types, (202 cases) 15.8% (32 cases) were malignant and (170 cases) 84.2% were benign. The mean age in malignant masses were 37.91 ± 18.52 (SD) and the mean age in benign masses were 31.33 ± 11.23 (SD) that it shows mean age in malignant masses is higher than benign masses. The mean size in malignant masses were 5.48 ± 4.67 (SD) cm that

shows mean size in malignant masses is higher than benign masses. Among 659 ovarian masses 567 masses were cystic and 80 masses were mixed and 12 masses were solid that 41.7% of solid masses and 20% of mixed masses and 1.9% of cystic masses were malignant that it shows risk of malignancy in solid and mixed masses is higher than cystic masses.

**Conclusion:** Ovarian masses are usually asymptomatic, in evaluation of them should be careful of all sings, because sometimes the malignant tumors are small or in young women.

***Keywords:*** Ovarian Masses, Malignant , Benign

## P-164: Comparision The Effect of GNRH Agonist Administration versus Vaginal Progesterone on Serum Progesterone in Luteal Phase in Ovarian Hyperstimulation and Intrauterine Insemination Cycles in Unexplained Infertility

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**Background:** To compare the effect of GnRH agonist administration with vaginal progesterone on serum progesterone in Luteal phase in control ovarian hyper stimulation and intrauterine insemination cycles

**Materials and Methods:** In this clinical trial, 242 infertile patients because of UEI (unexplained inferetility candidate for ovarian stimulation and intrauterine insemination reffering to infertility ward of Mirzakhoochak khan Hospital were recruited.The patients were randomly divided in two groups (A or Progesterone group) and (B or GnRH agonist group).The luteal phase was routinely supplemented in 121 patients (group A) with 400 mg/day/vaginally of natural micronized progesterone starting 1 days after the IUI (intra uterine insemination) for two weeks. In B group (121 patients) a single dose of GnRH agonist (triptorelin 0.1mg) subcutaneously was injected 4 days after IUI. Then serum progesterone level was compared 10 days after IUI in two groups.

**Results:** Totally we evaluated 240 patients with mean age 28.42 ± 4.02 (20-38) years in this trial. The mean serum level progesterone 10 days after IUI in A group was 33.45 ± 18.12ng/ml and in B group was 32.50 ± 23.82 ng/ml and difference between two groups was not significant (p=0.72). also, regarding results of IUI, we found BHCG positive in 20 patients in vaginal group and 19 patients in GnRH group( p=0.86) . Moreover, clinical pregnancy was detected in 15 patients in vaginal progesterone group and in 15 patients in GnRH group(p=NS).

**Conclusion:** We showed GnRH agonist are effective on luteal phase supporting and serum progesterone in patients with unexplained infertility, however GnRH agonists were not more effective than vaginal progesterone.

***Keywords:*** GnRH Agonist, Vaginal Progesterone, Controlled Ovarian Hyperstimulation, Intrauterine Insemination

## P-165: Results of Adding Recombinant LH in Normoresponder Patients for Assisted Reproductive Technology Treatment: A Prospective Randomized Control Trial

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**Background:** Based on classical two cell- two-gonadotropin theory, in the follicle, FSH and LH put on their main effects on the granulosa and theca cells. LH is essential for androgens production. Androgens as precursor, is used for estradiol production by granulosa cells under aromatase activity. Profound suppression of LH concentrations in some normogonadotropic patients can cause several adverse effects. In ART cycles, women undergoing ovarian stimulation can experience sever LH deficiency following oversuppression of endogenous pituitary secretion due to GnRH analogues suppression.

**Materials and Methods:** In this study, 40 patients who were candidates for ART were randomly selected. In all patients long luteal protocol was used for ovulation induction. After down regulation with GnRH agonist, FSH alone was administered for 40 normoresponder patients until the dominant follicle reached 14 mm, then patients were randomly divided into two groups: Group 1 (n=20) with standard long protocol (GnRH agonist ) and r-FSH alone, Group 2 (n=20) with standard long protocol (GnRH agonist) and r-FSH with r-LH. Results were statistically analyzed and compared in two groups.

**Results:** The mean of age in group 1 was 31.35 and in group 2 were 31.85. In group 2 the number of retrieved oocytes, mature oocytes, cleaved embryos, transferred embryos, estradiol levels in HCG administration day, implantation rate and clinical pregnancy rate were higher but not statistically significant.

**Conclusion:** In our study administration of rLH in late follicular phase there was no beneficial effect on outcomes in young women with mean age of 31 years. Maybe a greater sample size should be used to see the effects more accurately; also it is possible that rLH becomes useful in older patients.

***Keywords:*** FSH, IVF, LH, Ovarian Stimulation

## P-166: Obstetric and Neonatal Outcomes of Twin Pregnancies Conceived by Assisted Reproductive Techniques Compared with Natural Conceived Twins: A Prospective Follow-Up Study

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**Background:** Comparison of obstetric and neonatal outcomes of twin pregnancies conceived by assisted reproduction technology (ART) with those of spontaneously conceived (SC).
**Materials and Methods:** In this prospective cohort study, all dichorionic twin pregnancies following fresh IVF/ intra-cytoplasmic sperm injection (ICSI) or ICSI cycles (n=320) and (SC) (n=170) in nulliparous women in Royan Institute with those conceived spontaneously in Arash Women's hospital (university-based hospital) were evaluated from January 2008 to October 2010 and followed up until delivery. We compared the obstetric and neonatal outcomes of SC and ART twin pregnancies.
**Results:** Multivariable analysis, adjusted for maternal age and body mass index, revealed the obstetrics outcomes were similar between groups but in ART group, neonatal outcome was associated with a higher risk of very preterm birth (odds ratio (OR): 5.2, confidence interval (CI):2.1-12.9), extreme low birth weight (OR: 2.2, CI: 1.0-3.9), neonatal intensive care unit admission (OR: 2.0, CI: 1.2-3.2) and perinatal mortality (OR: 2.3, CI: 1.2-4.0).
**Conclusion:** The maternal outcomes of ART dichorionic twins are comparable to those of spontaneously conceived; despite of attempting to perform the same obstetric management, the rates of very preterm birth, Extreme low birth weight, neonatal intensive care unit admission and perinatal mortality were significantly higher in the ART group.
***Keywords:*** Assisted Reproductive Techniques, Twin Pregnancies, Dichorionic Placenta, Obstetrics Outcomes, Neonatal Outcomes

## P-167: Risk Factors for Ectopic Pregnancy: A Case-Control Study

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**Background:** Ectopic pregnancy (EP) is a common condition which presents a major health problem for women of childbearing age. This study aims to analyze and identify risk factors that have the potential for EP and evaluating the contribution of the risk factors associated EP.
**Materials and Methods:** In this case control study, performed from 2006 to 2011 at Arash hospital, the case-patients were a total of 83 women were diagnosed with EP. Control group consisting of 340 women who gave birth at the center at which the cases were treated. The basic recorded information included demographic characteristics, smoking habits, surgical, gynecological, obstetrics, sexual, contraceptive and infectious history and also fertility markers. The association between EP and the factors studied was measured by ORs.
**Results:** In aggregate maternal age, spouse's cigarette smoking, gravidity, prior spontaneous abortions, history of EP, tubal blockage, use of IUD, tubal damage, duration of marriage and first pregnancy interval, history of infertility were factors associated with increased risk of EP. BMI, menstrual bleeding pattern and induced abortion indicated no association with EP.
**Conclusion:** By detection of historical and clinical risk factors of EP, a successful risk-reduction counseling program before conception can be implemented. Additional studies are needed to be performed on hormonal and immunologic factors may be involved in EP
***Keywords:*** Ectopic Pregnancy, Risk Factors, Spontaneous Pregnancy

## P-168: To Increase The Success of Assisted Reproductive Techniques with Iranian Traditional Medicine (ITM)

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**Background:** The use of assisted reproductive technique has been common for more than two decades that the success of these methods depends on several factors. However, despite the advances of modern infertility treatment succeed rate remains low. And despite the fact that in many cases the cause of infertility is clear, but in many cases the cause is unknown. In Iranian Traditional Medicine the most important cause of health and disease is the body's temperament and the organ. Therefore correction of temperament of women that they candidate for assisted reproductive techniques has a significant impact on the acceptance of fetus. So that this correction can reduce the rate of infections and abnormal vaginal secretion, increase acceptance of endometer, correct the hormonal levels and can create a balance in woman's body. So the succeed rate increases.
**Materials and Methods:** There are some advices and protocols for infertile couples in the main sources of ITM. The explanation of these protocols can be recommended for increasing the rate of treatment of infertile couples.
**Results:** In ITM, uterus looks like living for fertilization beyond all forces must be healthy. So while reinforcing uterus and ovaries in women and increasing motility and

count of sperm in males, we can increase the chance of fertility and also delivery too.
**Conclusion:** ITM protocols can increase the ability of giving and taking between the couple, so the success in assisted fertility techniques will be increased clearly. It seems that research on the method open new horizons to help to increase the success of assisted fertility techniques.
***Keywords:*** Temperament, Iranian Traditional Medicine, Infertility

## P-169: Protective Effect of Vitamin E on Cypermethrin-Induced Damages Correlates with P53 Gene Expression and Nitrosative Stress

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**Background:** Because of fast rate of degradation and low mammalian cells toxicity the cypermethrin (CPM) is a wide used insecticide in domestic agriculture and in veterinary medicine applications. The compound exerts its pathological impact by down-regulating the antioxidant status. Therefore, current study was designed to evaluate the protective effect of vitamin E on CPM-induced apoptosis and nitrosative stress on ovarian tissue.
**Materials and Methods:** Eighteen Mature female rats were divided into three (N=6) test and control-sham groups. The animals in test group 1 received CPM (75 mg/kg) and in test group 2 received vitamin E (150 mg/kg) + CPM by gavages, for 24 constitutive days. The normal saline was administrated in control-sham group. After evaluating the total RNA content the P53 gene expression was evaluated by RT-PCR. The tissue total protein (TP), total antioxidant capacity (TAC) and nitric oxide (NO) were examined.
**Results:** Vitamin E administrated animals were manifested with significantly ($p<0.05$) higher total RNA ($3986.33 \pm 24.79$) and protein ($3.25 \pm 0.03$) contents versus the CPM alone-administrated ($1847.34 \pm 19.94$ for RNA and $2.79 \pm 0.12$ for protein) group. The P53 gene expression was detected in CPM alone group while it was not observed in vitamin E-administrated group. The vitamin E up-regulated TP and TAC and reduced NO level compared to CPM alone group.
**Conclusion:** Our data suggest that CPM impact the ovarian tissue by inducing the nitrosative stress which promotes the P53 gene which in turn leads to cellular apoptosis. On the other hand vitamin E by up-regulating antioxidant status inhibited the damages.
***Keywords:*** Cypermethrin, P53, Nitrosative Stress, Total Antioxidant Capacity, Total Protein

## P-170: Animal Models of Human Artificial Ovary, Valuable Tools for Fertility Preservation in Cancer Patients

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**Background:** With all the recent advances in cancer treatments, many young cancer patients find themselves facing the prospect of losing their fertility after aggressive chemotherapy or radiotherapy. Cryopreservation of ovarian cortical tissue has emerged as a potential option to restore fertility in these young women.
**Materials and Methods:** Because autotransplantation of cryopreserved ovarian cortex carries the risk of reintroducing cancer to the patient in remittance, xenotransplantation of frozen-thawed ovarian cortical tissue to immunodeficient animal hosts has been suggested as an alternative, whereby primordial follicles are activated in an immunocompromised animal model and after initial growth are transferred to an *in vitro* culture system. This approach eliminates the risk of cancer cell reintroduction, and in addition, the hitherto unaccomplished phase of primordial follicle culture is bypassed. This combination of *in vivo* transplantation and *in vitro* culture to trigger maturation of primordial follicles has already been achieved in mouse models.
**Results:** Several grafting techniques, including heterotopic or orthotopic, have been reported basically differing only in the location to which the ovarian grafts have been transplanted, such as the bursal cavity, the kidney capsule, and subcutaneous sites. Furthermore, several types of grafts have been reported, including xenotransplantation of human ovarian cortex or isolated primordial/ preantral follicles combined with extracellular matrix (artificial ovary) or without it, to immunodeficient mice.
**Conclusion:** Xenotransplantation of isolated primordial/ preantral follicles combined with extracellular matrix represents a valuable tool for the study of preantral follicular development and will continue as such as long as routine *in vitro* development of matured follicles derived from primordial follicles, which is unavailable for other species than the mouse. Given the low availability of human reproductive tissue for research purposes, animal models can offer interesting alternatives.
***Keywords:*** Animal Model, Cancer, Fertility Preservation, Artificial Ovary

## P-171: Expression of Vascular Endothelial Growth Factor Receptors In Endometriosis

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**Background:** Endometriosis is a disease which is defined by the growth of endometrium-like tissue outside of the uterine cavity. Literatures show that VEGF by interaction with their receptors, Flt-1 (Fms-like tyrosine kinase-1 or VEGFR-1) and Flk-1/KDR (fetal liver kinase/ kinase-insert domain receptor or VEGFR-2) is related to pathogenesis of endometriosis. The purpose of this study was to evaluate the expression of VEGF receptors and also their relationship with development and survival of endometriotic lesions in the endometriosis throughout the menstrual cycle.

**Materials and Methods:** This study contain three groups (n=30). In patients with endometriosis, ectopic (ovarian endometrioma) and eutopic biopsies were obtained by laparoscopic procedure and piplle respectively. In women with no sign of endometriosis, control biopsies gained with piplle. The samples in each group were obtained in different stages of the menstrual cycle. Gene expression of VEGF was determined by RT-PCR and the quantitative level of gene expression was tested by Real Time PCR.

**Results:** VEGF receptors were expressed in all groups. In eutopic endometrium from women without endometriosis, the expression of VEGFR1 and VEGFR2 were significantly higher during the menstruation than the secretory and proliferative phases. However, a significant increasing were observed in VEGFR1 level in secretory phase as well as VEGFR2 level in proliferative phase in eutopic endometrium from women with endometriosis ($p<0.05$).

**Conclusion:** It seems that different regulation of VEGF receptors in pathological condition depends on the site of endometriosis. Our data showed that ovarian endometriomas are lesions with low angiogenic activity and low ability of remodeling the surrounding tissue.

*Keywords:* Angiogenesis, Endometriosis, VEGF Receptors

## P-172: Teratogenic Effects of Imidacloprid and Precocene1 on Reproductive Ability in Female Wistar Rats

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**Background:** Embryonic development is one of the most critical stages in animal life. This step is strongly influenced by environmental and internal factors. The widespread uses of agricultural pesticide and their teratogenic effect on fetuses is one of the biggest health challenges facing the human. We examined the effects of Imidacloprid (IMI) and Precocene1 (PRE1) on reproductive ability in Wistar rats.

**Materials and Methods:** 15 female adult rats were maintained under standard conditions and their gestation were confirmed by observation of the vaginal plagues (VP). From seventh up to twentieth days of gestation, IMI and PRE1 were injected intravenously at 10mg/kg concentration. Fourteen days after birth their blood were collected through the heart of newborn rats. The analysis of Estradiol, Testosterone, Progesterone, LH and FSH in blood samples was done by electrochemical luminescence technique. Number of infants after they reach the age of fertility tested for reproductive ability. Waiting times for intercourse and the number of litter born was considered.

**Results:** Estradiol level in female rats which treated with IMI, PRE1 and IMI + PRE1 compared to control group respectively showed 1.2, 1.25 and 1.28 percent reduction. Progesterone levels in female rats which were treated with IMI, PRE1 and IMI + PRE1 compared to control group respectively showed 1.1, 1.15 and 1.17 percent reduction. In all groups LH level was less than 0.1 mlu/ml and FSH level was less than 0.1 iu/l.

**Conclusion:** We can consider that PRE1 which can cause sterility in some insects and IMI as an inhibitor of acetylcholine receptors which may be effective on the reproductive system through the CNS. Serological results confirm that IMI and PRE1 could have effects on infertility in Wistar rats.

*Keywords:* Theratogenic, Imidacloprid, Precocene

## P-173: Evaluation of The Follicular Growth after Mouse Ovarian Organ Culture in The Medium Supplemented with Growth Differentiation Factor-9B (GDF-9B)

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**Background:** Growth differentiation factor -9B (GDF-9B) is an oocyte derived growth factor, this protein is essential for development of ovarian follicles and act mainly by binding to its receptor on the surface of granulosa cells. The effect of this factor on the growth of follicles in various developmental stages particularly primordial and primary follicles is unknown. The aim of this study was to investigate the effects of GDF-9B on the growth of cultured mouse ovarian follicles in various developmental stage.

**Materials and Methods:** Ovaries from 14 days old mice were cultured for 7 days. Ovaries were cultured without or with recombinant GDF-9B (20, 40 ng/ml). At the end of this time, serial sections were prepared to count follicles in various developmental stages.

**Results:** The results of this study show that culture of ovaries in medium containing of GDF-9B significantly increased the percentage of antral follicles and decrease the percentage of preantral follicles.

**Conclusion:** Overall, this study showed that GDF-9B stimulate differentiation of preantral follicles to antral stage. However this factor had any effect on the growth of primary and primordial follicles.

*Keywords:* Growth Differentiation Factor-9B (GDF-9B), Mouse, Ovarian Organ Culture

## P-174: The Effective of Fennel (Foeniculum Vulgare) to Reduce The Severity of Pre-Menstrual Syndrome (PMS) Symptom in Young Girls

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**Background:** Premenstrual syndrome (PMS) is a condition characterized by a number of behavioral, psychological and physical symptoms recurring cyclically during the luteal phase of the menstrual cycle.The uncertainty in the pathogenesis of PMS has led to many treatment protocols being suggested as possible therapies.The present study was carried out to compare the effects of fennel extracts on the PMS on high school students, Iran in 2012.
**Materials and Methods:** In this single-blind randomized clinical trial, 200 students were requested to fill pre-menstrual recognition form and then 24 students with moderate to severe PMS were selected and were randomly divided into Two equal groups.first group received fennel extracts and the second group was our control group The severity of PMS was measured by Daily Record of Severity of Problems (DRSP) questionnaire at the end of the first and second menstrual cycles before the intervention and the results were compared with them after the intervention in first cycle and second cycle. Data was analyzed by SPSS (version 11.5) and $p<0.05$ was considered statistically significant.
**Results:** There were not any significant differences in the means of premenstrual syndrome scores before the intervention among the two groups (33.27 ± 16 in fennel group and 31.36 ± 17 in control group, $p>0.05$). But the differences were significant after the intervention (42.49 ± 16 in fennel group and 34.90±17 in control group, respectively, ($p<0.009$).
**Conclusion:** Fennel extracts could reduce the severity of PMS and Administration of the extracts of this herb is suggested for relieving the signs and symptoms of PMS.
***Keywords:*** Fennel (Foeniculum Vulgare), Premenstrual Syndrome (PMS)

## P-175: Herbal Regimes and OHSS

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**Background:** Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication that occurs in the luteal phase of an induced hormonal cycle. The aim of this study is to assess the association of herbal regimes and OHSS.
**Materials and Methods:** This is a clinical trial study. 110 married patients were referred to Dr. Rasekh clinic from Aug 2011 to Nov 2012. All patients had a vaginal ultrasound before and after the diagnosis of OHSS. Data was analyzed by SPSS 15 software.
**Results:** The mean age was 30.7. 52.7% of cases didn't have history of infertility, but 38.9 % primary infertility and 8.3 % with secondary infertility. The Herbal regimes include; black pepper) 61%, (ginger)25.5 %, (Cinnamon) 27.3 %, (Thyme) 19.3 %, (cumin) 14.8 %, (Chamomile) 14.5 %, (dill) 14/5%, (saffron) 14%. Consumption period was from third to eighth day of menstrual cycle for 1 to 4 months. 32(37.2%) patients were normal BMI (18.5-24.09), BMI 34(39.5%) patients <18.5, BMI >24.09 in 15(17.4%) patients, BMI >30 in 5(5.8%) patients. Dosage of herbal regimes is One tablespoon (3g). 80(93.02%) individuals consumers of herbal regimes were mild OHSS, 5(5.8%) moderate OHSS,1(1.1%)severe OHSS.
**Conclusion:** We concluded that the indiscriminate use of herbal regimens can lead to OHSS. Some of patients have been used herbal regimens in combination of chemical drugs. We hope to obtain the appropriate dosages of herbal regimes that is safe and could be replaced by synthetic drugs with high side effects.
***Keywords:*** Infertility, Herbal, Regimes, OHSS

## P-176: Infertility and Smoking

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**Background:** Approximately 30% of reproductive age women and 35% of reproductive age men in the United States smoke cigarettes. Substantial harmful effects of cigarette smoke on fecundity and reproduction have become apparent but are not generally appreciated. smoking is becoming increasingly popular in many parts of the world as in eastern Mediterranean and Arabic countries including Iran. The aim of this study is To evaluate the impact of smoking on fertility outcome.
**Materials and Methods:** A literature review was undertaken using the scientific resources on the Internet such as Pubmed, Science direct, google scholar, to find about studies With the search words: Infertility -smoking-conception and complication, from 1990 to 2012.
**Results:** Overall, the literature strongly supports an association between cigarette smoking and infertility. The association between smoking and increased risk for infertility is statistically significant but not particularly strong in most studies. The association between smoking and decreased fertility is generally quite consistent across most studies. A number of studies have demonstrated a dose dependent adverse effect of smoking on fertility .Even at one-half pack per day use, female cigarette consumption has been associated consistently with decreased fecundity. Smoking is associated with increased risks of spontaneous abortion and ectopic pregnancy. Available biologic, experimental, and epidemiological data indicate that up to 13% of infertility may be attributable to cigarette smoking. Smoking appears to accelerate the loss of reproductive function and may advance the time of menopause by 1 to 4 years.
**Conclusion:** The accumulated evidence supports the

value of taking a preventive approach to infertility by discouraging smoking and helping to eliminate exposure to tobacco smoke in both women and men.

*Keywords:* Infertility, Smoking, Conception, Complication

## P-177: Effect of Mannitol Therapy Protocol on Ovarian Hyperstimulation Syndrome Management; Comparison between PCO and Non-PCO Patients' Outcome

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**Background:** Ovulation induction is one of the main steps of ART procedures. Ovarian hyperstimulation syndrome (OHSS) cause as a serious side effect of some hormonal fertility drugs like HCG. Patients with polycystic ovary (PCO) are the high risk group for OHSS in these procedures and required more attention for their management. OHSS presentations are varying from mild symptoms (like abdominal distention and ascites) to severe symptoms (like hypodynamic instability, kidney failure and respiratory distress). The pivotal intervention of OHSS management, is prescribing plasma expanders. One of these drugs is Mannitol that maintaining hemodynamic stability in early stages of OHSS to prevent severe complications. In this study, we describe and compare the outcomes of PCO with non-PCO patients by Mannitol therapy for management of OHSS.

**Materials and Methods:** In this retrospective case-control study, out of 320 OHSS patients during March 2008 to March 2012 from the IVF/ICSI candidate referred to Sarem Women's Hospital, the outcome of 100 initially PCO patients (cases) compared with 220 non-PCO patients. The protocol was intravenous injection of Mannitol 20%, 1000 cc/day for all patients till all symptoms appropriately removed and adequate diuresis established. Group matching for "age" and "weight" variables was conducted between case and control groups.

**Results:** Mean age for case group was 29.76 ± 5.15 years and for control group was 30.33 ± 4.7 years ($p>0.05$). Mean Weights were 68.9 ± 9.2 kg and 68.53±9.9 kg for case and control groups, respectively ($p>0.05$). Infertility type (primary or secondary), ovulation induction protocol and Mannitol therapy for both groups are the same. Mean hospitalization periods for Mannitol therapy were 3.98 ± 1.7 days and 3.44 ± 1.2 days, respectively ($p>0.05$). PCO group had higher level of OHSS severity compare with the non-PCO group ($p=0.0001$). There were no significant statistical differences in pregnancy success rate (15.6% against 16.3%- $p>0.05$), as an outcome of the IVF/ICSI processes. Note that no serious complications have been seen in all studied patients in treatment protocol.

**Conclusion:** This study results suggest that the Mannitol therapy is a sufficient treatment option for managing of the OHSS condition even in the case of PCO patients as a high risk group for ovulation induction. Mannitol therapy also has some other advantages in term of availability and inexpensiveness, in addition to acceptable efficacy and lack of important side effects.

*Keywords:* Mannitol Therapy, Ovarian Hyperstimulation Syndrome, Polycystic Ovary, Outcome

## P-178: Separation and Identification of Alkaline Phosphatase Isozymes during Pregnancy

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**Background:** Alkaline phosphatase (ALP), (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from various molecules in the body. In humans, ALPis present in all tissues such as liver, bile duct, kidney, bone, and the placenta which detection of its activity is so useful in molecular biology. Pregnancy is associated with normal physiological changes that assist fetal survival as well as preparation for labor. The aim of this research was evaluating the role of various ALP isozymes in the first trimester abortion.

**Materials and Methods:** Enzyme Assay: Blood samples were collected from (60) pregnant and (30) aborted women who admitted in Rasht hospital. ALPase activity was measured by using Stopped Spectrophotometric method. The change in absorbance at 410 nm was determined that is indicative of enzyme activity. Total Protein Determination: Protein concentration was measured by bradford method and absorbance determined at 595nm using bovine serum albumin (BSA) as standard. Separation of ALP Isoenzymes by Gel electrophoresis and Partial Purification of Human ALPase: Using SDS-PAGE, various isoenzymes of ALP were detected in serum of our participant. The collected samples were subjected to different steps of purification including precipitation by ammonium sulphate $(NH_4)_2SO_4$, dialysis and ion-exchange chromatography.

**Results:** It was found that ALP in serum of abort person had fewer isozymes in comparison with the pregnant group ($p<0.05$).

**Conclusion:** Although assay of ALP activity in serum is a common clinical test, the physiological function of this enzyme remains uncertain. ALP activity: This article will focus on biochemical results to pregnancy, which fall into 2 different categories. On the basis of our results we conclude that isozymes of ALP in serum are a diagnostic marker for pregnant women in high risk for early abortion.

*Keywords:* Alkaline Phosphatase, Isozymes, First Trimester, Abortion

## P-179: The Role of Fetal Sex on Preterm Labor

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**Background:** Preterm labor is a major problem in obstetric practice. Rates of preterm birth vary between different populations and ethnic groups. In maternal fetal medicine, gender differences in outcome are often observed. Epidemiologic studies have suggested that the incidence of preterm birth is also higher in pregnancies carrying a male fetus. The purpose of this article is to review the evidence that the sex of the fetus may has a role in increasing preterm labor risk and need to be considered amongst the known compounding risk factors for threat of preterm labor.

**Materials and Methods:** A population-based study comparing all singleton 28 - 36 weeks gestational age deliveries that between 2010 and 2012 was performed. We compared pregnancies with male vs. female fetuses. Patients with a previous cesarean section, preterm labor and vaginal bacterial vaginosis infection were excluded from the study. Statistical analysis was performed with the SPSS software and differences were considered significant at p values < 0.05.

**Results:** During the study period 2450 deliveries were occurred in our hospital. Ten percent of births were less than 36 weeks gestational age. Male infants had a greater incidence of preterm birth. The male/female ratios were 1.71. Rate of neonatal mortality was also consistently higher in male offspring (30 vs. 21%).

**Conclusion:** These results showed greater male susceptibility to preterm labor. Rate of mortality was also consistently higher in male offspring.

*Keywords:* Preterm Labor, Fetal Sex, Neonatal Mortality

## P-180: The Protective Effects of Exogenous Melatonin on Nicotine-Induced Changes in Mouse Uterus and Fallopian Tube

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**Background:** The aim of this study was to evaluate protective effects of melatonin on uterus and fallopian tube in female mice under treatment with nicotine.

**Materials and Methods:** Adult Female mice were divided into four groups. Group A: control animals received normal saline, group B: were injected with nicotine 40 µg/kg. Group C:were injected with melatonin, 10 µg/night. Group D:were injected with nicotine 40 µg/kg and melatonin 10 µg/night. All animals were treated 15 days intraperitoneally On the 16th day, all animals were dissected and uterus and oviducts were removed from the mice. Evaluations were made using KI-67, ER-ά for determining of proliferative activity and estrogen receptors and ELISA for assay of serum estradiol level.

**Results:** Administration of nicotine in group B, showed a decrease in estradiol level and ER-ά numbers both in uterus and oviduct ($p<0.05$), Melatonin alone, were not effective on serum estradiol level however it significantly increased ER-ά numbers in uterus in compare with controls ($p<0.05$). Melatonin was not affective on proliferative activity of epithelial cells both in uterus and oviducts. Administration of melatonin in last group preserved histology of uterus or oviduct better and increased ER-ά numbers in uterus and oviduct in compare with nicotine group($p<0.05$) .

**Conclusion:** This study indicates nicotine impairs histology of uterus and oviduct and melatonin ameliorate them partly trough alteration in ER-ά numbers without affect on proliferative activity.

*Keywords:* Nicotine, Melatonin, Uterus, Oviduct, Proliferative Activity, Estradiol

## P-181: Protective Role of Vitamin E As An Alternative Treatment for Ovariectomized Osteoporotic Rats

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**Background:** Osteoporosis one of the postmenopausal symptoms is characterized by bone loss. There is a link between excessive reactive oxygen species (ROS) formation, estrogen deficiency due to cessation of ovarian function and bone loss. Free radicals are responsible for causing osteoblast apoptosis and reducing osteoblastogenesis in bone remodeling. Vitamin E is a potent antioxidant with the ability to protect bone cells from damage by neutralizing free radicals .Its role to Scavenge ROS including in the process of osteoporosis is important. The present study was carried out in search for an alternative treatment of osteoporosis using vitamin E.

**Materials and Methods:** The ovariectomized rat model was used in this study. Thirty mature female Wistar rats weighing approximately 200 g were selected and randomly divided into ovariectomized control, and ovariectomized rats treated with vitamin E. in experimental group treated daily at the dose of 80 IU per kg dietary. It was administrated for two months. Then the femur bone of the animals was collected and tissue bone investigated under a light and electron microscopic level.

**Results:** The ovariectomized rats showed a significant decrease in bone mass density of femur in control group with an increase in resorption and reduction in both trabecular volume and number. Bone loss induced deterioration of bone trabecula in comparison with normal tissue bone they showed inappropriate lamellar structure and a large uncalcified bone matrix. In experimental group vitamin E prevented the reduction in trabecular bone content and decreased trabecular separation bone. Administration of vitamin E reversed bone loss and prevented destruction of bone tissue and trabecular formation. Serum biochemical assays revealed that vita-

min E. prevent osteoporosis induced in ovariectomized group ($p<0.05$).

**Conclusion:** In this study, we investigated the effects of vitamin E which act against of bone degeneration and alternation in bone after osteoporosis. It is suggested that vitamin E has the potential effect as an alternative and effective treatment for prevention of bone loss in postmenopausal osteoporosis especially order with other routine treatment methods.

***Keywords:*** Postmenopausal, Osteoporosis, Vitamin E, Ovariectomized Treatment

## P-182: The Comparison of Clinical and Laboratory Characteristics in Obese and Normal Weight Women with Polycystic Ovary Syndrome

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**Background:** The main characteristic of polycystic ovary syndrome (PCOS) is oligomenorrhea with clinical and biochemical signs of hyperandrogenism(HA) and the phenotypic spectrum of PCOS is very broad. There are very limited studies about the features of patients with PCOS in Iran. The purpose of this study is to compare the clinical and laboratory characteristics of PCOS between the overweight women and normal.

**Materials and Methods:** In this cross sectional study with control group, 368 patients with PCOS, aged 15-39 year, were investigated PCOS was diagnosed by Rotterdam criteria. The patients were divided in two groups [with body mass index (BMI) ≥25 and with BMI <25]. The demographic, clinical, biochemical and sonographic features of patients were registered in the questionnaires, then four presented phenotypes were determined by rotterdam criteria [irregular menes (IM)/PCO/HA, IM/PCO, IM/HA, PCO/HA]. Data were analyzed by statistical software of SPSS.

**Results:** The most common phenotype in both groups was IM/HA/PCO (54.89%) and the most common clinical signs were oligomenorrhea (81.2%) and hirsutism (65.67%). Differences between overweight and normal weight groups for hirsutism, irregular means, Galactorrhea, acne, alopecia, living place, family history of DM, acanthosis nigricans, polycystic ovary, the volume of ovary, testosterone, DHEAS, and the phenotypes of PCOS. In over weight group, the mean age (26.8 vs. 25.59. PV:0), the mean systolic blood pressure (114.4 vs. 106.66. PV: 0.01) the mean diastolic blood pressure (73.24 vs. 67.29, PV: 0.01), the prevalence of infertility (45.5% vs. 22.61% PV: 0) and the prevalence of android obesity (PV:0) were significantly higher than normal weight group.

**Conclusion:** In over weight group, the prevalence of infertility and android obesity and mean of age and SBP and DBP, were significantly higher than normal weight group.

***Keywords:*** Ideal Body Weight, Obesity, Polycystic Ovary Syndrome, Women

## P-183: TGF-β Level and Insulin Resistance during Normal Pregnancy

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**Background:** Pregnancy is associated with glucose metabolism disorders and insulin resistance (IR). The purpose of this study was to evaluate the role of tumor growth factor-β (TGF-β ) in IR during normal pregnancy.

**Materials and Methods:** This cross sectional study was carried out on 86 healthy pregnant women including 26, 23 and 37 of them in the 1st, 2nd and 3rd trimesters, respectively, and in 21 healthy non pregnant women. Serum TGF-β, resistin and insulin concentrations were measured by enzyme linked immunosorbent assay (ELISA) method. insulin resistance value were calculated using the homeostasis model assessment (HOMA- IR).

**Results:** Serum TGF-β level was also significantly increased in pregnant women as compared with maternal healthy controls. There was significant correlation between gestational age and body mass index (BMI) ($r = 0.28$, $p = 0.01$). There was not significant correlation between gestational age and insulin resistance (IR). We also did not find correlations between IR and TGF-β in pregnant women.

**Conclusion:** TGF-β does not appear to contribute significantly to pregnancy induced insulin resistance in healthy pregnancy.

***Keywords:*** TGF-β, Insulin Resistance, Pregnancy

## P-184: Investigation of Electronic Fetal Monitoring Application during Labor with Cesarean Section and Apgar Score

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**Background:** Electronic fetal monitoring was used in many hospitals before labor and during delivery . The objective of this study was to investigate whether the use of fetal monitoring had effect on neonatal outcomes and cesarean section rate.

**Materials and Methods:** A prospective study on monitoring neonatal Apgar and kind of delivery data, from deliveries occurring at Shahid Sadughi hospital in 1389-1390 were done.

**Results:** Four houndred pregnant cases were investigated. Cesarean section rate was 53% and vaginal delivery was 46.8%. The most of cesarean section cases were in fetal bradicardia (100%) and late deceleration (83.3%).The most of vaginal delivery was in acceleration(59.1%). The incidence of Apgar score less than 7

in tachycardia was 75% and the rest were in acceleration(9.5%) and other conditions.
**Conclusion:** Electronic fetal monitoring can be associated with fetal distress. Use of electronic fetal monitoring was associated with the increase of the cesarean section rate.
***Keywords:*** Electronic Fetal Monitoring, Apgar Score, Cesarean Section

## P-185: A Clinical Randomized Single Blind Trial of Medical Therapies for Menorrhagia Using Ibuprofen and Tranexamic Acid

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**Background:** To compare the clinical efficacy tranxamic acid and Ibuprofen in the treatment of ovulatory menorrhagia.
**Materials and Methods:** This randomized controlled trial was performed at my office one hundred women with ovulatory hemorrhagic were randomized to take one of two treatment regimens during four consecutive cycles: tranexamic acid 3gr daily on day 1-5, ibuprofen 1200 mg daily.
**Results:** Menstrual blood loss was reduced in tow treatment groups The success rate during the second treatment cycle was observed as 49% in trnexamic acid and 27% in Ibuprofen group.
**Conclusion:** Tranexamic acid was shown in this comparative study to have the highest efficacy and the most beneficial effect in the treatment of ovulatory menorrhagia.
***Keywords:*** Tranexamic Acid, Ovulatory Menorrhagia, Ibuprofen

## P-186: The Expression of IL-6Ra in Fallopian Tubes and Ectopic Pregnancy

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**Background:** IL-6 is a proinflammatory and immunoregulatory cytokine to be very high in endometriosis and pelvic inflammatory disease, which are known risk factors for ectopic pregnancy(EP). The effects of IL-6 on target cells occur via the formation of a high-affinity complex with IL-6Ra that expressed in the cilia of the epithelial cells. In the present study,we compared expression of IL-6Ra in the human fallopian tube(FT) of women with ectopic pregnancy and normal pregnancy.
**Materials and Methods:** In this study, a total of 24 FTs samples obtained from two groups including: 12 FTs of women that bearing an ectopic pregnancy(aged 22-35), 12 FTs of healthy pregnant women (aged 32-38). Ampullaris parts of Fallopian tubes were evaluated from specimens taken during tubal ligation in normal pregnancies or at operation for tubal surgery in ectopic pregnancies. Samples were processed for quantitative reverse transcriptase polymerase chain reaction to determine mRNA expressions of IL-6Ra.
**Results:** IL-6Rα mRNA is expressed in in both the FT from women with ectopic pregnancies and normal pregnancies .Expression of IL-6Rα mRNAs was lower in fallopian tube from women with ectopic pregnancies than in normal pregnant women but it was not significant ($p>0.05$).
**Conclusion:** There is not an association between expression of IL-6Rα and ectopic pregnancy. However, it was not possible to compare normal fallopian tube of women at comparable gestational age also it requires further study.
***Keywords:*** IL-6Ra, Fallopian Tubes, Ectopic Pregnancy

## P-187: Challenges of Commissioning Mothers in Iran for Selection of Surrogate Mothers

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**Background:** Surrogacy is one of the most controversial procedures in infertility treatment. Despite increasing of using this technology in Iran, there are few practical data about surrogacy. There isn't any study assessing experiences of commissioning mothers about surrogate mother selection. The purpose of this study was exploring of commissioning mothers' experiences in selection of surrogate mother.
**Materials and Methods:** This study has been done with qualitative approach from August 2010 to Jun 2012. The method of data collection was unstructured interview and sampling was purposeful. Generally 32 interviews were done with 15 commissioning mothers with gestational surrogacy and 5 infertility centers' personnel. Environment of research was Royan Research Center in Tehran and other governmental or nongovernmental infertility centers, hospitals and clinics that have done surrogacy in Iran. Data analysis has been done by use of conventional content analysis method.
**Results:** Finally the main theme of "Selection dilemma" was revealed that were indicative of partnerships' essence of their experiences about choosing the surrogate mother. This theme was including three sub themes: 1. Difficulty of finding surrogate Mothers" 2. "Encounter

with free market of surrogacy" 3. "Encounter with unsuitable womb renter candidates".
**Conclusion:** Selection of surrogate mother is the most challenging stage of surrogate motherhood and also the supportive system isn't efficient. Presentation of context based findings could help to attendants for accurate planning in future.
***Keywords:*** Commissioning Mothers, Iran, Surrogacy, Surrogate Mother

## P-188: Pregnancy Rates after Abdominal Myomectomy in Infertile Women

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**Background:** Uterine myoma is the most common benign pathology during reproductive age. It has been suggested that the presence of uterine myoma may cause infertility and recurrent pregnancy loss. The purpose of this study was to determine the effect of abdominal myomectomy as a therapy on pregnancy rate in infertile women.
**Materials and Methods:** We considered eligible for this study only the 82 infertile patients that have at least one intramural myoma > 5cm diameter and wishing to conceive after surgery. They did not present any plausible infertility factor, apart from the removed myoma. We have evaluated the pregnancy outcome following abdominal myomectomy.
**Results:** Pregnancy occurred in 44 women (53.7%). The pregnancy rates in women with <2 years versus ≥2 years of infertility were 67.1 and 47.7%, respectively. The localization of the myoma and the total number of tumors removed did not influence the outcome.
**Conclusion:** Our results suggest a benefit of myomectomy in infertile patients.The main determinant of pregnancy rate after surgery is the diameter of myoma and not its localization and number
***Keywords:*** Abdominal Myomectomy, Infertility, Pregnancy Rate

## P-189: Effect of Piroxicam Administration on Pregnancy Rate in Intrauterine Insemination (IUI) Cycles: A Randomized Clinical Trial

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**Background:** To investigate the effect of administering piroxicam on improving the success rate of IUI cycles in those suffering from unexplained infertility.
**Materials and Methods:** This was a randomized clinical trial being performed at Ghadir Mother and Child Hospital and Motahari clinics of Shiraz University of Medical Sciences. We included 260 women with unexplained infertility selected for IUI cycles. Patients were randomly assigned to receive piroxicam 10 mg/day at days 4-6 after IUI cycle or nothing (control group). Main outcomes included number of IUI cycles, pregnancy, abortion and multiple pregnancy rates.
**Results:** The mean age was found to be 28.8 ± 4.7 years and 28.9 ± 5.3 years in piroxicam and control groups respectively (p=0.873). The pregnancy rate (detected by positive β-hCG) was found to be 25 (19.2%) and 16 (12.3%) in piroxicam and control groups respectively (p=0.039). The prevalence of abortion was found to be 5 (3.8%) in piroxicam group and 5 (3.8%) in control groups (p=0.823). Five (3.8%) patients in piroxicam group experienced twin pregnancy while only 3 (2.3%) patients in control group had twin pregnancy (p=0.361). The pregnancy rate per cycle was also significantly higher in those who received piroxicam compared to controls (11.16 vs. 6.66; p=0.021).
**Conclusion:** Administration of piroxicam after IUI cycles is associated with decreased number of cycles and increased pregnancy rate and pregnancy rate per cycle in IUI cycles. However piroxicam does not have any effect on abortion, multiple pregnancy and ongoing pregnancy rates.
***Keywords:*** Piroxicam, Intrauterine Insemination (IUI), Pregnancy Rate, Abortion Rate, Multiple Pregnancy Rate

## P-190: Regression of Endometrial Hyperplasia after Treatment with Drug and Conservative Treatment in Early Abortion: A Case Series Study

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**Background:** The aim of the conservative treatment is the regression of the hyperplastic to normal endometrium. Conservative treatment of endometrial hyperplasia is based on the administration of agents with either antioestrogenic action or direct anti-proliferative effect on the endometrium. Progestagens have been used widely in the treatment of endometrial hyperplasias.
**Materials and Methods:** 19 patients with recurrent abortion were enrolled in this trial at the Mother and Child Hospital. In the primary visit, transvaginal ultrasound performed for all patients. If endometrial thickness was more than 12 mm, Before initiating of treatment, endometrial biopcy was done. Then, the patients were divided to 4 groups: group I took ocp for 3 months, group II took 200 mg (BID) danazol for 3 months, group III took medroxyprogesterone, 10 mg/d for 3 cycles and group IV had conservative treatment. After ending the treatments, the patients were allowed to become pregnant.

**Results:** Six of 7 patients with conservative treatment and 2 of 12 patients with drug treatment had abortion. 1 of 7 patients with conservative treatment and 10 of 12 patients with drug treatment had clinical pregnancy more than 12 weeks. The result of clinical pregnancy were statistically significant between conservative (14.3%) and drug treatment (83.3%) groups (p=0.006).

**Conclusion:** The use of drug treatment compare with conservative treatment in recurrent abortion with endometrial hyperplasia patients statistically significantly improved the pregnancy rate and decrease early pregnancy loss.

**Keywords:** Endometrial Hyperplasia, Early Abortion, Drug Treatment, Conservative Treatment, Clinical Pregnancy

# Genetics

## P-191: Association of STK11 Gene Polymorphisms with Methformin Treatment in Patients with Polycystic Ovary Syndrome

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**Background:** Metformin is an insulin sensitizing drug, which is co-administered with Clominophene citrate or gonadotropins in most cases of polycystic ovary syndrome (PCOS), to improve ovulation. STK11 gene is necessary for action of metformin. Therefore the study of STK11 polymorphisms is important as pharmacogenetics aspects of metformin. The aim of our study was to investigate polymorphism of STK11 gene in PCOS patients (40 people) in comparison to 2 other groups used as fertile (40 people) and drug response-control (40 people) groups to clarify whether any differences between three groups exists in observed single nucleotide polymorphisms (SNPs).

**Materials and Methods:** Multiple polymorphisms of STK11 were examined on DNA isolated from blood samples. In exons 6, 8 we used Polymerase chain reaction-restriction fragment length polymorphism (PCR- RFLP) while sequencing was undertaken to study exon 4, introns 1and 3 to detect known SNPs. Two amino acids of active site in exon 4 were also studied.

**Results:** The results indicated that polymorphism in intron 1 of STK11 was associated with PCOS.The highest percentage of individuals with GG genotype (37.5%) was in PCOS group. Individuals with this genotype had high insulin level and showed better response to treatment with metformin. Our fertile control group has showed 7.5% of GG genotype.

**Conclusion:** In total, 40% (48 out of 120) of our studied population, were shown SNP (rs2075606) (T>C) in intron 3. Data showed SNPs of exons 4, 6, 8 were not associated with PCOS. No amino acid change was observed in active site of this gene.

***Keywords:*** STK11, Polycystic Ovary Syndrome, Metformin, Polymorphism

## P-192: Association of Cytochrome P450 2D6 (CYP2D6) Gene Polymorphism with Clomiphene Citrate Treatment in Iranian Infertile Women with Polycystic Ovary Syndrome

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**Background:** Clomiphene Citrate (CC) is the most frequently administered drug for the treatment of female infertility [e.g. polycystic ovary syndrome (PCOS)]; which aims at restoring ovulation. Clomiphene is metabolized by CYP2D6, an important enzyme responsible for the metabolism of approximately 25% of clinically used drugs. CYP2D6 is very polymorphic and thought to result in inter-individual differences in metabolic activity. The aim of our study was to define allelic variants of CYP2D6 gene in PCOS patients in comparison to 2 other groups used as fertile and drug response-control groups to determine whether an association between patients' response to Clomiphene and CYP2D6 genotype exists in PCOS patients.

**Materials and Methods:** Blood samples were obtained from 40 PCOS infertile women referred to Royan Institute. Our control groups include 40 healthy fertile women and 40 women (with male factor infertility) under intra uterine insemination (IUI) treatment. PCR- RFLP (exons 1, 6) was undertaken on extracted DNA to detect known functional polymorphisms of CYP2D6 gene. Alterations of amino acids in the active site of exons 3, 4 and 9 were also studied by sequencing.

**Results:** Alleles *10 (C100T) and *2 showed most significant association with Clomiphene response in our patients. Patients who had allele *10 (a poor metabolizer phenotype, PM) showed some resistance to Clomiphene therapy while better response to drug was observed in CC genotypes (follicle diameter and number of follicles in these patients were higher). No amino acid change was observed in active site of CYP2D6. Other SNPs have detected also reported in our study.

**Conclusion:** We report for the first time that Clomiphene treatment is associated with CYP2D6 polymorphism in Iranian PCO patients. We have identified a new

polymorphism in intron 4 (+90 G>A) in Iranian women (13.5%).

*Keywords:* Cytochrome P450 2D6, Polycystic Ovary Syndrome, Clomiphene Citrate, Polymorphism

## P-193: Chlamydia Trachomatis Can Increase Sperm DNA Fragmentation in Infertile Men

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**Background:** Chlamydia Trachomatis (CT) is an obligate intracellular bacteria, that requires living cells to replicate itself and is responsible for lower urinary tract disease in men and women. It can cause urethritis, epididymitis, orchitis and complications such as chronic prostatitis in men. Chlamydia infections may also directly affect male fertility by damaging the sperm, because sperm parameters, proportion of DNA fragmentation and acrosome reaction capacity can be impaired with chlamydial infection. The aim of this study was to determine sperm DNA Fragmentation Index (DFI) in men with chlamydia trachomatis infection compared to control group.
**Materials and Methods:** This research was a case-control study. 258 males with abnormal sperm parameters and leukocyto spermia reffered to Royan Institute were examined for CT infection by ELISA technique, in which 20 samples were CT positive. The results were confirmed by molecular test using PCR for CT genome. DFI was evaluated by SCSA (Sperm Chromatin Structure Assay).30 males with normal sperm parameters considered as a control group.
**Results:** Means of DFI in CT infected patients and control group was 16.95 and 10.16 %, respectively. The DFI in CT infected group is significantly higher than that of in control group (p=0.013).
**Conclusion:** According to the results, patients with CT infection have higher sperm DNA fragmentation in comparison with fertile controls. This increase is commensurately greater than the influence on classical semen parameters and could result in a decreased fertility capability.

*Keywords:* Chlamydia Trachomatis (CT), DNA Fragmentation, Sperm Parameters

## P-194: Investigation The Association of Leukemia Inhibitory Factor Gene Polymorphism with IVF Outcome in Infertile Women

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**Background:** Clinical infertility is defined as the inability to become pregnant after 12 months of unprotected intercourse. Worldwide, more than 80 million couples suffer from infertility;the majority of this population are residents of developing countries. *In vitro* fertilization (IVF) is the most successful of the infertility treatments, and for many people is the last possibility for pregnancy. The implantation process is currently considered as the most relevant limiting factor for successful pregnancy. Thus, implantation failure of the trophoblast should be a major cause of repeated unsuccessful attempts at IVF. The embryo Leukemia inhibitory factor (LIF) is one of the key cytokines in the embryo implantation regulation and it is located at 22q12.2. The aim of this study was to investigate the C/T polymorphism in LIF gene for its involvement in IVF outcome.
**Materials and Methods:** This study included 60 infertile women who underwent an IVF cycle and 57 healthy volunteers. Genomic DNA was extracted from peripheral blood leukocytes. Genotypes were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Statistical analysis was performed using the MedCalc program for Windows version 12.
**Results:** No significant difference of the frequency of LIF gen polymorphism was observed between patients and control groups (p>0.05).
**Conclusion:** LIF gene polymorphism may not be associated with IVF outcome in this population.

*Keywords:* Gene Polymorphism, IVF Outcome, Leukemia Inhibitory Factor

## P-195: The Relationship between PAI-1 Polymorphism with Recurrent Implantation Failure (RIF)

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**Background:** RIF is the most common cause of unsuccessful pregnancy after IVF. Among the various causes of RIF, the role of maternal genetic factors is of great significance. PAI-1 polymorphism is one of the notable polymorphisms in this field. In this study, the relationship between this polymorphism and the recurrent spontaneous abortions and infertility in different populations has been investigated. In other words, the relationship between this polymorphism and the occurrence of RIF has been investigated because there have been no studies

on the patients suffering from New definitions of RIF in Iran.

**Materials and Methods:** This descriptive-analytical study was conducted in Montaserieh Research and Clinical Center for Infertility of Mashhad University of Medical Sciences. It was carried out on 80 infertile couples after IVF from 2006 to 2011. Based on the new definitions of RIF, participants were divided into three groups: 1. Control group: Forty participants whose transferred embryos were successfully implanted. The selection criterion was the observation of gestational sac in ultrasonography twenty days after IVF 2. Patient group RIF 1: Those participants who received IVF two times and six embryos. In this group, no sign of pregnancy or forming gestational sac was found. 3. Patient group RIF 2: Those participants who received IVF at least three times and ten embryos. Again no sign of pregnancy and gestational sac was found. After receiving 5cc blood containing EDTA from participants, the process of DNA extraction was performed. Genotype of PAI-1 gene was determined by using PCR-ARMS technique. Gained frequencies in different groups were compared with each other using chi-square statistical analysis.

**Results:** The frequency of 4G/5G genotype in control group, total participants, RIF1 group and RIF2 was 75,87, 80 and 95%, respectively. The frequency of 5G/5G genotype in control group, total participants patient group RIF 1, and patient group RIF 2 was orderly 25, 5, 5 and 5%. 4G/4G genotype was observed in none of the control groups and patient group RIF 1, But the observed 15% of patient RIF 2.

**Conclusion:** According to our results, 4G/4G Polymorphism of PAI-1, is seen just in the second group of the patient (RIF 2), with the new definition of recurrent implantation failure. Hypofibrinolysis as a result of the 4G allele of the PAI-1 gene appears to be a risk factor for implantation failure by limiting trophoblastic invasion. So, with increasing number of IVF failure role of genetic factors become more significant.

***Keywords:*** *In Vitro* Fertilization, Recurrent Implantation Failure, Polymorphism

## P-196: Lactation and mRNAs Expression of RFRP-3 and KiSS-1 in Hypothalamus of Rat

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**Background:** Comparison of the effect of lactation on expression of RFRP-3 mRNA in dorsomedial hypothalamic nucleus (DMH) and KiSS-1 mRNA in arcuate nucleus (ARC) of hypothalamus in lactating and non-lactating rat

**Materials and Methods:** Fourteen rats of the Sprague-Dawley strain were randomly allotted into three groups. The non-lactating rats (n=5) were separated from their pups immediately upon parturition. The lactating rats were allowed to suckle five pups for eight days (the period of increasing of lactation). Four ovariectomized rats were selected as control group. Relative expression of RFRP-3 and KiSS-1 mRNAs (compared to the control group) respectively in DMH and ARC of hypothalamus was determined using real-time PCR. Mean of data in lactating and non-lactating groups were compared by independent sample test (SPSS 11.5; $p<0.01$).

**Results:** Mean and SE of relative expression of RFRP-3 mRNA in DMH in the lactating group (62.0 ± 10.0%) was higher than that of the non-lactating rats (0.4 ± 0.1%, $p=0.001$). Relative expression of KiSS-1 mRNA in ARC was not different between the lactating (2.2 ± 0.3%) and the non-lactating rats (1.8 ± 0.4%, $p=0.4$).

**Conclusion:** Increased of RFRP-3 mRNA expression in DMH of hypothalamus while increasing of milk production in the rats may be the inhibitory factor of GnRH secretion. Whereas no change in KiSS-1 mRNA expression in the ARC of rats may indicate lower activity of the nucleus in production of the gene in both groups postpartum.

***Keywords:*** Lactation, RFRP-3 mRNA, KiSS-1 mRNA, Hypothalamus, Rat

## P-197: Aberrant Gene Expression Profile in Blastocyst Embryos As Consequences of Assisted Reproductive Technologies

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**Background:** Currently, ARTs are of indispensable importance in treatment of human infertility. While ARTs are considered to be safe, some studies suggest that ARTs manipulations may perturb the epigenetic information and subsequently the health of the offspring. To examine these events further, this study was conducted to assess the effect of superovulation, *in vitro* culture and vitrification on gene expression of several histone modifier enzymes and pluripotency factors at blastocyst stage.

**Materials and Methods:** In this regard female mice were randomly assigned to four experimental groups: in groups control (C) and superovulation (S), blastocysts were collected on day 3.5 after *in vivo* fertilization and development without and with superovulation females in groups C and S, respectively. In group superovulation + *in vitro* culture (SI), blastocysts were obtained from superovulated females after *in vivo* fertilization and *in*

*vitro* development in G1/G2 media from 2-cell to hatched blastocyst stage. In group superovulation + vitrified + *in vitro* culture (SVI) blastocysts were obtained from superovulated mice after *in vivo* fertilization, vitrification/warming at 2-cell stage and *in vitro* development in G1/G2 media from 2-cell to hatched blastocyst stage.

**Results:** The mRNA expression of Mll1 and Ash1l which mediate tri-methylation of H3K4 was the highest in C group which was not significant in compare to other groups ($p>0.05$). The analysis of mRNA expression of Tip60 and Gcn5, which acetylate H4K12 and H3K9, respectively, revealed that the level of mRNA was significantly lower in manipulated groups in compare to control ($p<0.05$) except in SVI group for Tip60. The mRNA expression of pluripotent markers including Sox2 and Nanog was significantly higher in C group in compare to other groups ($p<0.05$). The mRNA expression of Pou5f1 was significantly higher than S group, while it was not significant for SI and SVI groups.

**Conclusion:** ARTs manipulations may alter the epigenome of the resultant offspring.

*Keywords:* ARTs, Histone Modifier Enzymes

## P-198: Analysis of Expression Level of TEX12 Gene in Testis Tissues of Severe Oligozoospermic and Non-Obstructive Azoospermic Men

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**Background:** During the first meiotic prophase, alignment and synapsis of the homologous chromosomes are mediated by the synaptonemal complex. Incorrect assembly of the synaptonemal complex leads to impaired recombination and cell death, which in humans, causes infertility in males. Testis-expressed gene 12 (TEX12) is a germ cell-specific gene that is located on the chromosome 11 (11q22) in humans.TEX12 is exclusively expressed in mice and humans testis. TEX12 protein is small (14 kDa) and does not contain any known protein domains. TEX12 is a component of the central element structure of the synaptonemal complex required for propagation of synapses and maturation of early recombination events into crossovers. The absence of TEX12 results in a disrupted central element and only partial synapsis of the meiotic chromosomes, which could have consequences for the progression of meiotic recombination and results in male infertility.

**Materials and Methods:** Testis tissue samples were obtained from 10 patients with severe oligozoospermia and 10 patients with non-obstructive azoospermia who were referred to the Royan institute. Total RNA was extracted and cDNA was synthesized. Quantitative real-time RT-PCR was performed using Power SYBR Green kit.

**Results:** Normalizing the relative amount of TEX12 transcript by the amount of GAPDH transcript in the same sample, indicated that expression of TEX12 in the testis samples of patients with non-obstructive azoospermia(arrested in spermatosytic level) is significantly reduced as compared with oligozoospermic patients (p value= 0.05).

**Conclusion:** According to the results, we can conclude that TEX12 expression levels are essential for normal spermatogenesis and deficiency in this gene can cause spermatogenic failure and infertility in men.

*Keywords:* Male Infertility, Non-Obstructive Azoospermia, Oligozoospermia, TEX12

## P-199: Genetic Variation Analysis of MIF in Endometriosis Patients

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**Background:** Macrophage migration inhibitory factor (MIF) is a key pro-inflammatory cytokine that is secreted by active macrophages accumulated in ectopic tissue of endometriosis. It involves in pathophysiological events of endometriosis such as angiogenesis, cell proliferation and it can stimulate the synthesis of PGE2 that are necessary for survival and establishment of ectopic endometriosis tissue. There are two polymorphisms in MIF promoter at positions -794 (CATT)5-8 and -173 (G/C) which both are related to promoter activity.

**Materials and Methods:** Genomic DNA of 42 patients with endometriosis, who had undergone laparoscopy during 2012, and 50 unrelated controls women without endometriosis were amplified via PCR. Restriction fragment length polymorphism (RFLP) was applied to determine -173G/C polymorphism and -794 (CATT) 5-8 was detected by sequencing in all samples.

**Results:** MIF -173G/C was identified in both patient and control groups, all probable genotypes (G/G, G/C and C/C) were observed in patients group, however G/G and G/C were only detected in control group. The distribution frequencies of G/G, G/C and C/C genotypes in patient group were 69, 10 and 21%, respectively, although in control group, frequencies of G/G, G/C were 78 and 22%, respectively, that were different. Detected number of -794 CATT repeats was 5-7 in our study. Homozygote of -794(CATT)5 only observed in control group (19%). Haplotype of -794(CATT) 5/-173G in control group was 92% and -794(CATT) 7/-173C in patient group was 50%.

**Conclusion:** We report for the first time that endometriosis is associated with C/C genotype in -173 and -794(CATT) 7. Since homozygote of -794(CATT) 5 and -794(CATT) 5/-173G demonstrated in control samples thus low-expression of MIF is associated with healthy group. -794(CATT) 7/-173C was observed more fre-

quently in patients group (50% vs. 31%). Therefore it seems that polymorphism of MIF promoter might be considered as an important factor in pathophysiology of endometriosis.

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

## P-200: Effects of The ω3 Fatty Acid Eicosapentaenoic Acid on PPARa and Aromatase in Human Granulosa Cells

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**Background:** The ω3 fatty acid eicosapentaenoic acid is currently used in the clinic as lipid-lowering and anti-inflammatory supplements. The present study was designed to further explore the effect of EPA on PPARα and aromatase gene expression of human granulosa cells.

**Materials and Methods:** Human luteal granulosa cells cultured *in vitro* were exposed to a range of concentrations (from 25 up to 100 μM) of the EPA, and investigated with respect to PPARα and aromatase gene expression by Real-Time PCR.

**Results:** EPA induced both PPARα and aromatase gene expression *in vitro* at a concentration as low as 50 μM ($p<0.01$). Exposure of cells to the EPA agonist induced a time and dose-dependent increase in aromatase gene expression (+36%; $p=0.006$).

**Conclusion:** The present study indicates that EPA treatment of human granulosa cells induces PPARα mRNA, which is accompanied by increased aromatase gene expression. These data provide evidence for a EPA-induced increase in estrogen production in granulosa cells. We hypothesize that pharmacological effects of EPA may involve actions on granulosa cells hormone secretion.

***Keywords:*** EPA, PPARa, Aromatase, Granulosa

## P-201: The Role of P53 Family Members in Infertility

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**Background:** P53, p63, and p73 transcription factors which are belong to The p53 family, are conserved during evolution. They have important roles in many molecular and cellular functions, including tumor suppression, the development of epithelial cell layers, and the development of central nervous system and immune system. Studies show these molecules also have role in maintaining the genomic integrity of germ cells and help faithful development and reproduction. Embryonic implantation are regulated by p53 through transcriptional regulation of leukemia inhibitory factor (LIF), a cytokine crucial for blastocyst implantation. TAp63 regulates cell cycles in female germ cells during meiotic arrest and protect them. TAp73 is regulator of function in ovary and oocytes. In this article we have reviewed studies regarding role of these molecules in male and female fertility and gathered their results in order to gain a better understanding of relationship of these molecules and fertility success.

**Materials and Methods:** We have conducted a thorough literature search using search engines in the medical databases and collected the data and research results regarding these molecules and fertility. We focused on polymorphisms, mutations in genes of these protein, expression patterns of the proteins and interactions with each other, and they have been evaluated to elucidate whether these variations are involved in the infertility.

**Results:** There are polymorphisms and mutations in these genes that may alter expression of them and may have role in human fertility. These variations may affect maturation of egg, normal mitosis in the developing blastocyst and Embryonic implantation.

**Conclusion:** To clarify the functions of The p53 family molecules, future studies need to investigate the polymorphisms in these genes and protein levels, and examine the role of these proteins in human fertility success. Understanding functions of the p53 family proteins and their variations may lead to new strategies for infertility treatment.

***Keywords:*** Infertility, P53 Family, Polymorphisms, Mutation, Gene Expression

## P-202: Reduced Expression of JMJD1A Histone Demethylase Gene in Testis Tissues of Infertile Men Referred to Royan Institute

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**Background:** Epigenetic modifications are involved in different cellular processes through regulating chromatin dynamics. histone methylation is an important modification that can be dynamically regulated by histone methyltransferase and histone demethylase enzymes. JMJD1A (also known as JHDM2A and KDM3A) is a histone demethylase specific for H3K9me2/me1. JMJD1A is a key epigenetic regulator that highly expresses in the testis and has an important role in male germ cell development. It activates expression of genes involved in sperm chromatin condensation and maturation by removing

the repressive H3K9me2/me1 modification from their promoters.This study aimed to evaluate the expression profile of JMJD1A gene in testis tissues of infertile men.

**Materials and Methods:** Ethical approval and informed patient consent was gained for the use of tissue samples. Testicular biopsies were collected from 20 infertile men referred to Royan Institute and underwent testicular sperm extraction (TESE). Through pathological and spermogram analyses, these samples distributed into 4 groups: hypospermatogenesis (positive control), sever oligoasthenoteratozoospermia, complete maturation arrest at spermatid level and sertoli cell only syndrome (negative control). Total RNA was extraced from the tissue samples. After synthesis of first-strand cDNA, quantitative real-time PCR was performed using designed JMJD1A primer pairs.

**Results:** qRT-PCR analysis significantly revealed lower expression of JMJD1A in all 3 sample groups with spermatogenesis defect in comparison to positive control.

**Conclusion:** These results indicate that JMJD1A deficiency can cause to defective spermiogenesis, and JMJD1A demethylase may be considered as an epigenetic biomarker in male infertility.

*Keywords:* Male infertility, JMJD1A, Epigenetics

## P-203: Examination of FMR1 Gene Transcription and Protein Expression in Patients with Diminished Ovarian Reserve Reffered to Royan institute

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**Background:** Diminished ovarian reserve (DOR) is a primary infertility disorder characterized by a reduction in the number and/or quality of oocytes, usually accompanied by high follicle-stimulating hormone (FSH) levels and regular menses. DOR aetiology factors are different, such as genetic factors, ageing, autoimmune disorders, adrenal gland impairment factors and iatrogenic causes, e.g. chemotherapy. Premature ovarian failure (POF) is related to DOR and both are diagnosed by high levels of FSH. Furthermore, DOR has been described as a feature of POF. The association between FMR1 premutations and the POF disease has suggested that the FMR1 gene acts as a risk factor for the POF and recently for the DOR. In this study mRNA and protein expression of FMR1 gene in granulosa cells of women with DOR were analyzed.

**Materials and Methods:** Local ethical approval was gained for this study and informed consent was given by patients. Granulosa cells were collected from 30 infertile women referred to Royan Institute. Samples distributed into two groups(DOR patients and non-DOR patients) based on the number of follicles, FSH levels and the number of CGG repeat. The DOR patients group included samples that had higher levels of FSH(>11), number of CGG repeats between 50-200 and follicles number lower than three .Each group contained 15 samples. Using reverse transcription and quantitative real-time PCR reaction(qRT-PCR) methods, the expression profile of FMR1 gene was evaluated. Also expression of the FMR1 encoded protein(FMRP) was analyzed by immunocytochemistry on ovarian granulosa cells biopsy.

**Results:** Significantly higher expression levels of FMR1 gene in granulosa cells of DOR patients compared to non-DOR patients was shown. FMR1 protein expression was also shown in granulosa cells of DOR patients.

**Conclusion:** Our data suggest that within the permutation range, a strong correlation is observed between translation of FMR1 mRNA and the number of CGG repeats in premutation alleles.

*Keywords:* DOR, FMR1 Gene, POF

## P-204: Evaluation of FMR1 Gene Regulatory Region for The Epigenetic Mark of H3K9 Acetylation in Blood Cells of Patients with Diminished Ovarian Reserve Reffered to Royan Institute

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**Background:** Diminished ovarian reserve (DOR) is a heterogeneous disorder causing infertility, characterized by a decreased number of oocytes and high FSH level, the genetic cause of which is still unknown. The association between FMR1 premutations(50-200 CGG repeats) and the premature ovarian failure( POF) disease has suggested that FMR1 gene acts as a risk factor for POF and recently for DOR pathogenesis. Expansion of CGG repeat numbers results in silencing of FMR1 gene expression. This expansion triggers methylation of FMR1 CpG island, hypoacetylation of associated histones, and chromatin condensation, all characteristics of a transcriptionally inactive gene. This study aimed to evaluate the acetylation level of lysine 9 of histone 3(H3K9ac), on the regulatory region of FMR1 gene in blood cells of patients with DOR

**Materials and Methods:** Local ethical approval was gained for this study and informed consent was given by patients. Blood samples were collected from 30 infertile women referred to Royan Institute. These samples distributed into two groups (DOR patients and non-DOR patients), based on the number of follicles, serum FSH level and number of CGG repeats. The patients group included samples that had higher levels of FSH (>11), number of CGG repeats between 50-200 and follicles number lower than three. Each group contained 15 samples. Using chromatin immunoprecipitation(ChIP) coupled with real-time PCR, H3K9 acetylation changes in the promoter and exon1 regions of FMR1 gene were quantitatively compared

**Results:** The data clearly demonstrated that incorporation of H3K9ac were significantly higher in the regulatory region of FMR1 in DOR patient in comparison with non-DOR patients

**Conclusion:** Our experiments indicate that H3K9 acetylation can be considered as a dynamic epigenetic switch in regulation of FMR1 gene in DOR, causing female infertility. This indicates that the promoter and exon 1 regions are the critical sites for epigenetic regulation of the FMR1 gene in DOR

*Keywords:* DOR, FMR1 Gene, H3K9 Acetylation

## P-205: Production of Recombinant Fish FSH Hormone in Pichia Pastoris

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**Background:** Follicle-stimulating hormone (FSH) belongs to the family of glycoprotein hormones that composing alpha and beta subunits with non-covalently bonds. This hormone involve in regulation of the reproductive processes such as gamete generation and follicular growth. Injection of the hormone in most of fish species increases 17 beta-estradiol production by ovarian tissue and also stimulates 11-keto testosterone production in testes. The purpose of this research is production of recombinant fish FSH hormone (rfFSH) by Pichia pastoris expression system under post-translational modification.

**Materials and Methods:** The alpha and beta fish FSH chains was separately cloned in pTZ57R / T vector and was subcloned into pHILS1 expression vector. This vector (pHILS1) was transformed into competent cell (Pichia pastoris yeast) by electroporation. Transformed clones were suspended in BMMY liquid medium and induced by 0.5% methanol to high levels of expression. The medium was centrifuged at 14000 rpm for 4 minutes for separating Yeast cell from media. Finally, the separated media containing target protein concentrated by Amicon Ultra centrifugal filter for detecting protein by SDS-PAGE and Western blotting techniques.

**Results:** The integration of both vector including alpha and beta FSH genes in the yeast genome was confirmed by PCR with pHILS1 vector-specific primers (5'AOXI and 3'AOXI). Also expression and secretion of rfFSH hormone was confirmed by SDS-PAGE and Western blotting techniques.

**Conclusion:** Pichia pastoris can be an ideal protein expression system for producing a protein with high specific biological activity and correct folding pattern, also it can be used as a model for increasing expression level of glycoprotein hormones by recombinant DNA technology.

*Keywords:* Follicle Stimulating Hormone (FSH), Pichia Pastoris, SDS-PAGE, Western Blotting

## P-206: Genetic Variations of FSH Receptor Gene in Patients with Premature Ovarian Failure and Diminished Ovarian Reserve Referred to Royan Institute

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**Background:** Immaturity of the ovarian follicles results in an infertility citation called Premature Ovarian Failure (POF), affecting approximately 1% of women under the age of 40. Women with this disorder go through early menopause and have high levels of gonadotropin hormones (FSH & LH). Diminished Ovarian Reserve (DOR) is another infertility disorder in which women's ovaries have the ability to go through early menopause. On the other hand, follicle stimulating hormone has a critical role in the maturation of the ovarian follicles. FSH will start a signaling cascade in the granulosa cells after sitting on its receptor. Inactivating of this receptor may arrest follicle maturation and therefore result in POF development.

**Materials and Methods:** The presence of 566C>T, 1555C>A and 1043C>G inactivating mutations and 1572C>G and 1993A>G polymorphisms were analyzed in a case control study. 43 Iranian POF and 27 Iranian DOR patients who had been referred to Royan Institute and had high levels of FSH hormone, normal karyotype and wild type FMR1 gene were selected as the case group. 40 Iranian fertile women were enrolled as the control group. The patients DNA were extracted from their peripheral blood and amplified by relevant primers. For determining allelic variant status, RFLP and SSCP with Sequencing were done with the amplified PCR products.

**Results:** Wild type genotype was seen in all cases. A common polymorphism (919G>A) was seen in all three groups. There was no significant difference between them. Hence no inactivating mutations were seen in Iranian POF and DOR patients.

**Conclusion:** Although these mutations and polymorphisms, especially 566C>T were seen in some other populations, this study showed that FSHR gene inactivating mutations and polymorphisms are not frequent in Iranian POF and DOR patients.

*Keywords:* Follicle Stimulating Hormone Receptor, Polymorphisms, Inactivating Mutations, Premature Ovarian Failure, Diminished Ovarian Reserve

## P-207: Pharmacogenetic Study of CYP19A1 (Aromatase) in Ovarian Induction in Iranian Polycystic Ovarian Syndrome Patients

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**Background:** Hyper-androgenemia is one of the main clinical features of polycystic ovarian syndrome (PCOS). Letrozol is an aromatase inhibitor drug, which is co-administered with gonadotropins in most cases of PCOS, to improve ovulation. Aromatase has a critical role in catalyzing the conversion of androgens to estrogens and is responsible for keeping the homeostatic balance between them. Hence variations in its gene, CYP19A1, might be associated to syndromes of androgen excess such as PCOS.
**Materials and Methods:** The aim of our study was to investigate polymorphisms of CYP19A1 gene in PCOS patients (55 people). The comparison was against 2 control groups which included 40 healthy fertile women and 45 women (with male factor infertility) under intra uterine insemination (IUI) treatment (drug response-control group). The study investigated the associations between above three groups and observed SNPs of CYP19. The expressions of CYP19A1 mRNA and protein in patients who had reached IVF treatment cycle was studied to compare with the observed polymorphisms.
**Results:** PCR- RFLP was undertaken (exons 7, 9 and 10) on extracted DNA from blood samples to detect known functional polymorphisms of CYP19A1. Alterations of amino acids in the active site of exon 8, a TCT ins/del and a polymorphic TTTA repeat in intron 4 were also studied by sequencing. Finally the mRNA and protein expressions of 5 PCOS patients in comparison to 5 drug response-control patients was studied with real time and western blot techniques, respectively.
**Conclusion:** No association was observed between SNPs of exons 7, 9 and 10 with PCOS. However, an association between polymorphism rs700519 in exon 7 and improvement of treatment (Follicular Diameter) was seen in cases who had taken Letrozol. No amino acid changes were observed in the active site of this gene. In addition, more studies must be done on the expression of CYP19A1 gene in both mRNA and protein levels.
***Keywords:*** Aromatase, CYP19A1, Polycystic Ovarian Syndrome, Letrozol, Polymorphisms

## P-208: Analysis of H2BFWT Gene Alterations in Severe Oligospermic and Azoospermic Infertile Men Referred to Royan Institute

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**Background:** Telomeres play a dramatic role in sperm pronuclei formation and subsequently successful fertilization. The H2B family, member W, testis specific (H2BFWT) gene encodes a testis specific histone that co-localized with telomeric sequences and interfere in the dynamic rearrangement of telomeres at late stages of spermatogenesis. H2BFWT is essential for transmission of the telomeric chromatin through generations. This protein is also important in regulation of spermatogenesis and early chromatin remodeling at fertilization. Two recent allelic association studies on −9C>T (rs7885967) and 368A>G (rs553509) polymorphisms in H2BFWT gene among different populations suggested that genetic variations of this gene could influence the susceptibility to spermatogenesis impairment. In this study the correlation of H2BFWT gene polymorphisms with male infertility was investigated in Iranian population.
**Materials and Methods:** To study genetic alterations of two single-nucleotide polymorphisms (SNP) loci, −9C>T and 368A>G in H2BFWT gene, genomic DNA from the peripheral blood samples of 92 infertile men suffering from azoospermia and severe oligospermia and 46 fertile men with normal semen parameters referred to Royan Institute were extracted by salting-out method. Extracted DNA amplified using Polymerase Chain Reaction (PCR). PCR products were then digested with position specific restriction enzymes and analyzed by electrophoresis on 3% agarose gel.
**Results:** Statistical studies indicated that the distribution frequencies of −9C>T (p=0.840) and 368A>G (p=0.864) had no significant difference between the infertile groups and control.
**Conclusion:** These findings showed no notable association between the −9C>T and 368A>G polymorphisms of H2BFWT gene and the risk of male infertility in Iranian population. However, direct sequencing is now ongoing to get new SNPs of H2BFWT gene in this population.
***Keywords:*** H2BFWT, Male Infertility, Spermatogenesis Impairment

## P-209: Decreased Expression of Histone Acetyltransferase CDY1 Gene in Testis Tissue May Lead to Decreased Expression of Transition Protein (TNP) and Protamine (PRM) Genes, Causing Male Infertility

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**Background:** Infertility is a complex medical problem. About 15% of couples are infertile, and male infertility being involved in roughly 50% of the cases. Among these, many cases are associated with a severe impairment of spermatogenesis. During the last stage of spermatogenesis (spermiogenesis), sperm chromatin endures complex modifications in which histones are lost and depositioned with transition proteins (TNPs) and protamines (PRMs), respectively. These processes include chromatin modifications mediated by different histone modifying enzymes resulting in compaction and protection of chromatin. A testis specific histone acetyltransferase named CDY1, which encodes a member of Chromodomain Y family of genes, causes hyperacetylation of TNP and PRM genes involved in sperm chromatin condensation. This study aimed to evaluate the relationship of CDY1 gene expression, with expression of TNP1, TNP2, PRM1 and PRM2 genes in testis tissues of subfertile men.
**Materials and Methods:** Local ethical approval was gained for this study and informed consent was given by patients. Testicular biopsies were collected from 20 infertile men referred to Royan Institute and underwent testicular sperm extraction (TESE). Through pathological and spermogram analyses, these samples distributed into 4 groups: hypospermatogenesis (positive control), severe oligoasthenoteratozoospermia, compelete maturation arrest, and Sertoli cell only syndrome (negative control). Each group contained 5 samples. Using reverse transcription and quantitative real-time PCR (qRT-PCR) methods, the expression profile of CDY1، TNP1 ،TNP2، PRM1 and PRM2 genes were evaluated.
**Results:** The data significantly showed lower expression levels of CDY1، TNP1 ،TNP2، PRM1 and PRM2 genes in testis tissues of all four patient groups compared to positive control.
**Conclusion:** This data demonstrated that defective expression of the histone acetyltranferase CDY1 gene, can cause to lower expression of TNP and PRM genes during spermiogenesis, leading to impairment in condensation of chromatin of sperm and subsequently male infertility.
***Keywords:*** Spermiogenesis, CDY1, TNP, PRM

## P-210: Estrogen Receptor Beta Gene Polymorphisms and Recurrent Pregnancy Loss: A Case- Control Study in A Population of Iranian Women

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**Background:** Recurrent pregnancy loss (RPL), that affects 1-5% of couples, is a multifactorial disorder as both genetic and environmental factors are involved. The aim of this study was to determine association of single nucleotide polymorphisms (SNPs) located on estrogen receptor beta (ERβ) gene with the risk of RPL in a population of Iranian women; +1082G>A (rs1256049) in exon 5, +1730G>A (rs4986938) in exon 8, and rs1256030 C/T in intron 2.
**Materials and Methods:** In this case-control study, two hundred and fifty women with a history of three or more consecutive pregnancy losses before 20th week of gestation and a hundred and five healthy women with at least two live births and no history of pregnancy loss were included. Genotyping was performed through polymerase chain reaction- restriction fragment length polymorphism analysis.
**Results:** Among the three SNPs examined, there was a significant difference in the genotype distribution of the +1082G>A polymorphism; the GG, AG genotypes was different between the case and control groups ($p< 0.05$). However, we found no significant differences in the +1730G>A and rs1256030 C/T polymorphisms ($\chi(2)=0.07$, $p=0.421$; $\chi(2)=0.233$, $p=0.895$, respectively) between the subjects with RPL and controls.
**Conclusion:** Our current findings suggest that +1082G>A and +1730G>A polymorphisms which are located on functional region of ERβ gene may influence pregnancy outcome in Iranian women with RPL.
***Keywords:*** Estrogen Receptor, Polymorphism, RPL, RFLP

## P-211: Quantitative Changes of Fetal DNA in Maternal Circulation during Pregnancy Based on Detection of SRY Gene in Ovine Species

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**Background:** It is well documented that fetal DNA can cross the placenta and is present in peripheral maternal blood during pregnancy in human. This fetal DNA also named circulating cell free fetal DNA, has emerged as a valuable source for genetic evaluation. Compared with humans, ovine species have a different structure of placental (synepitheliochorial) with no direct contact between the trophoblast and the maternal blood, hence, it is likely that the passage of fetal DNA to the ovine maternal blood would be scarce. This study is carried out to quantify the amount of fetal DNA in maternal blood plasma during pregnancy using quantitative real-time PCR (qPCR).
**Materials and Methods:** Fetal DNA was isolated from blood plasma of 46 pregnant ewes during the second to fifth month of gestation. Real-time quantitative PCR technique was used to evaluate the quantitative changes of fetal DNA during pregnancy based on detection of SRY gene.

**Results:** The SRY 286-base pair fragment was detected in all samples with male pregnancies, but no female pregnancies. The sensitivity and specificity of tests were 100% with no false negative or false positive results. It was also determined that fetal DNA levels are significantly increased during pregnancy, up to approximately 1.65-fold in the last 2 months of pregnancy ($p < 0.05$).

**Conclusion:** The results of quantitative analysis demonstrated a significant increase in the amount of fetal DNA in maternal circulation in later stages of pregnancy.

*Keywords:* Circulating Fetal DNA, SRY, PCR, Ewe

## P-212: Ala 307 Thr Polymorphism of FSHR in Iranian Patients with Polycystic Ovary Syndrome (PCOS)

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**Background:** Follicle stimulating hormone (FSH) receptor plays an important role in FSH signaling during reproductive development. Any changes in FSHR gene is resulted in alteration of amino acid in protein chain, consequence to affect the binding affinity, intracellular signaling and expression levels of FSHR. FSHR polymorphisms can be involved in etiology of many reproductive disorders such as polycystic ovary syndrome. We aimed to investigate the association of rs6165 polymorphism (Thr307Ala) which can influence the FSH receptor binding affinity to FSH, with Iranian PCOS patients and correlation with drug (exogenous FSH) response.

**Materials and Methods:** A case control study including 90 individuals (30 fertile controls, 30 control 1 and 30 PCOS patients) was performed to investigate the association of SNP rs6165 with drug response in PCO patients. The control group 1 (patients with male factor infertility) and PCO patients were candidates for Intra Uterine Insemination. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was implemented on the DNA isolated from blood samples to genotype this SNP.

**Results:** There was significant difference between the percentage of individuals carrying this polymorphism in patients (70%) and control groups (23%). PCO patients with this SNP were shown more response to ovulation induction.

**Conclusion:** There is association between PCOS and rs6165 polymorphism. Further studies are needed to clarify the mechanism of action of this polymorphism. Although, it seems the change of amino acid at position 307 consequences to increase the sensitivity of FSH receptor, which can be considered in ovulation induction of patients to avoid of activeness of OHSS.

*Keywords:* Follicle Stimulating Hormone Receptor, Polycystic Ovary Syndrome, Polymorphism

## P-213: Mutation Analysis of Mitochondrial ND4L Gene in Iranian Infertile Men with Varicocele

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**Background:** Varicocele is the abnormal tortuosity and dilatation of the veins of the pampiniform plexus within the spermatic cord. Varicocele-related pathology is suspected in infertility as it leads to elevated temperatures in the scrotum and testes, which has a deleterious effect on spermatogenesis. In Varicocele patients, ROS production is enhanced and total antioxidant capacity (TAC) is reduced. This imbalance between enhanced ROS production and diminished antioxidant capacity results in a condition called oxidative stress, which damages spermatozoa. The presence of oxidative stress could cause molecular and genetic defects leading to infertility. Mitochondria and especially mitochondrial complex I plays an important role in reducing oxidative stress. Since the mitochondrial complex I is one of the most essential complexes in respiratory chain for reduced in ROS, we analyzed the mutations in ND4L gene in infertile patients with varicocele.

**Materials and Methods:** Total genomic DNA was isolated from peripheral blood of 72 infertile patients with varicocele and 54 fertile men. Molecular analysis of mitochondrial ND4L gene mutations was performed by PCR-SSCP. Direct sequencing of DNA from samples with altered band pattern in the SSCP was used to identify any mitochondrial DNA mutations.

**Results:** We found a homoplasmic synonym polymorphism m.10550A>G in the mitochondrial ND4L gene in 12 patients. The mutated site was conserved among other species during evolution. In this position any mutation was not reported previously in infertility.

**Conclusion:** Based on previous studies, the high level of ROS may cause mtDNA mutations and also, as ROS production in infertility was enhanced, it can be concluded that this mutation may increase frequency of mutations in ND4L gene. Nonetheless, involvement of this mutation in infertility remains to be determined and need for more researches.

*Keywords:* Varicocele, mtDNA, ND4L Gene Mutation, PCR-SSCP

## P-214: Parental Contribution of HLA-G*0106 and G*0105N to Repeated Implantation Failure

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**Background:** HLA-G, as a tolerogenic molecule, expressed in cytotrophoblast cells and playing an important role in the suppression of the immune response in maternal decidua. Today Interaction of the extracellular domains of HLA-G protein with cell receptors of immune system, including CD8, LILRB1, LILRB2 and killer cell immunoglobulin-like receptor KIR2DL4, was well known.
**Materials and Methods:** To investigation of association between HLA-G *0106 and G*0105N with repeated implantation failure (RIF), we used polymerase chain reaction (PCR) - sequencing technique for exon 2, 3, 4 and intron 2 of HLA-G gene in 100 couples with two or more failed assisted reproductive technology (ART) in their history and 50 couples with normal fertility and had one or more child that referred to Royan institute of Iran.
**Results:** The obtained results indicate that HLA-G *0106 and G*0105N alleles were significantly higher in the patients group compared to controls group (G *0106: p=0.002; OR=3.98; 95% CI = 1.54-10.28 and G*0105N: p=0.01; OR=0).
**Conclusion:** Although HLA-G gene polymorphisms do not clearly affect on the risk for implantation failure in most couples who undergone ART, but allelic variation, specially, in exon3, exon4 of HLA-G gene can led to ART failure in human embryos, and also exist of *0106 and G*0105N alleles in the parents could be a prognostic factor for implantation failure.
***Keywords:*** HLA-G, Implantation, ART, RIF

## P-215: Discovery of A Novel APA Variant of A Human Potential Gene Based on Expressed Sequenced Tags Analysis

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**Background:** Expressed sequence tags (ESTs) are sequences of cDNA fragments prepared from different tissue sources. There are over one million of these sequences in the publicly available database, and these sequences are believed to represent more than half of all human genes. The ESTs belong to different cDNA libraries, was prepared from one particular cell type, organ, or tumor. Therefore, the presence or absence of ESTs in different libraries provides information about the organ, cell type, or tumor specificity of expressed genes. Also, a gene is often represented by many ESTs; generally, the more a gene is expressed in a given tissue, the more ESTs for that gene will be found in the library.
**Materials and Methods:** 1. Computational study: Bioinformatics Analysis showed some important ESTs can be representation of a new potential gene. Important ESTs were selected to design specific primers. 2. Cell Culturing: U87-MG cell line cultured in RPMI medium. RNA was extracted and then single strand cDNA was prepared based on oligo dT primer. RT-PCR was performed by nested primers for our based EST and then its sequence confirmed by DNA sequencing.
**Results:** We have discovered a novel alternative polyadenylation variant that encoded by our potential gene, experimentally. This variant has a potential role in tumorigenesis. Our sequencing data revealed this variant can misses a spicific region that potentially has a binding site for a known micro-RNA.
**Conclusion:** Based on bioinformatics approaches, our study revealed this novel variant that is longer than our based EST has a target site for has-miR-130a-5p but it needs an experimental validation. Expression of this variant in U87-MG cell line but not in normal tissues, supports this hypothesis that 3'UTR of mRNA involves in tumorigenesis.
***Keywords:*** EST, Potential Gene, Novel Variant, 3'UTR of mRNA, U87-MG Cell Line

## P-216: Common Polymorphisms of StAR Gene Are Not Associated with Polycystic Ovary Syndrome in Iranian Patients

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**Background:** Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting 5-10% of reproductive aged women. High levels of androgen hormone can be accounted as the most important symptoms in PCOS. Androgen is the precursor of steroid biosynthesis in ovary. The transport of cholesterol to the inner mitochondrial membrane through StAR protein is also necessary for androgen and steroid biosynthesis. Therefore StAR gene can be a candidate for surveying the etiology of PCO syndrome.
**Materials and Methods:** Seven common polymorphisms of StAR (exons 5,6 and 7) were studied in 45 PCOS patients who has referred to royan Institute, 40 women (with male factor infertility) under intra uterine insemination (IUI) treatment (as drug-response control group) and 40 healthy fertile women as control group. We used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method on the DNA isolated from blood samples.
**Results:** No association was observed between SNPs of exons 5, 6 and 7 with PCOS. However, heterozygote genotype for polymorphism rs28938471 (Ala218Val) in exon 6 was only seen in 7 PCO patients, 1 drug-response control and 5 fertile controls that was not significant.
**Conclusion:** Although these common polymorphisms have been reported in other cholesterol metabolism disorders, this study showed there is no association between these common StAR polymorphism and PCOS in Iranian patients.

*Keywords:* Steroidogenic Acute Regulatory (StAR), Polycystic Ovary Syndrome, Polymorphism

## P-217: Expression Analysis of The Histone Variant H2A.Z in Endometrium Tissue during The Menstrual Cycle

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**Background:** The human endometrium undergoes cyclical changes including proliferation, differentiation, tissue breakdown and shedding (menstruation) throughout a woman's reproductive life. The postovulatory rise in ovarian progestrone induces profound remodeling in chromatin structure of cells, and consequently differentiation of estradiol-primed endometrium. This change is crucial for embryo implantation and maintenance of pregnancy. H2A.Z is a histone variant that plays role in cellular proliferation. Some studies has recently demonstrated the importance of the presence of H2A.Z in chromatin to cell-cycle progression via the p53/p21 pathway. H2A.Z overexpression has been associated functionally with the proliferation state of cells. It is an important regulator of gene expression, and its deregulation may lead to the increased proliferation of mammalian cells. in the current work, expression analysis H2A.Z of histone variant has been evaluated in women in prolifrative and secretory phases.
**Materials and Methods:** Local ethical approval was gained for this study and informed consent was given by patients. All the women taking part in the investigation had regular cycles, showed no evidence of any pathological uterine disorder and had not used oral contraception or an intrauterine device in the previous 3 months. Cellular RNA was extracted from tissue samples and cDNA synthesis was performed on them. The expression level of H2A.Z was evaluated by the use of qRT-PCR techniqe.
**Results:** The results showed that the mRNA level of H2A.Z was significantly higher in prolifrative phase compared to secretory phase.
**Conclusion:** This finding implies for the first time that the histone variant H2A.Z can be considered as an epigenetic factor in endometrium reconstruction during the menstrual cycle.
*Keywords:* H2A.Z, Endometrium, Menstrual Cycle

## P-218: Investigation of Association between Angiotensin II Type 1 and 2 Receptor (AT1R & AT2R) Gene Polymorphisms and Susceptibility to Pre-Eclampsia (PE) in Iranian Women

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**Background:** Hypertension during pregnancy period along with the presence of protein in the urine, after the 20th weeks of gestation is called preeclampsia (PE). About five percent of all pregnancies are manifested with PE and its exact etiology has still remained unknown. The renin-angiotensin system (RAS) has an important role in the regulation of blood pressure during pregnancy and so pathophysiology of PE. In this system, angiotensin II via two distinct receptors, angiotensin II type-1 (AT1R) and angiotensin II type-2 (AT2R) regulates blood pressure.Two single nucleotide gene polymorphisms (SNPs), including AT1R (+1166 A/C) and AT2R (+1675 G/A), which are associated with receptor function have been detected.
**Materials and Methods:** This Case-control study was performed on 97 (25 severe and 72 mild) women with pre-eclampsia and 100 healthy normal Iranian women. Cases and controls were selected age & ethnic matched from the same geographic region (Fars province, south west of Iran). After DNA extraction, PCR-RFLP method was used for genotyping of AT1R at position +1166 A/C and AT2R at position +1675 G/A. Chi-square test was used for analysis the results.
**Results:** Statistical analysis did not showed any significant differences between cases and controls regarding the distribution of genotype and allele frequencies at position +1166 A/C in AT1R or position +1675 G/A in AT2R gene ( p=0/64, p=0/4 respectively). Moreover no significant correlation between both polymorphisms and the severity of the disease was also observed.
**Conclusion:** The results of the present study indicated that the genetic polymorphisms in AT1R and AT2R genes are not associated with susceptibility or severity of pre-eclampsia in south west Iranian women.
*Keywords:* Preeclampsia, AT1R, AT2R, Polymorphism, Renin-Angiotensin System

## P-219: The Role of E-Cadherin Coding Gene (CDH1) in Pathogenesis of Endometriosis

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**Background:** Endometriosis, a gyncological disorder,

benign and common cause of infertility, is defined as the presence of endometrial glands and stroma at ectopic locations outside the uterine cavity. Clinical observations have led to the assessment that endometriosis is an invasive disease. Abnormal expression of adhesion molecules such as cadherins is likely to be an important determinant of local invasion and dissemination. Cadherins are major cell-cell adhesion molecules involved in the development and maintenance of all solid tissues. E-cadherin is a transmembrane glycoprotein and a member of the multigene superfamily of cadherins and it is a crucial adhesion molecule which hampers tumor invasion and metastasis. In the current work, the expression of E-cadherin gene (CDH1) has been evaluated in eutopic and ectopic endometrium of women with endometriosis.

**Materials and Methods:** Local ethical approval was gained for this study and informed consent was given by patients. The levels of CDH1 gene expression in ectopic and eutopic endometrium in women with endometriosis were investigated and compared with the endometrium samples of women without endometriosis. Ectopic biopsies were obtained by laparoscopic procedure and eutopic biopsies were obtained by piplle. In women without endometriosis, control biopsies were gained with piplle. Quantitative PCR was performed on cDNA samples using CDH1 primers, and GAPDH was used as housekeeping gene.

**Results:** This study showed that CDH1 gene expression in eutopic and ectopic endometrium was significantly lower than normal endometrium. On the other side, mRNA level of CDH1 was significantly higher in eutopic endometrium compared to ectopic endometrium.

**Conclusion:** These findings suggest that CDH1 gene could be involved in pathogenesis of endometriosis, in the way that decrease of CDH1 gene expression could lead to invasiveness and metastasis in patients with endometriosis.

*Keywords:* E-Cadherin, CDH1, Endometriosis

## P-220: Co-Administration of Testosterone and Vitamin E Inhibited The Atrazine-Induced Damages on Testis; Evidence for RNA Damage and Leydig Cells Steroidogenesis

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**Background:** Atrazine (ATR), an herbicide, is used in order to control broadleaf and grassy weed. ATR has been estimated to impact the testicular tissue both by inhibiting the endocrine system and partly by reducing the antioxidant status. Thus, current study was designed to evaluate the protective effect of vitamin E and testosterone against ATR-induced damages in testicular tissue.

**Materials and Methods:** Thirty Mature male rats were used. The test group subdivided into three (n=6) groups as; ATR-induced (200 mg/kg/day by gavages), ATR+testosterone (250 mg/kg per week, intraperitonealy), ATR+vitamin E (150 mg/kg, every 48 hours, by gavages) and ATR+testosterone+vitamin E. The control-sham animals received corn oil (0.2 mg/kg/day by gavages). The epi-fluorescent and fluorescent analyses were performed to examine the germinal cells RNA damage and leydig cells biosteroid activity. The leydig cells distribution in interstitial tissue, tissue total thiol molecules (TTM) and total antioxidant capacity (TAC) were estimated.

**Results:** The testosterone and vitamin E-received animals were manifested with significantly ($p<0.05$) lowered RNA damage in germinal cells accomplished with remarkable increase in steroid synthesis of leydig cells. Moreover, co-administration of testosterone and vitamin E promoted the antioxidant status. Qua, the animals in these groups revealed with significant increase in TAC and TTM levels.

**Conclusion:** Our data suggest that, the testosterone by promoting endocrine system both by protecting leydig cells and compensating the testosterone level and the vitamin E by up-regulating antioxidant status could protect germinal cells RNA content against ATR-induced damages.

*Keywords:* Atrazine, Testosterone, Vitamin E, RNA Damage, Oxidative Stress

## P-221: Impact of Prunus Cerasus on The Expression of HAS2 in Cumulus Cells on Mice

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**Background:** Cherries are an important source of phyto-chemicals, especially anthocyanin. They have many potent biological Properties such as antioxidant activity. Oxidative stress is mainly insert their effect through decreasing of cell proliferation and viability. Infertility problem affect more than 15% of young couples in different societies. Oocyte expansion plays an important role in fertility and needs some factors such as hyaluronic acid. The objective of this research is to find if prunes' cercus have an effect on the expression of HAS2 in Cumulus cells

**Materials and Methods:** Thirty female bulbc/ mice aging 6-8 weeks, were superovulated with 10 unit hMG and 48 hours later 10 unit hCG as IP injection. For the collection of cumulus- oocyte complexes(COCs) mice were killed 36 hours later by cervical dislocation and COCs were collected from oviduct by flashing method .COCs were cultured in 20cc universal medium supplemented with 20 ג prunes' cercus and overlaid with mineral oil and incubated for 4-6 hours at 5% $CO_2$ and 37°C. Surrounding cumulus cells were removed mechanically by using 20 ג hyaluronidase .The cumulus cells was stored at-80°C until real-time PCR. PCR was performed by using

a master mix containing CYBR green. The expansion level HAS2 of mRNA was evaluated by real-time PCR.
**Results:** The result showed that in the presence of Prunus cerasus the expression of HAS2 gene is increased significantly in comparison to control group
**Conclusion:** Prunus cerasus enhances the expression of HAS2 gene in cultured cumulus cells from bulbc/mice
***Keywords:*** HAS2, Real Time PCR, Prunus Cerasus

### P-222: Expression of RFamide-Related Peptide-3 and KiSS-1 mRNA in The Hypothalamus of Pregnant Rat

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**Background:** There is no follicular development during pregnancy in the rat comparable to the changes of the estrous cycle. RFamide-related peptide-3 (RFRP-3) and kisspeptin were recognized as regulator of gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in several species, including the rat. The aim of the present study was to evaluate the relative expression of RFRP-3 and KiSS-1 mRNA in the hypothalamus of pregnant rats.
**Materials and Methods:** Eighteen adult pregnant Sprague-Dawley rats (3-4 months old) were decapitated, and the whole hypothalamus was dissected at the age of 7, 14, and 21 days of pregnancy (n=6). The exact day of the rat pregnancy was confirmed using the vaginal smear method2. Four female non-pregnant rats were ovariectomized and assigned as control group. The dorsomedial hypothalamic nucleus (DMH) for detection of RFRP-3 mRNA and arcuate nucleus (ARC) for detection of KiSS-1 mRNA were separated from hypothalamus. Then relative expression of RFRP-3 and KiSS-1 mRNA were compared using real-time PCR method between control and pregnant groups. The data were subjected to the test of normality and analyzed by one-way ANOVA (SAS 9.1, SAS Institute Inc., NC), and mean separation was performed by Tukey's test at p=0.01.
**Results:** The mean and SE of relative expression of RFRP-3 in DMH did not change during pregnancy (day 7, 40.8 ± 16.2%; day 14, 73.9 ± 17.7%; day 21, 49.9 ± 13.2%; p>0.01). However, the relative expression of KiSS-1 in ARC was the highest in day 7 of pregnancy and decreased up to day 21 of pregnancy (day 7, 17.1 ± 4.4%; day 14, 13.8 ± 5.3%; day 21, 4.3 ± 4.0%; p<0.01).
**Conclusion:** Decrease of the GnRH and LH secretion during the pregnancy of rat may be controlled by constant expression of RFRP-3 mRNA and decreasing pattern of KiSS-1 mRNA expression.
***Keywords:*** KiSS-1, RFamide-Related Peptide-3, Pregnancy, Hypothalamus, Rat

### P-223: The Expression of Gp130 in Fallopian Tubes with Ectopic Tubal Gestation

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**Background:** The transmembrane protein gp130 plays a central role in cytokine action as a signal transducing receptor subunit common to all interleukin-6 type cytokines.The expression of IL-6 is significantly increased near the implantation site in tubes with ectopic gestation as compared with normal.Thus, current study was designed to investigated the expression of gp130 in fallopian tubes with ectopic gestation
**Materials and Methods:** Quantitative reverse transcriptase polymerase chain reaction to determine mRNA expressions of gp130 was performed in ampullaris parts of the fallopian tube of 12 women with ectopic tubal gestation at operation for tubal surgery. 12 non-pregnant women at luteal phase underwent hysterectomy and bilateral salpingo-oophorectomy for benign disease which did not affect the fallopian tubes , histological dating was carried out according to the criteria of Noyes et al. to confirm the luteal phase of the menstrual cycle women .
**Results:** Expression of gp130 mRNAs was significantly lower in fallopian tube from women with ectopic tubal gestation compared to the nonpregnant woman (p<0.05).
**Conclusion:** It is suggested that the decrease in gp130 expression in fallopian tube may be a risk factor for increased ectopic tubal gestation. gp130 expression was obviously high in midluteal phase fallopian tube which suggests that high levels of gp130 may contribute to embryo transportation into the uterus
***Keywords:*** Gp130, Fallopian Tubes, Ectopic Tubal Gestation

## Reproductive Imaging

### P-224: Mechanisms That Reduce Endometrial Receptivity in Endometriosis

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**Background:** Several studies indicate clear relationship between infertility and endometriosis. However, the etiology of this association is uncertain. Endometrial dysfunction is a possible cause of infertility in women with endometriosis, which could explain the high rates of implantation failure and miscarriages in these women. Delay in endometrial maturation and intrauterine biochemical abnormalities may be causing this problem.
**Materials and Methods:** Review study
**Results:** It has been shown that in patients with endometriosis, during implantation window, express of the ligands that mediated embryo- endometrium attachment (like α5β integrin), are low. On the other hand, in the many of these cases, the production of enzymes involved in the synthesis of the ligands for L-Selectin (the protein that coats the trophoblasts in the maternal blood level) are lower than the control group. It seems that the presence of ectopic endometrium and its secreted inflammatory mediators can reduce endometrial reception, directly and indirectly. For example, secretion of specific types of prostaglandin by the peritoneal endometrium, indeed of inhibition of the binding of trophoblasts to their endometrial receptors, can also induce uterine contractions, that excrete embryo.
**Conclusion:** In this paper, the mechanisms involved in reducing endometrial receptivity in the women with endometriosis, will be reviewed.
***Keywords:*** Endometrial Dysfunction, Endometriosis, Implantation

## P-225: The Assessment of Hemocysteine and Gherelin in IUGR Fetuses with Abnormal Doppler

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**Background:** To compare of two factors of Hemocysteine and Gherelin in IUGR and normal growth fetues.
**Materials and Methods:** In this study, forthy fetuses were assessed for growth by ultrasound as IUGR with abnormal doppler and AGA fetus, Hemocysteine and Gherelin from cord blood were compared after delivery.
**Results:** Two factors of Hemocysteine and Gherelin were increased in IUGR fetuses.
**Conclusion:** The level of Hemocysteine and Gherelin may allow to predict compromised fetuses for IUGR .
***Keywords:*** Gherelin, Hemocysteine, AGA, SGA, IUGR

## P-226: Non-Invasive Prenatal Screening for Fetal Chromosomal Anomalies in South of Iran

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**Background:** Prenatal diagnosis for Fetal Chromosomal anomalies currently relies on assessment of risk followed by a combination of biochemical and nuchal translucency. Trisomy 21 is the most common trisomy that is associated with intellectual disability. Pregnant women who receive a prenatal diagnosis of trisomy 21 currently have the option of continuing or terminating their pregnancy, but no fetal treatment is available.
**Materials and Methods:** A total of 157 women referred to social welfare organization participated in the first trimester screening for trisomy of chromosomes 13,18,21 based on biochemistry and nuchal translucency in 11-14 week of gestational age. Screen positive women elected for amniocentesis and karyotyping.
**Results:** A total of 157 women participated in first trimester screening based on biochemistry and nuchal translucency. 61 women (38.8%) had age of more than 35 years. 125 women (61.6%) had consanguineous marriage, 52 women (25.8%) had at least one abortion. 45 women (22.3%) had positive screening result for trisomies. 31 women decided to do amniocentesis and cytogenetic tests that from them 6 fetus (19.3%) were affected (5 trisomy 21 and 1 trisomy 18) and referred for termination of pregnancy.
**Conclusion:** First trimester screening is advantageous for detecting pregnancies with increased risk of fetal chromosomal anomalies and reffering them for amniocentesis and cytogenetic evaluation to find affected pregnancies and terminate them on time.
**Keywords:** Prenatal, Screening

## P-227: The Survey on Endometrial Thickness and Echo Pattern Changes in Consecutive Cycles of Clomiphene Citrate Therapy in Infertile Women

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**Background:** One of the most common causes of infertility in women is ovulatory dysfunction (PCOS) and the choice of treatment is clomiphene citrate. One of the mechanism of dichotomy between the rate of ovulation and pregnancy is anti estrogenic effects of clomiphene citrate on endometrium. According to its long half life, the anti estrogenic effects probably exacerbate in consecutive cycles. We studied the effect of consecutive cycles of clomiphene citrate on endometrial thickness and echo pattern.
**Materials and Methods:** Only patients with PCOS were included in this study. Patients received clomiphene citrate with dose of 100 mg from day 3 to day 7. Each woman was evaluated using Trans vaginal ultrasound on 13th

to 16th. For patients who had at least one mature follicle, endometrial thickness and echo pattern were evaluated. During treatment period, if patients didn't have at least one follicle with 18mm in diameter, excluded from this study. Results were analyzed using Repeat Measure test for quantity variable and square k for quality variable.

**Results:** One hundred forty seven patients included in this study. Fifty patients were treated with clomiphene citrate for at least 4 consecutive cycles,19 patients 5cycles and7patients 6cycles.Analysis of data demonstrated that there was no difference in mean endometrial thickness in consecutive cycles of clomiphene citrate therapy. There was also no change in distribution of endometrial echo pattern in consecutive cycles of clomiphene citrate therapy.

**Conclusion:** Considering the result, there is no additive harmful effect on endometrium with consecutive cycles of clomiphene citrate therapy.

***Keywords:*** Endometrial Thickness, Echo Pattern Changes in Consecutive Cycles, Clomiphene Citrate , Infertile Women

Abstracts of

# 8th Royan Nursing and Midwifery Seminar

4-6 September 2013

# Royan Institute

## Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

# Invited Speakers

## $I_{nm}$-1: Indication of First Trimester Sonongraphy

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Improvement in ultrasound technology, including transvaginalsonography and higher frequency probes, has led to a better understanding of early pregnancy development. The first trimester ultrasound have been identified that can be used to reassure women that their pregnancy is progressing normally or abnormal them that their pregnancy will fail. The practicing radiologist and obstetrician must be familiar with basic embryology and sonography of the first trimester to interpret studies accurately and counsel women about the status of their early pregnancies. Sonographic appearance depends on the stage of development and size of size of the conceptus. Therefore,sonographic examination should be relevant to developmental stage. Most examinations are needed for vaginal bleeding or pelvic pain, or a palpable mass which has revealed in physical exam. The primary goals of early first trimester sonography examination are as follows: 1. Site of implantation (intrauterine pregnancy or ectopic), 2. shape and size of gestational sac, 3. Presence of yolk sac and embryo, 4. Presence of cardiac activity, 5. Assessment of gestational age, 6. presence and size of sub-chorionic hematoma, 7. Assessment of uterine or adnexal masses and 8. Detection of embryonic fetal anomalies.

*Keywords:* First Trimester, Ultrasound

## $I_{nm}$-2: Polycystic Ovarian Syndrome is a Potent Risk Factor for GDM in Infertile Patient

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**Objective:** Gestational diabetes mellitus (GDM), a common medical complication of pregnancy, is defined as "any degree of glucose intolerance with onset or first recognition during pregnancy.Risk factors for GDM: a previous GDM or Pre diabetes , a family history revealing a first degree relative with type 2 diabetes , maternal age (>35yr), ethnic background , being overweight , a previous pregnancy which resulted in a child with a high birth weight , Poly cystic ovary syndrome (PCOS).
The aim of this study is influence of PCOS on GDM without consideration of obesity.
**Materials and Methods:** Our retrospective study subjects were530 patients with different Diagnosis of infertility who offered to prenatal Clinic after pregnancy with assisted reproductive technique (ART) in Royan Institute during 12month(March2012-February2013).Diagnosis of PCOS patients has been with Roterdom criteria . Diagnosis of GDM was based on glucose challenge test (GCT) (with 50 gr Glucose) and then confirmed with glucose tolerance test (GTT) (with 100 gr Glucose) in 24-28 week of pregnancy. Weight and Height were measured and body mass index (BMI) was calculated for each patient .The prevalence of GDM in PCOS and non PCOS were compared.
**Result:** Mean age: 29.8±4.5, Mean BMI: 25.2±3.9.Total number of patient were 530, 204 and 326 were PCOS and Non PCOS respectively.100 of 204 patients with PCOS had GDM (49.4%) and 111 of 326 patients with non PCOS had GDM (34%), it shows that prevalence of GDM were significantly higher in PCOS subjects. (P=0.001) Mean BMI and age were (BMI: 26.1 ± 3.5, Age: 29.8 ± 4.1) in PCOS and (BMI: 25.5 ± 4.1, Age: 30.9 ± 4.8) in Non PCOS woman respectively. There were no statistically significant differences in BMI and age factors between two groups.
**Conclusion:** PCOS is one of the most important risk factors for GDM without consideration of obesity. We recommend infertile patients with history of PCOS should be investigated for GDM as soon as possible in pregnancy.

*Key Words:* Gestational Diabetes, Polycystic Ovarian Syndrom, Obesity, Infertility

## $I_{nm}$-3: Concept of Sexual Satisfaction: A New Look

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The process of sexual response is defined in four phases: Desire, Excitement or arousal, Orgasm and Resolution. This model was introduced by Mr. Masters and Mrs. Johnsons for the first time and then completed by Mrs. Kaplan. At that time, this classification was so practical and valuable for understanding sexual response and thereupon defining sexual dysfunctions, even being still used as a criterion for classification of sexual dysfunctions.
After some decades this model was criticized by experts. This model being somehow determined as a linear model, has limited sexual function to physiological phases, whereas in practice sexual response and behavior is extremely related to mental aspects in human. Even in physiologic changes, linear model is mostly focused on responses of sexual organs.
Another critical issue in this model is lack of attention to the concept of sexual relationship and limiting it to intercourse and also disregard for the difference of sexual relationship concept between some men and women. Different studies have shown that sexual demands and considerations in females can be totally different from males. Intimacy, emotions, relation and appropriate dialogue, commitment and pregnancy are some examples for those considerations that have been ignored in physiological models whilst all of mentioned factors are

somehow involved in sexual satisfaction concept.
Specially the difference between male and female is more prominent in sexual desire and this should not be misinterpreted as more desire in male or female as it is wrongly mentioned in some writings. The nature of sexual desire in women and even some men is different from the current stereotype or even modified expression of DSM classification.
For the mentioned reasons, in the recent years other different models have been presented and the most prominent of them is Mrs. Basson's suggested model. This model has a non-linear nature and in many aspects answers to some of the criticisms to the linear models.
The Non-linear Basson model provides this possibility to remove the label of disorder from the people only slightly different from others, and also making it possible to find definitions for some subgroups of dysfunctions like arousal disorders.
Due to this model many women may have Neutral sexual desire in normal conditions. These conditions may change when an emotional factor like love expression from partner or even non-sexual motive trigger the desire in that person. Therefore, despite the former models considering sexual desire a spontaneous and permanent feeling, Basson model defines it as a responsive feeling towards environmental stimulants and motivations. In other words, sexual desire could be achieved following and in response to arousal.
***Keywords:*** Sexual Satisfaction, Basson, Linear Model, Non-Linear Model, Sexual Response

## $I_{nm}$-4: Supportive and Educational Role of The Nurse and Midwife with A Focus on Patient-Centered Infertility Centers

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Delivering high-quality care is the ultimate but challenging goal of healthcare. In all fields of healthcare, effectiveness and safety are the most acknowledged quality dimensions. Although crucial, true high quality care goes beyond this scope, and is also patient-centered. Patient centeredness is defined as 'care that is respectful of and responsive to individual patient preferences and needs and that is guided by patient values.' Patient centeredness "encompasses qualities of compassion, empathy, and responsiveness to the needs, values, and expressed preferences of the individual patient." In addition, translation and interpretation services facilitate communication between the provider and the patient and are often a legal requirement. The patient-centered approach includes viewing the patient as a unique person, rather than focusing strictly on the illness, building a therapeutic alliance based on the patient's and the provider's perspectives.
Providing relevant information to patients, respecting their wishes and considering their capacity to make treatment decisions is crucial for high-quality and patient-centered fertility cares, resulting in patients who are well-informed, better adjusted to their circumstances and is compliant with their treatment. Fertility patients generally appear to be satisfied with the information they are given but a significant minority is not. Giving information to infertile patients is complicated by the nature of their condition, desire for a child and complexity of treatment options. Patients need detailed, well-timed information to support difficult decision-making, such as when to end treatment. Four reasons clearly indicate the need for reproductive medicine to focus on other quality dimensions besides 'effectiveness' (pregnancy rate), in particular the 'patient centeredness' of care. First, 'patient-centeredness' is important to all segments of health care, and it is defined as one of the six dimensions of quality of care. Secondly, despite the success of current Medically Assisted Reproduction, one-third of the infertile couples finally do not deliver a child. Hence, process indicators such as patient-centeredness are very important in addition to outcome indicators. Thirdly, recent reports confirm that besides effective medical treatment, patients also want patient-centered infertility care. Fourthly, infertility and its treatment involve a physical and emotional burden for both women and men and that burden contributes to high drop-out rates from treatmentFor instance,patients who voluntary dropped out from treatment have reportedthe impact of the psychological burden (72%) and the lack of staffempathy (32%) on their decision. Education, often delivered by nurses and midwives, is an important part of all management programmers for infertile patients, both in clinical practice and research. Most infertile clients at different times during their treatment also need emotional and social support, which nurses can provide. So, emotional and educational support and interventions from nurses would complement and reinforce all other interventions for infertile clients undergoing treatment.
Keywords: Patient-Centeredness, Health Care, Emotional and Educational Support

## $I_{nm}$-5: Outcomes of Human Vitrified Embryos Transfer

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The techniques of cryopreservation let embryos which cannot be immediately transferred to the patient be stored for future use. The first successful pregnancy after transfer of cryopreserved human embryos was described in 1983 by Trounson et al. and the first birth after cryopreservation was later reported in 1984 by Zeilmaker et al.). The technique has rapidly been applied in most in-vitro fertilization (IVF) programmes and a large number of children have been born.
For a long time, slow cooling was the predominant method for cryopreservation of human embryos, however, vitrification has naturally become the preferred method in

recent years. The first birth after transfer of vitrified human embryos was reported at Royan institute in 2008. Vitrification has been successfully utilized in freezing human oocytes as well as cleavage-stage human embryos and human blastocyst-stage. One of the draw backs of traditional slow freezing is the formation of intracellular ice, which can result in cell damage. Vitrification is an ultrarapid method of cooling cells into a glass-like state, which prevents ice crystal formation and as a result it reduces associated cell injuries. However, for achieving these results, higher concentrations of cryoprotectants must be utilized. Many published reports have indicated better post-thawing survival rates and encouraging pregnancy rates when compared with slow freezing. Although vitrification is now used widely in assisted reproduction technology (ART) clinics, there are still concerns considering the safety of vitrification. Most published reports looking at the neonatal outcomes after transfer of vitrified embryos have used blastocyst-stage embryos. Reported data on children born after transfer of Day 3 vitrified embryos are relatively rare. Our data suggest that the vitrification process did not seem to adversely influence fetal development and no increased perinatal risk was found. Long-term follow-up studies are needed to assure that there are no late consequences for the children conceived from cryopreserved and thawed embryos.

***Keywords:*** Cryopreservation, Vitrification, Slow Freezing, Neonatal Outcomes,

## $I_{nm}$-6: Evaluate The Supportive Role of Spouse in Infertile Couples Training

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Infertility is a life crisis and the most important problem for infertile couples. The inability to bear children is a stressful situation. The most infertile couple experience emotional suffering from infertility. The relation of stress to infertility is long-standing and ambiguous. The reaction of infertile couple in the stressful situations is influenced by the coping strategies for infertility adjustment. Counseling could give adopting coping strategies in order to reduce stress. Better understanding of coping strategies at the initial visit may help it identify individuals who may benefit from counseling earlier and inform the direction of therapeutic work. Since, the identification of coping strategies for stressful events in infertility is important. And evaluating of adaptive coping strategies may help in recognition need cognitive intervention for mental health. Psycho-educational interventions that teach couples what to expect at the beginning of treatment and provide fundamental strategies for coping are highly valuable; they help to improve and maintain relationship integrity and equip couples with the communication skills to navigate unpredictable terrain. It may be possible to influence men to participate in seeking mental health support earlier in the course of ART treatment by providing patient handouts with prominent testimonials from men that convey the experience of infertility in a way with which male patients can identify. Typically, patients in a pre-contemplative stage of change will see less benefit to counseling than someone who is at a later stage in their desire to transform behavior. Women, who typically struggle with intervention schedules and medication protocols, may feel that they live with their body under a form of continual physical and emotional siege. They are far more likely than their male partner to discuss their difficulties with other women, friends, and relatives, and they may more readily perceive counseling as a part of the support they need. Women, frequently, attend their first consultative infertility appointment without their partner. Many men report that their initial exposure to gynecological medicine is as mysterious as their understanding of menstruation. They are neither prepared to know more nor comfortable being asked to contribute beyond the initial diagnostic sperm sample, and for many men this is where their desire for regular participation in infertility treatment is inclined to stop. When couples present for treatment they are often unaware of the pervasive impact that assisted reproductive technologies (ART) can have on their lives. Although it might seem appropriate to describe infertility as a “couple problem,” men and women generally experience treatment as observer and participant, respectively. Studies demonstrating gender differences in response to fertility treatment highlight the need to educate individuals about ways to traverse these differences and, in fact, it is often lack of preparation—rather than unwillingness—that inhibits the participation of the male partner.

Evidence shows that men who receive pretreatment educational brochures are more likely to attend infertility appointments than those who do not. Along with clinical protocols, referral packets should include a description of available counseling services and an explanation of their potential benefits; literature that addresses the needs of couples as well as individuals may encourage the participation of both partners in counseling. Finally, pre-appointment telephone calls directed toward each partner establish the expectation that the consult is intended for the couple rather than the woman only, thereby setting a precedent for follow-up visits.

***Keywords:*** Communication Skills, Infertility, Spouse

## $I_{nm}$-7: Genetic Etiologies of Premature Ovarian Failure

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Premature Ovarian Failure (POF) defined as functional stop of ovaries before the age of 40. It is a common cause of infertility in women that characterized by primary or secondary amenorrhea, high gonadotropin levels and estrogen level declining in patients. Factors that reduce follicle or defect in the follicle growth stimulating mechanism defined as numerous complication factors that they can cause POF. According to reports several genetic factors considered to cause POF syndrome. Cy-

togenetic and molecular genetic studies should be done in order to identify the altered gene or numerical and structural chromosomal defects. The Chromosomal defects involving the X-linked defects, displacement and autosomal chromosome abnormalities. Chromosomal abnormalities included Turner syndrome, deletions, rearrangements and translocations are involved in near 5% of the genetic causes of the POF. Genes on the X-chromosome and autosomal genes are detected in this disorder too. FMR1 gene that is on X-chromosome is the most important gene related with POF. Premutation in this gene are more common in these patients. According to studies, many genes are involved in the development of POF, although some of the genes responsible for causing POF are often viewed as the genes under investigation in human or other animals with POF. Some of them are BMP15, FMR2, LHR, FSHR, INHA, FS1, FOXL2, FOXO3a, ER, LIN28A, PGRMC1, POF1, HSD17B, TG, LAMC1, POU5F1, TGFBR3, FOXE1, FOXO4, CITED2, SALL4, CXCL12, PTHB1, Wnt4, BRSK1, HK3, ADAMTS19, NOBOX, FIGLA, KDR, BMPRIB, BMPRII, C1galt1, Mgat1 and FGFR.
Keywords: Premature Ovarian Failure (POF), abnormality, Genes

## $I_{nm}$-8: Assisted Reproductive Technologies and Genetics

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Genetic causes have a considerable involvement in infertility. Well-known examples are some chromosomal translocations or sex-chromosomal abnormalities and Y-chromosome deletions. The most common chromosomal aberrations associated especially with severe oligo- and azoospermia are sex chromosome aneuploidies and chromosomal translocations. Consequently, occurrence of aneuploid embryos will lower the success rate of the IVF treatment, and offspring have a risk of an unbalanced translocation or an aneuploidy. Advanced maternal age has increased sporadic chromosomal anomalies with conception. Special consideration is needed when treating infertile men, since infertility may be caused by abnormalities that may cause infertility and/or potential serious diseases to the offspring. Also, a number of causes behind female infertility may lie in chromosomal aberrations and gene mutations. The possibility of single or multiple gene defects in common clinical conditions, such as polycystic ovarian syndrome, or premature ovarian failure, has been described. A woman's age and her supply of eggs all contribute to the success rate — but additional genetic factors are also thought important. In addition, other genetic causes like mutations in LH and FSH receptor genes as well as structural abnormalities of the female genital organs may cause female infertility. There are a number of reports on adverse outcomes in children born as the result of ART. Numerically, multiple gestations are clearly the major risk to the future child's health. There is also a growing concern for structural anomalies and long-term health effects.Genetics is expanding into the domains of national screening program, disease prediction and pharmacogenetics means that a growing range of health professionals, including midwives need skills and knowledge in genetics in order to take on new roles. Specialist genetic services would also benefit from midwives having sufficient skills to understand the processes of genetic counseling, which would enable them to discuss the decision to undertake a test, help women to cope with the result and minimize its effects on the family. Educational provision on genetics for midwives is insufficient and has not been coherently planned. Counseling is an essential part of all the treatments. Genetic counseling is a communication process that deals with the occurrence, or risk of occurrence, of a genetic disorder in the family. Counseling session should be characterized by openness for discussion. The counselor should give the couple a general understanding of the principles of ART (e.g., ovarian stimulation and IVF/ICS and potential risks). It might be considered advantageous to be able to perform genetic counseling and offer a selection of genetic tests before all IVF treatments, because many genetic causes of infertility still remain unrecognized.
***Keywords:*** Genetic, Infertility, ART

## $I_{nm}$-9: Cord Blood Banks and Cord Blood Donors Selection

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Cord blood is a sample of blood taken from a newborn baby's umbilical cord.Cord blood is collected because it is rich in stem cells content, such as hematopoietic cells, which can be used to treat hematopoietic and genetic disorders.
Cord Blood Transplantation has been used in the treatment of over 80 diseases, including leukemia, lymphoma and anemia.
Advantages of Cord Blood Treatment:
• Ease of collection
• No risk for mother or child
• Less time needed for processing (more quickly available for use)
• Less risk for transmission of infection
• Less need for stringent antigen matching
• Less rejection
Disadvantages of Cord Blood Treatment:
• Slow engraftment
• Limited cell dose as a result of small volume of unit and additional cell doses unavailable
• Autologous donation may have limited benefit due to hereditary disorders

A cord blood bank is a facility which stores umbilicalcord blood for future use andcould befamilial (private)or public.Public banks accept donations to be used for anyone in need, whereas, private banks register the identity of donors, so that the donors' families may retrieve the samplesin the future.

There are 4cord blood banks in Iran:
1. Royan institute Private cord blood bank
2. Royan institute Public cord blood bank
3. National cord blood bank of Iranian blood transfusion organization
4. Shariati hospital cord blood bank
Cord Blood Donor Selection:
The donors must be evaluated for the suitability of their infant's cord blood for donation based on their medical history (Former and current diseases),drug history,maternal tests (RPR, HBsAg, HBsAb, HBcAb, HCVAb, HIV (I, II), HTLV (I, II), CMV (IgM, IgG)), and etc. They are selected in advance to ensure that their baby's cord blood stem cells are unlikely to harm any recipients.
***Keywords:*** Cord Blood, Donor Selection, Stem Cell

## $I_{nm}$-10: OHSS Management Procedure and Results

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The ovarian hyperstimulation syndrome (OHSS) is a consequence of superovulation therapy for assisted conception procedures. This potentiallyfatal condition is avoidable by the judicious use of gonadotropins and carefulmonitoring of stimulation regimens. Women who are at particular risk ofdeveloping the syndrome include those who have polycystic ovaries and thosewho are young (under 30 years).
We have clear protocols for identifying patients at risk both before and during ovarian stimulation which are in place for the management of patients who develop symptoms. Information is provided to patients within the general pre-treatment information leaflets and also after the egg collection, so that they are aware of the risk and the symptoms to be aware of.
We keep a record of cases of OHSS, with particular note of patients who require hospitalization incorporated in standard risk management protocols.
We ensure appropriate follow up of patients after embryo transfer and during pregnancy.
Mild ovarian hyperstimulation is managed expectantly; Patients with Grade 2 hyperstimulation need reassurance and explanation, together with bed rest in hospital. The development of clinically detectable and usually painful ascites, together with deterioration in respiration, circulation and renal function indicates the development of severe Grade 3 hyperstimulation and may require admissionto an intensive care unit. The intravascular volume is monitoredby measurements of central venous pressure, renal function by meticulousattention to input and urine output and haemoconcentration by measurementof haematocrit, whose level reflects intravascular volume depletion and bloodviscosity. Infusion of colloid (e.g. human albumen or 6% hydoxyethyl starch (HES) isrequired to maintain intravascular volume, as indicated by restoration ofnormal central venous pressure. Crystalloid (normal saline usually) is administered for rehydration with careful monitoring of fluid balance. Prophylactic heparin is given to prevent thromboembolism and continues up to the end of the first trimester of pregnancy.
We try to minimize the risk of OHSS by using low doses of gonadotropinsand reducing doses in women with polycystic ovaries. If an exuberant ovarianresponse is observed then the dose of gonadotropin would be reduced furtherand the dose of hCG also reduced or hCG not administered.
***Keywords:*** PCOS, OHSS, Treatment

# Oral Presentations

## $O_{nm}$-1: Mental Health of Women with Polycystic Ovary Syndrome (PCOS) and Some of Its Socio-Demographic Determinants

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**Background:** Polycystic ovary syndrome (PCOS) is among the common endocrine disorders that not only affect physical aspects of women's health but also can create manifestations such as anxiety and depression. In this study, we examined the mental health and some of its socio- demographic determinants among Iranian women suffering from PCOS.
**Materials and Methods:** It was a descriptive cross-sectional study. The sample consisted of 184 women suffering from PCOS who referred to selected infertility centers of Tehran in 2011. Mental health status of the subjects was evaluated by the General Health Questionnaire-28 (GHQ-28) and analyzed using chi-squared test and Pearson correlation coefficient using SPSS-PC (v. 14).
**Results:** The mean (SD) age of the women was 27.75 (5.37) years. Considering a cut-off point of 24 for the GHQ-28 questionnaire, 91.3% of the subjects had an abnormal score. The abnormal scores were in the subscales of social withdrawal (91.3%), somatic symptom (89.1), anxiety (75%), and depression (33.7%). There were a significant relationship between age and anxiety subscale, occupation and depression subscale, and body mass index and depression subscale($p<0.05$).
**Conclusion:** This study revealed that women with PCOS are at risk of psychological disorders, especially in social withdrawal domain. Thus referring these women to appropriate consultation centers for screening psychological disorders is strongly recommended.
***Keywords:*** PCOS, Mental Health, Socio- Demographic Determinants

## $O_{nm}$-2: Seroprevalence of Cytomegalovirus Infection in Pregnant Women and Associated Role in Spontaneous Abortion in Gonabad, East of Iran

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**Background:** Cytomegalovirus (CMV) is the most common main cause of congenital viral infection in prenatal period. It is also the leading cause for congenital infection with a permanent hearing/vision loss and neurological impairment. The risk of intrauterine infection strictly depends on the time of maternal infection during pregnancy. Seemingly, primary CMV infection in early pregnancy bears higher risk of fetal damage. The aim of this study was to compare the women with spontaneous abortion and in pregnant women with no previous history of abortion regarding the seropositvity of CMV-IgG and CMV-IgM in the east region of Iran.
**Materials and Methods:** In this case-control study 350 serum samples were collected from women with spontaneous abortion (n = 160) and pregnant women at third trimester with no history of abortion (n = 240) as case and control groups. Accordingly, ELISA method was used for detection of CMV IgG and IgM markers during February-October 2011. IgG avidity test has been used for all patients whom were CMV-IgM+ and CMV-IgG+ to distinguish primary and recurrent CMV infection.
**Results:** The majority of women were CMV-IgG+ in case and control groups (67.3 compared to 72.1% respectively). The rate of CMV-IgM+ were 10.0 and 2.1% in case and control groups respectively ($p= 0.001$). In the IgM+ women, 5.5% in case group and 0.4% in control group had low IgG avidity index ($p= 0.002$), indicating primary infection. There was significant relationship between residence place and personal hygiene status with IgGseropositivity rate ($p<0.05$).
**Conclusion:** Our study showed higher prevalence of primary and recurrent CMV infection in women with spontaneous abortion comparing to pregnant women with no previous history of abortion. It could be concluded that recent active CMV infection may be considered as a risk factor for spontaneous abortion. Noninvasive serological test could be a valuable parameter to recognize active CMV infection in preconception counseling.
***Keywords:*** Cytomegalovirus (CMV), Spontaneous Abortion, Pregnancy, Seroprevalence

## $O_{nm}$-3: Patient-Centeredness Fertility Care; A Solution Toward Pleasant Infertility Treatment: A Qualitative Study

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**Background:** Infertility and its treatment involve a physical and emotional burden for both women and men, and that burden contributes to high drop-out rates from treatment. Lack of patient-centredness of care can cause patients to drop out from treatment for non-medical rea-

sons. However, in order to provide patient-centred fertility care, an insight into the patient's perspective on fertility care is required. Therefore a qualitative study aimed at exploring infertile couples' experiences and understanding their needs during infertility treatment process was conducted.

**Materials and Methods:** A qualitative design, based on a content analysis approach, was used to reach this study aim. After employing purposive sampling to choose 17 infertile couples who were seeking infertility treatment at the public and private infertility centers and infertility specialists clinics in Isfahan and Rasht, unstructured interviews were carried out to gather data.Also, taking field note were used to collect the data. All the interviews were recorded and transcribed verbatim.

**Results:** During the data analysis, five main categories emerged including "sence of competency and self-efficacy ", "sense of being valued and self-esteem", "sense of confidence, trust and satisfaction", "improve information, reinforce knowledge and understanding", "financial support and facilitate the use of services". The main common themes of categories were"empowerment and sense of adequacy".

**Conclusion:** This study showed that infertile couples beside of medical needs have a variety of problems and needs in the process of evaluating and treating infertility. Also through listening to patients, we learned that fertility clinics currently do not sufficiently meet patient's needs. Recognizing these needs by health care providers and medical staffs could improve their interaction with patients and the patient-centeredness of their care.

***Keywords:*** Infertility, Patient-Centeredness, Fertility Care, Qualitative Research, Infertile Couples

## $O_{nm}$-4: Maternal Psychological State during The Transition to Motherhood: A Longitudinal Study

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**Background:** The aim of this study was to investigate the psychological state of women and its associated factors in the third trimester of pregnancy and at eight weeks postpartum and to explore the changes in psychological state between these two time points.

**Material and Methods:** This survey study was carried out on 358 pregnant women visiting urban health centers in Shahroud, northeast Iran, in 2011. The women were followed at 8 weeks postpartum. In the third trimester of pregnancy, the participants completed the General Health Questionnaire (GHQ-28) and two other questionnaires on socio-demographic characteristics and frequent psychosocial stressors. They also completed the GHQ-28 and an obstetrical information form at 8 weeks postpartum. The cut-off point for GHQ-28 in Iran has been calculated at 24, which denotes probable psychological health problems.

**Results:** The mean total GHQ score decreased from 23.7 in the third trimester of pregnancy to 18.8 in the postpartum (p<.001).The proportion of women with GHQ-28 score >= 24 in the third trimester of pregnancy and postpartum were 42 and 26%, respectively. Multiparity, caring for other family members, financial problems, and anxiety about personal and fetal health were the predictors of psychological health problems in the third trimester of pregnancy. The two predictors for postpartum psychological health problems were psychological health problems in the third trimester of pregnancy and the method of infant feeding at 8 weeks postpartum.

**Conclusion:** The prevalence of psychological symptoms was high among women in the third trimester of pregnancy. Psychological state of women improved after childbirth.

***Keywords:*** Psychological State, Postpartum Period, Pregnant Women

## $O_{nm}$-5: Evaluation of Patients with Ovarian Hyper Stimulation Syndrome and Medical Care in Infertility Treatment Course in Royan Research Center from 2010 to 2012

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**Background:** Ovarian Hyper stimulation syndrome (OHSS) is infrequent and iatrogenic complication of ovary stimulation by Follicular stimulating hormone (FSH). OHSS is a probable complication of ovulation stimulating drugs, including clomiphene citrate and gonadotropins. Development of infertility treatment with the aim of reaching the desirable number of oocyte and embryo has led to ovary stimulation and increased pregnancy rates and risk of OHSS. On the basis of recent reports, the prevalence of short-term complications after infertility treatment is estimated 2%, which half on them is caused by OHSS.We performed this study is to evaluate the basic characteristic and treatment methods of OHSS patients

**Materials and Methods:** This study has been designed as a cross-sectional study to assess demographic information, medical history and treatment protocols of patients that developed moderate or severe OHSS during infertility treatment cycles in Royan research center, Tehran, Iran; from January 2010 to January 2012.In this study we used census method to collect samples.

**Results:** From 10000 referred patients to Royan research center, 261 patients (2.61%) developed OHSS. 101 (38.7%) of them had mild OHSS; 125 (47.8%) had

moderate OHSS and 33 patients (12.6%) had severe OHSS and 2(0. 9%) had critical OHSS. 84.9% of patients had primary infertility. Mean of ovulation stimulation days was 9.9 in moderate cases and 10.31 in severe cases. (p=0.356). Totally, 18.8% of infertilities were caused by ovarian factors, 2.5% by fallopian factors, 49.4% by male factors, and 3.8 by unknown causes. Infertility type (primary vs. secondary), type of OHSS (early vs. late), history of EP and abortion, thyroid hormones, allergy and menstruation regulation were not different significantly between the moderate and severe/critical OHSS groups. Totally, 5.6% of cases undergo IUI, 70% ICSI and 24.4% IVF/ICSI.

**Conclusion:** OHSS is a complication of ovulation. which can be life threatening. It is significantly more prevalent in young patients. Despite previous reports, PCOS and allergy did not correlate significantly with OHSS in this study.

***Keywords:*** Ovarian Hyper Stimulation Syndrome, Poly Cystic Ovary Syndrome, Assisted Reproductive Technology, Infertility

## $O_{nm}$-6: Seeking Security in Surrogacy Motherhood: A Grounded Theory

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**Background:** Surrogacy is one of the scientific revolutions in infertility domain in recent decades. Despite the widespread use of this technique especially surrogacy in Iran, studies about experiences of its clients are rare. Optimal care of commissioning mothers in surrogacy, requires proper understanding the process of motherhood. The aim of this study was exploration of the motherhood process in commissioning mothers.

**Materials and Methods:** A qualitative research approach using grounded theory approach was done. The experiences of commissioning mothers were assessed from decisive decision to use surrogacy to after child birth. Data were collected initially as purposeful sampling from Royan Institute and continued as theoretical sampling for 20 month from other infertility centers, offices and hospitals in Tehran and other provinces of Iran. After acquisition of informed consent, 39 unstructured interviews were done with 15 commissioning mother, 2 husband, 4 surrogate mother and 5 personnel of infertility centers. Data were analyzed with Strauss and Corbin (1998) approach.

**Results:** The data analysis showed that becoming a mother in surrogacy happen in a cultural dilemma context and the main issues expressed by participants was "insecurity in motherhood". "Seeking security" was basic strategy of commissioning mothers that emerged as a core concept. "Having the support", "Non-effective support", "Couples Interactions", "Individual and environmental characteristics" that can influence this process may play a role in facilitating or inhibiting. Finally, the outcome of implementing strategies and affecting factors is "to achieve relative peace" and "physical and mental exhaustion" that occur because of discomfort stability.

**Conclusion:** These findings suggest an interactive effect of multiple factors on the process of becoming a mother. With a greater understanding of the motherhood process in particular mothers, nurses can have more personal and professional care for this special population of mothers.

***Keywords:*** Becoming A Mother, Commissioning Mothers, Iran, Motherhood, Surrogacy

# Poster Presentations

## $P_{nm}$-1: Attitudes to Surrogacy in Women Attending to The Selected Shahrekord Public Health Centers in 1392

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**Background:** There has been little interest in the research literature on public opinions regarding assisted conception and surrogacy, particularly in Iran. Nevertheles, the growing evidence shows that problems in adaptation and coping may be related to perceived normative values.
**Materials and Methods:** In this cross sectional study, questionnaires on attitudes to surrogacy were completed by 187 women referring to health centers of Shahrekord-Iran.
**Results:** Significant socio-demographic differences were found between women who were possibly willing (n = 76) and those who were unwilling (n = 111) to become surrogate mothers. General attitudes to surrogacy also differed between groups (p = 0.004). This study differentiated adequately between groups on attitudes to recruitment for surrogacy (p = 0.005), the consequences of surrogacy (p = 0.002), factors that induce people to become surrogates (p = 0.004), social support (p = 0.005), age (p = 0.003) and predicted (UN) willingness to become a potential surrogate mother.
**Conclusion:** Further research is needed with larger sample sizes of potential surrogates to determine whether the predictive attitudes reported here translate to actual behaviors. The larger group which was not interested in considering becoming a surrogate scored significantly more negatively on all attitudes towards surrogacy. The negative attitudes reported by the 'unwilling to consider being a surrogate' group may reflect attitudes held by the majority of the population and are likely to be influenced by reports of stigma associated with surrogacy.
*Keywords:* Attitudes, Surrogacy

## $P_{nm}$-2: The Impact of Behavioral Health Sleep Education on The Quality of Life in The Pregnant Women with Sleep Disorder: A randomaized Control Trial, Year 2012

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**Background:** 79% of the pregnant women suffer from sleep disorders. These disorders are the result of physiological, hormonal and physical changes. Sleep disorders before, during and after childbirth can affect the quality of life of pregnant women. This study aimed to evaluate the impact of behavioral health sleep education on the quality of life in the pregnant women with sleep disorder in the second trimester of pregnancy.
**Materials and Methods:** This study is a randomized clinical trial, which was on 112 pregnant women with sleep disorder (According to the Pittsburgh Sleep Quality questionnaire). Tools for data collection included demographic questionnaire and summarized in the World Health Organization Quality of Life Questionnaire (WHOQOL: BREF). Sampling was done in an easy and accessible way. After sampling , women were randomly divided into control and case groups: the case group, health behavior sleep education were presented during a four-hour session in weeks 22, 23, 24 and 25 then followed up by filling out the summarized in the World Health Organization Quality of Life (WHOQOL: BREF) questionnaire in the first visit after intervention in week 29 of pregnancy and the second follow-up session two months later at week 33 by the subjects. The control group received no intervention and only routine prenatal care was provided for them. The results were assessed by chi-square tests, t independent test and Fischer by SPSS version 18.
**Results:** A statistically significant change was reported in the quality of life in the intervention group (case) in comparison to the control group [29 weeks ($p < 0.000$), 33 weeks ($p < 0.001$)].
**Conclusion:** Behavioral health sleep education improves the quality of life in pregnant women who are experiencing insomnia. Findings from this study add support to the reported effectiveness of behavioral health sleep education in the prenatal care and clinical management of insomnia in pregnancy.
*Keywords:* Quality of Life, Pregnant Women, Sleep Disorder

## $P_{nm}$-3: Quality of Life in Pregnant Women with Sleep Disorder

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**Background:** 79% of the pregnant women suffer from sleep disorders. Sleep disorders before, during, and after childbirth can affect the quality of life of pregnant women. This study aimed to evaluate the quality of life of pregnant women with sleep disorders in the second trimester.
**Materials and Methods:** This study was cross-sectional study. Data was collected with continuous sampling method ,from 100 pregnant women with sleep disturbances (Pittsburgh Sleep Quality based on standard questionnaires ) in second trimester referring to two elected health centers in order to get prenatal care at Maku (Western Azarbayjan University of Medical Sciences ). Data was collected by using the personal information forms and the questionnaires summarized in the World Health Organization Quality of Life (WHOQOL: BREF). Statistical methods (frequency tables, Pearson, Spearman and ANOVA) were used for data analysis.
**Results:** The results indicate that mean of sleep quality was $8.62 \pm 2.81$ in pregnant women with sleep disorders or poor quality of sleep in the second trimester. Quality

of life and four domains include: physical health, psychological health, social and environmental health related quality of sleep.
**Conclusion:** Given that a large percentage of pregnant women suffer from sleep disorders, particularly in the second trimester, Hope this period, in addition to usual care, special programs for research, diagnostics and troubleshooting the cause of the disturbance takes place.
*Keywords:* Quality of Life, Pregnant Women, Sleep Disorder

## $P_{nm}$-4: Educational Effectiveness on The Emotion Recovery in Spontaneous Abortion

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**Background:** Recurrent spontaneous abortion (RSA), as a heterogeneous condition affects approximately 1-3% of women in reproductive ages. Etiology of this frustrating disease in 40-50% is often unexplained. RSA as a traumatic event, especially with unclear etiology, could be followed by psychological squeal particularly anxiety. Emotion recovery, alongside investigation and medical treatment should be considered for these women. Coping strategies is one of apparatus to provide this aim. The study was carried out to assess whether education might induce coping strategies efficacy or not.
**Materials and Methods:** Volunteers were 50 women with RSA history. They were between the 5th and 10th weeks of gestation. Participants answered "Cattlle's Anxiety Questionnaire (CAQ)" and "Ways of Coping Questionnaire" .We compared their education levels with their anxiety level and coping manner. Statistical analysis was performed by SPSS (version 13).
**Results:** Surveying coping style strategies and anxiety level by Pearson test, show that there is significant negative relation between them (p=0.008 and R= -0.411). Using ANOVA, guided us to significant relation between anxiety score and education (p=0.021).According to the statistical analysis revealed the positive relation between education levels and coping style strategies (p=0.001 and R=0.486).
**Conclusion:** Problem - focused coping strategies associated with low anxiety. Considering to positive relation between coping and education, it seems that promoting personal insight helps to reduce the anxiety in these patients.It is well known that some patients suffering from RSA may present with psychological disorders especially anxiety. In summary, coping strategies and education as a network has been proposed to contribute to decline emotion storm.
*Keywords:* Recurrent Spontaneous Abortion, Anxiety, Coping Style Strategies, Education

## $P_{nm}$-5: Assessment of Anxiety among Infertile Women Undergoing First IVF- ICSI Treatment Cycle

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**Background:** Infertility is one of the worst stressful experiences of marital life. As a Bio-psycho-social crisis, it may affect infertile couple's mental health. The most common mental complain is anxiety. On the other hand, women who try to treat their infertility problem are more agitated. Aim of this study was to determine anxiety rates among infertile women undergoing first IVF-ICSI treatment cycle.
**Materials and Methods:** 90 infertile women undergoing their first IVF-ICSI cycle in Royan Infertility clinic was entered this descriptive study. Samples were selected randomly based on available sampling. Data was collected by "demographic" and "Spielberger State Anxiety Inventory" questionnaires and was analyzed via SPSS18 software by using descriptive tests.
**Results:** Women aged 29.95 ± 4.7 years. Average duration of marriage and infertility were 6.5 ± 3.54 and 5.45 ± 3.6. Education degree was diploma in 44.4% of them and 85.6% of samples were housekeeper. Average anxiety rate in first day of ovulation stimulation was 42.5 ± 10.1 which mean moderate anxiety according to Spielberger Scale.
**Conclusion:** Our study demonstrates that women undergoing first IVF-ICSI cycle suffer from anxiety interdependent to treatment and need to be helped to reduce it. Thus, extra studies are recommended to evaluate if any intervention can decrease treatment related anxiety.
*Keywords:* Anxiety, Infertility, IVF, ICSI, Assisted Reproductive Technologies

## $P_{nm}$-6: Eight Sonographic Key Points That Every Midwife or Nurse Needs to Know

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**Background:** Ultrasound is preferred as first imaging modality in the study of the female pelvis. In this article, we have tried to mention 8 points that each midwife or nurse needs to know for planning the time of sonogra-

phy and analysis of findings.
**Results:** During the menstrual cycle, all pelvic organs are influenced by cyclic changes. Thus, pathologic conditions of pelvis need to be well assessed depending on the day of the cycle. Therefore, best timing of the sonography is the key point for diagnosis and decision making about the patients. Here are 8 key points to facilitate this: 1. If you suspect a patient to have an ovarian cyst, best time is 2-5thdays of the menstrual cycle, when the follicles have not grown yet. 2. PCOS, suspected in women with irregular menses, is best diagnosed at days 2-5th. 3. Uterine polyps are best recognized during days 5-8th. 4. Fibromas are well assessed at days 11-14, to evaluate: type of which, location of placement and if they cause endometrial distortion or not. 5. Uterine malformations which are usually raised in recurrent miscarriages are best evaluated during days 11-14 at 2D sonography, and days 17-21 on 4D sonography. 6. Endometerial thickness changes cyclically during menstrual cycle and best time to assessing endometrial pathologies(hyperplasia …) depend on the situation. 7. Fallopian tubes are not visualized at TVS naturally, but they get recognizable in some pathologic conditions (hydrosalpinx...). Thus, TVS will be helpful if there is clinical suspicion. 8. Infertility workup: A- Base sonography for overall evaluation of the pelvis and determining any pathologic condition (days 5-8th). B- Monitoring sonography during treatment cycle to investigate ovarian and endometrial responses to drugs.
**Conclusion:** While ultrasound has a pivotal role to confirm diagnosis and options of different treatment regimes, considering these key points improves work skills, accuracy and efficiency.
***Keywords:*** Timing of Sonography, Menstrual Cycle, Pelvic Pathologies

## $P_{nm}$-7: Cross-Border Reproductive Care or Fertility Tourism

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**Background:** The fertility tourism is a term refers to the cross-border movements made by infertile patients in order to receive reproductive treatment through assisted reproductive technology. In recent years the number of countries involved in medical tourism has been increased. The aim of this study is to provide an overview of the research that has explored aspects of cross-border reproductive care.
**Materials and Methods:** A literature search was conducted on PubMed.
**Results:** The main interest of travelling to another country for infertility treatments arises from different ethical, religious and legal attitudes concerning reproductive health, such as: high cost, long waiting lists, law evasion when a certain type of treatment is forbidden due to specific demographic or social characteristics, limited access to the technology due to a lack of expertise or lack authoritative evidence of safety and efficacy, the quest for equity of access to high quality, safety and effective health care. Since fertility tourism is a growing phenomenon, some of its problematic aspects including high risk of health complications, disappointment and discrepancies need to be resolved. The European Society of Human Reproduction and Embryology established a good practice guide for centers and health practitioners which focuses on patients, third-party reproduction, future children and professionals. This is obtained by including equally important operational principles of fairness and justice in healthcare, safety, evidence-based care, efficiency, being timely and patient centeredness. In order to prepare the treatment, IVF stimulation monitoring and following of complications, planning to setup a supporting clinics network is suggested.
**Conclusion:** The reproductive care for cross-border patients is a fast-growing industry in both developed and developing countries and helps contribute to economic growth and enhances patient's autonomy, thus should be promoted at all levels.
***Keywords:*** Fertility Tourism, Infertility, Reproductive Care

## $P_{nm}$-8: The Effect of Pregnant Women Empowerment Program on Elements of Empowerment and Their Satisfaction

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**Background:** To determine the effectiveness of pregnant women empowerment program on elements of empowerment and their satisfaction of prenatal cares.
**Materials and Methods:** A randomized controlled trial was conducted at a prenatal clinic. The total number of samples were 70; that pregnant women were randomly assigned to either control (n=35) or experimental group (n=35). Women in the experimental group received their prenatal care (5 sessions) through four groups of women with the same gestational age. Data collected by two scales as follows: empowerment scale for pregnant women, and participation and satisfaction questionnaire.
**Results:** Seventy women were recruited to the study and 61 ultimately received all their prenatal care (5 sessions). There were no significant differences between control and experimental groups, with whole demographic variables. There were significant differences between control and experimental groups, whit empowerment score ($p= 0.013$), and satisfaction score ($p<0.001$).
**Conclusion:** Pregnant women empowerment program provided three components of care as follows: prenatal assessment, education, and social support. These components enhanced empowerment of pregnant women.

*Keywords:* Empowerment, Self-Efficacy, Social Support, Prenatal Care, Satisfaction

## $P_{nm}$-9: Serum and Peritoneal Fluid Level of Vascular Endothelial Growth Factor in Endometriosis

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**Background:** Endometriosis is known as one of the most common disease in women of reproductive age. Due to importance role of vascular endothelial growth factor (VEGF) in neo-vascularization for the implantation of endometrial cell and different reported results for VEGF level in the serum and peritoneal fluid (PF) in endometriosis patients, this study was designed to determine the serum and PF levels of VEGF in endometriosis patients, and to compare it with normal subjects.
**Materials and Methods:** Women who were subjected to laparoscopy for the evaluation of infertility or pelvic pain were allocated into two groups; women with (group I, n= 90) and without (group II, n=89) endometriosis. The PF from pelvis and venous blood samples were obtained. The VEGF concentration of the serum and PF were measured using enzyme immunoassay kit and were compared using t-Student test.
**Results:** The level of VEGF level in serum was significantly less than in PF in both groups (p=0.00). However endometriosis patients had a higher level of VEGF in peritoneal fluid significantly when compared with non-endometriosis patients (p=0.043).
**Conclusion:** Angiogenic activity is increased by the elevated level of VEGF in the PF of endometriosis patients. This elevated level of VEGF also promotes neovascularization within the peritoneal environment.This disease is associated with only pelvic inflammation, and is not associated change in the level of circulating VEGF.
*Keywords:* Endometriosis, Vascular Endothelial Growth Factor, Peritoneal Fluid

## $P_{nm}$-10: The Relationship between Work Ability and Work Satisfaction in Midwives Employed at Public Hospitals and Health-Care Centers in Iran (Mashhad) in The Year 2011

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**Background**: Work ability and work satisfaction are the key factors in staff efficiency in organizations with physical and psychological impacts on employees especially in nursing and midwifery jobs. So the aim of this study was to determine the relationship between work ability and work satisfaction in midwives.
**Materials and Methods:** This cross sectional study was carried on 123 midwives employed in the public hospitals and health centers of Mashhad with two stage sampling method (cluster-convenience) in Mashhad in the year 2011. Demographic characteristics, Work Ability Index (WAI) and Herzberg work satisfaction questionnaires were also completed by the participants. The statistical analysis was performed with various statistical tests such as Pearson correlation coefficient, and linear regression using SPSS statistical software (version 11.5).
**Results:** The mean of ± SD and work ability and work satisfaction score were 38.81 ± 0.05 and 134.69 ± 1.21 respectively. The study results showed that work ability was well in 49.6% of the midwives, and there was a positive correlation between the rate of work ability and work satisfaction scaling. (p=0.001, r=0.761).
**Conclusion:** Regarding the level of work ability in midwives and its positive correlation with work satisfaction, evaluation of circumstances in their work environment and making efforts to increase their work ability seem necessary.
*Keywords:* Work, Ability, Satisfaction, Midwives

## $P_{nm}$-11: *In Vitro* Fertilization and The Challenges Ahead

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**Background:** Infertility is defined as inability in pregnancy after one year of regular intercourse without using contraceptive methods. Approximately15-10% of couples in childbearing appears infertile. It is the cause of infertility in men and women and infertility treatment depends on type of failure and is one of the methods of treating infertility *in vitro* fertilization. This research is review articles in recent years, with a critical approach.
**Materials and Methods:** A pub-med and Googlescholar search limited to "*In vitro* fertilization" (IVF) and "Infertility" was conducted from 2008 to 2012. Studies regarding challenges and risks of IVF were investigated in details.
**Results:** Using the technique of *in vitro* fertilization for infertility treatment passes more than 25 years. IVF is a method that offers the possibility of fertilization of eggs in vitro. Scientists who fallowed up to 845 cases found that in vitro fertilization pregnancy risks and complications of every 3 people have risen to 16 per thousand and mothers who become pregnant through in vitro fertilization than those that normally are pregnant, are three times more risk of complications. Placenta-Prevails complication of pregnancy with IVF can cause bleeding in the mother and increase the risk of preterm delivery and complications during pregnancy. IVF increases the likeli-

hood of Multiple Pregnancy (20 to 40%) and the rate of cesarean section and as we know in multiple pregnancies, prenatal complications than are singletons.
**Conclusion:** Since the risk factors for infertility has increased in the modern world and human tries to solve this problem, it should be understood the advantages and disadvantages of various methods of artificial fertilization, and will provide the necessary care in order to better resolve the disadvantages.
***Keywords:*** IVF, Challenge, Review

## $P_{nm}$-12: Nutrition and Endometriosis

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**Background:** Endometriosis has a significant impact on women's quality of life. The aim of this study by reviewing the literature is to discover the impact of diet on endometriosis.
**Materials and Methods:** Searching databases was Cochrane, Pub-med, library studies, and research website of leading researchers in the world from 2002 to early 2012.
**Results:** The literature search shows that certain components of the diet may be associated with the risk of endometriosis. Recent study of the relationship between endometriosis and low intake of fruits and vegetables and dairy products, and high consumption of saturated fats, ham, beef and other red meats are raised. Endometriosis is an estrogen-dependent disease.A vegetarian diet would supposedly raise serum ligand and sex hormone carrier protein levels, thus reducing the available estrogen concentration. Vitamins, especially vitamin B complex, magnesium and omega-3 play an anti-inflammatory in patients with endometriosis. Diets based on fruits, vegetables, vitamins, magnesium, and omega-3 reduces animal protein intake and therefore reduces the excess of body fat and estrogen peripheral production. On the other hand another study showed an increased risk of endometriosis with β-carotene and higher servings of fruit/d, but these findings have not been confirmed elsewhere and require further evaluation in a prospective investigation.
**Conclusion:** According to the impact of endometriosis on quality of life in women and its relation to diet, prospective studies on various diets recommended.
***Keywords:*** Diet, Endometriosis, High-Fiber Diet

## $P_{nm}$-13: Effect of Electronic Education on The Awareness of Women about Post-Partum Nutrition Education

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**Background:** With regard to general advantages of electronic education and the necessity to pay attention to this issue, and with consideration of postpartum maternal care promotion, especially nutrition education, this study was conducted to define the effect of electronic education on the awareness of women about post-partum nutrition education.
**Materials and Methods:** This is a quasi- experimental study conducted on 72 primiparous(36 electronic, 36 booklet) women during postpartum period referring to Shahid Beheshti hospital in 2012-2013. In order to educate the subjects through electronic package and booklet methods, the subjects were selected through random allocation. Pre- test before and post- test after educations conducted with a self-instructed questionnaire to measure women's awareness about nutrition. The data were analyzed by descriptive statistical tests, and T-test and paired t test through SPSS (version 11.5). Significance level was considered <0.05 .
**Results:** Mean scores of awareness about postpartum nutrition before and after intervention in electronic education and control (booklet) groups were 1.02(0.01), 1.1(0.01), 3.42 (0.03), and 2.22 (0.2) respectively, which showed a significant difference (p=0.031). Levels of increase of awareness in electronic education and control groups were 82 and 45% respectively.
**Conclusion:** As electronic education method had a higher effect on level of awareness about postpartum nutrition among mothers compared to booklet method, health providers are suggested to apply this method to increase mothers' knowledge and to provide them with care.
***Keywords:*** Postpartum, Electronic, Learning, Nutrition

## $P_{nm}$-14: Knowledge and Attitude of Iranian Infertile Women toward Surrogacy

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**Background:** Infertility as a stressful life crisis devastates the person's mental health.One of the latest methods of ART is surrogacy. The unique aspects of surrogacy have led to it becoming the most controversial of all the assisted reproductive techniques in recent years and arises ethnical, moral, psychological and social issues. So this study performed with the purpose of determining the knowledge and attitudes of infertile women about surrogacy.
**Materials and Methods:** This is the descriptive study done on 150 infertile women who referred to Fatemieh infertility treatment center in Hamadan city in Iran between 2010-2011 years. Samples were selected by systematic randomized method. Data gathering was on the base of questionnaire and interview. Data analyzing performed with SPSS (version 16) software.
**Results:** The rate of using surrogacy if it was necessary

was 43.33% between infertile women. Approximately half of respondent (49.3%) preferred using known surrogate mother. 68.6% of infertile women agreed with disclosure to child. 67.33% of women did not have received any educational information about surrogacy. However, only 10% of infertile women did not haveenough knowledge toward surrogacy. The positive attitudes of the infertile women toward surrogacy were 53.33%.

**Conclusion:** Although we have good knowledge of infertile women toward surrogacy, their attitudes about this technology aren't so positive; on the other hand changing the cultural background in society for better acceptability of this method by people is needed.

*Keywords:* Surrogacy, Attitude, Infertility

## $P_{nm}$-15: IUD Insertion and Alterations of Candida Species in Cervicovaginal Specimens by Polymerase Chain Reaction Technique

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**Background:** Vulvovaginal Candidiasis is one of the most common vaginal infections and identification of candida species can be helpful in medical treatment. Intra uterine devices (IUDs) are effective, long-term methods of contraception, which in some cases may lead to Vulvovaginal Candidiasis. This study was developed to assess the effect of IUD insertion on Candida fungal flora in cervicovaginal specimens.

**Materials and Methods:** This longitudinal prospective study was performed in 2012 on 95 Iranian women clients attending Health Centers of Tehran, who selected Copper T-380-A IUDs for contraception and who had no history of local or systemic antibiotic or antifungal medications use during the last 2 weeks before sampling. Cervico-vaginal specimens were collected and cultured on Sabouraud dextrose agar and CHROMagar Candida, before and 3 months after IUD insertion. Finally, a molecular method, PCR-RFLP was performed for definite identification of candida species. The statistical software used was: SPSS v.16. Pvalues <0.05 were considered significant.

**Results:** Positive candida cultures were significantly increased 3 months after IUD insertion (25 vs. 11.6%, Man-Whitney pvalue: 0.007). There were no significant alterations in the identified species of candida after IUD insertion. The most common species before and after IUD insertion, were Albicans, Glabrata and then both Albicans and Glabrata simultaneously. The prevalence of C.albicans and C.glabrata decreased, but simultaneous C.albicans and C.glabrata increased insignificantly.

**Conclusion:** There was a substantial increase in the prevalence of cervicovaginal candidiasis after IUD insertion. As the prevalence of simultaneous infection with Albicans and Glabrata species which are more resistant to treatment, was increased- although insignificantly- this finding needs further investigation.

*Keywords:* CopperT-380-A IUD, Candidiasis, Candida Species, PCR Technique

## $P_{nm}$-16: The Effect of Childbirth Preparation Classes on Delivery Outcome

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**Background:** Childbirth education has existed as a formal structure and has provided preparation for childbirth with a focus on natural birth. Over time, the classes have continued to evolve but the underlying purpose remains the same: to provide prenatal preparation for pregnancy, labor and birth. The aim of this study was to determine the effectiveness of antenatal preparation for child birth courses on delivery outcome.

**Materials and Methods:** This is a quasi-experimental study. 600 pregnant women who attend the antenatal clinic at Al-Zahra Hospital, Rasht, were assigned randomly to either on experimental or control group. Experimental group was trained both theoretically and practically for eight sessions while 300 pregnant women in control group received standard antenatal care. The following criteria were used to select the study sample: Iranian pregnant women who primiparas,18-35years old, over 20 weeks of pregnancy, with no medical problems, with no multiple pregnancies, not smoking, and not threatened to abortion in their history and the fetus had vertex presentation. Women in both groups were followed until their delivery and then, the deliveries between them were investigated by using SPSS software.

**Results:** There were no significant differences in sociodemographics and obstetrics characteristics (p>0.05). The rate of vaginal delivery in experimental group (62.5%) was significantly higher than control group (47.3%) in Chi-squared test. Moreover, duration of active labor and the second stage of it were shorter in trained women (p<0.001).

**Conclusion:** Our findings suggested that educating pregnant women about labor and delivery may result in increasing the rate of natural delivery. Thus, implementing this program as a continuous intervention during pregnancy is recommended.

*Keywords:* Childbirth Preparation Class, Prenatal Care, Natural Delivery

## $P_{nm}$-17: Cost Effectiveness of Human Papillomavirus Testing in Cervical Cancer Diagnosis

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**Background:** Epidemiological studies are shown robust association between human papillomavirus (HPV) and cervical cancer. From 1990 HPV was known as a necessary but insufficient cause of cervical invasive cancer. The purpose of this study was to evaluate the cost effectiveness of HPV test in cervical cancer screening.
**Materials and Methods:** This is a systematic review article; the newest scientific references were used for data gathering.
**Results:** Henry Kitchener in Manchester University compared screening results of 24000 women who were randomly assigned to either on Pap smear and HPV testing group or only Pap smear group and shown that there is no significant difference for diagnosing cervical high grade lesion between groups. In another prospective study compared cost effectiveness of four strategies that were conducted on 100000 women (Pap smear and HPV testing; only Pap smear; colposcopy and HPV testing and only HPV testing). They concluded that HPV testing is cost effectiveness for screening cervical cancer. Mettenorf et al. (2008) in a review study indicated that sensitivity of HPV testing for cervical intraepithelial neoplasia (CIN) 2, 3 was 94.6% while, sensitivity of Pap testing was 55.4%. Specificity of HPV testing (1.94%) was lower than Pap testing (96.8%). Although HPV testing detected more cases of high grade CIN compared to Pap, but this may increase false positive. Maximum sensitivity was achieved by using both HPV and Pap testing for each woman but false positive and utilization of colposcoy were increased. Researcher concluded that HPV testing was cost effective for cervical cancer screening. Flores et al. (2011) showed that using HPV testing or the combination of it and Pap testing is always more cost effective than using Pap test alone.
**Conclusion:** Strategies incorporating HPV testing are not only more effective than screening based on cytology alone but are also highly cost-effective. These results may help policy makers implement HPV testing as part of the cervical cancer screening program.
***Keywords:*** Cost-Effectiveness, HPV Test, Cervical Cancer Screening

## $P_{nm}$-18: Is There Relation between Newborns' Sex and NICU Admission or Mode of Delivery?

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**Background:** Newborns' health is one of the important indexes in health services. The aim of this study was to assess the relationship between newborns' sex and rate of admission at NICU and mode of delivery.
**Materials and Methods:** This descriptive and analytic study was confirmed on 492 newborn that was admitted to NICU of Rouhani Hospital of Babol University from March 2012 to 2013. Data collection tools included an information record paper of newborns' medical file in NICU. The data were analyzed by SPSS.
**Results:** Of the 492 newborns,215(43.7%) were term (male 107, female108) and 277(56.3%) were preterm (male156, female 121).These results did not show any significant difference between newborns' sex and rate of admission at NICU ($p>0.05$).In this study 110 newborns were delivered by normal vaginal delivery (male 60, female 50) and 382 newborns were delivered by cesarean section (male 203, female 179).The results showed cesarean section in male sex is more than female sex in this unit ($p<0.05$).
**Conclusion:** It appears there is not any association between newborn's sex and rate of admission at NICU but there is relation between newborns' sex and mode of delivery.
***Keywords:*** Newborn's sex, NICU, Delivery

## $P_{nm}$-19: The Effect of Black Cohosh (CimicifugaRacemosa) on Vasomotor Symptoms in Postmenopausal Women: A Randomized Clinical Trial

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**Background:** Hot flash is considered to be an early and common symptom of menopause. This symptom can interfere with the social and personal life of women; therefore, it demands a secure and effective intervention. Therefore, the present study aimed to determine the impact of black cohosh (Cimicifugaracemosa or actaearacemosa) on vasomotor symptoms in postmenopausal women.
**Materials and Methods:** This was a randomized, double-blind, placebo-controlled clinical trial. This study was performed on 84 postmenopausal women. The participants were randomly divided into control and intervention groups. The participants of the intervention group received one black cohosh tablet per day (6.5mg of black cohosh dry root extract), and the control group received one placebo tablet per day for eight weeks. The severity of vasomotor symptoms and number of hot flashes were recorded during the pre-intervention phase, and 4 and 8 weeks after the intervention. The data were analyzed using repeated measures ANOVA and ANCOVA tests. The level of significance was considered lower than 0.05.
**Results:** There was a significant difference between the two groups in terms of severity and number of hot flashes in weeks 4 and 8 by controlling the intensity of vasomotor symptoms and number of hot flashes before the intervention. Intragroup comparison showed a significant difference between the intensity of vasomotor symptoms and number of hot flashes during the two intervals. Moreover, using repeated measures ANOVA, the intergroup comparison indicated a significant dif-

ference between the test and control groups in terms of severity of vasomotor symptoms and number of hot flashes.
**Conclusion:** According to the findings of the study, it seems that black cohosh can be used as an effective alternative medicine in relieving menopausal vasomotor symptoms.
***Keywords:*** Menopausal Vasomotor Symptoms, Black Cohosh, Herbal Therapy

## $P_{nm}$-20: Assessment of Infertility Effect on Mental Health of Infertile People

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**Background:** In recent years, particular attention is the mental health of infertile couples. In fact, for many infertile couples experiencing infertility are severe distresses. The aim of this study is Assessment of infertility effect on various aspects of mental health of infertile people.
**Materials and Methods:** In this review study, books, articles and some sites that are related to mental health of infertile people were used. Then, the subjects were studied and the obtained findings were compiled as an essay.
**Results:** According to this study, several aspects of the Performance Couples are affected by infertility, such as disorder in sexual activity, self-esteem, communication between couples, sexual identity and feelings of rejection, cognitive function, costly and laborious Encounter therapy, anxiety and depression, guilt and failure noted. On the other hand, the prevalence of infertility in men and women are approximately equal, but women endure more pressure and discomfort.
**Conclusion:** According to multiple effects inappropriate of infertility on infertile people and presence of factors such as of social and economic factors in this problem, attention and planning authorities for further decreases psychological symptoms in these people seems necessary.
***Keywords:*** Infertility, Mental Health, Infertile

## $P_{nm}$-21: The Use and Adverse Reactions of Herbal Medicines during Pregnancy among Iranian Women

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**Background:** The use of herbal remedies has increased despite the lack of scientific evidences about their safety and efficacy during pregnancy. This cross-sectional study aimed to explore the usage of herbal products during pregnancy amongst Iranian women based on their socio-demographic characteristics, assessment of side effects in mothers, and ultimately their possible effects on neonates.
**Materials and Methods:** In total, 400 postpartum Iranian women and their singleton newborns were recruited during the first few days of postnatal period at Arash Hospital in Tehran, Iran. Data were collected in the course of a face-to-face interview by a pharmacist and completion of a questionnaire including maternal socio-demographic characteristics, medical history, pregnancy-related conditions, and all medicines used during pregnancy. Questions were used to gather the relevant information for up to one month before pregnancy. Medical notes were scrutinized for neonatal characteristics.
**Results:** Of all 409 eligible women, 400 (97.8%) agreed to participate. At least one herbal medicine was used by 325 (81.2%) of the interviewed subjects. The use of herbal drugs was significantly ($p<0.05$) higher amongst the higher-educated, employed, and primiparous women. Mint, frankincense, flixweed, and olive oil were the most common used herbal preparations. Vitamins and minerals, anti-infectives, and gastrointestinal drugs were on the top of the list of non-herbal drugs. Nausea, vomiting, and heartburn were the most reported side effects with herbal remedies. Regarding neonates, jaundice and respiratory distresses were the most prevalent problems detected.
**Conclusion:** This study indicates that taking herbal and non-herbal medications during pregnancy is common amongst Iranian women. Although not always safe, herbals are mostly used according to personal judgment without informing health care professionals. Pregnancy care providers should be aware of the potential risks and benefits of the commonly used herbal drugs by the pregnant women.
***Keywords:*** Pregnancy, Herbal Medicines, Adverse Drug Reactions

## $P_{nm}$-22: Breast Screening in Patient Undergoing ART Cycles

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The fact that the development of breast cancer is linked to repeated or sustained exposure to high blood estrogen levels has raised obvious concern that reproductive hormones particularly estrogen, might increase the risk of breast cancer development. While, the general consensus is that overall; IVF is not associated with an increased risk for development of breast cancer, a recent report published by Stewart LM (Fertil Steril. 2012) on a large Western Australian study, comprising data from 21,025 women and spanning 20 years revealed that women who initiated their 1st IVF attempt in their

mid-twenties were approximately one-and-a-half times more likely to develop breast cancer than were controls , who underwent other (non-IVF) forms of fertility treatment. The American College of Obstetricians and Gynecologists recommend that women be offered annual screening for breast cancer starting at age 40; the American Cancer Society endorses mammograms starting at the same age. However, women who have risk factors that increase the chance of breast cancer should undergo earlier screening, especially if they are planning to undergo fertility treatment. Other risk factors used in the modified GAIL MODEL are: current age, age at menarche, age at the first live birth or nulliparity, number of previous benign breast biopsies, atypical hyperplasia in a previous breast biopsy, race and a positive family history. Most major medical organizations recommended starting routine mammography (MMG) for women at age 40. MMG is not a stand-alone procedure and physical findings must be considered along with the radiographic and histological assessment. There are several studies supporting the use of US for breast cancer screening as an adjunct to MMG for high risk women. Breast MRI can be considered in addition to MMG for screening in high risk patient.

***Keywords:*** Breast Screening, ART

## $P_{nm}$-23: The Relationship between Fetal Sex and Maternal Blood Group with Sleep Disorders during Pregnancy

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**Background:** 79% of the pregnant women suffer from sleep disorders. These disorders are the result of physiological, hormonal, physical changes, that occur in pregnancy due to different causes including fetal sex and maternal blood group. This study aimed to evaluate the relationship between fetal sex and maternal blood group with sleep disorders during pregnancy in the second and third trimester.

**Materials and Methods:** This study was cross-sectional and data was collected with continuous sampling method ,from 312 pregnant women with sleep disturbances (Pittsburgh Sleep Quality based on standard questionnaires ) in second and third trimester referring to two elected health centers in order to get prenatal care by the city of Maku in the Persian month Azar Bayjan Western University of Medical Sciences (University) and with using the personal and prenatal information forms completed by units research , and statistical methods to analyze data and analysis (frequency tables, Pearson, Spearman, ANOVA and Chi-squear ) was used .

**Results:** There was significant association between subjective sleep quality ($p \leq 0.000$), sleep duration ($p \leq 0.04$), sleep efficiency ($p \leq 0.025$), sleep disturbance ($p \leq 0.045$) and sleep quality score ($p \leq 0.015$) with maternal blood group. But among the sleep components only Subjective Sleep Quality ($p = 0.21$) and day time Say time dysfunction ($p = 0.06$) did not have relationship with fetal sex.

**Conclusion:** Fetal gender is independently associated with adverse pregnancy outcome. Although the added risk is relatively small, further investigation of the mechanisms underlying this association may contribute to our understanding of the pathophysiology of pregnancy complications such as sleep disorders.

***Keywords:*** Pregnant Women, Fetal Sex, Blood Group, Sleep Disorder

## $P_{nm}$-24: The Impact of Behavioral Health Sleep Education on The Depression of Pregnant Women with Disorders Sleep: A Randomaized Control Trial, Year 2012

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**Background:** 79% of the pregnant women suffer from sleep disorders and 70% of pregnant women have some of the depression symptoms. These disorders are the result of physiological changes, hormonal, physical, pregnancy which occur from different causes and can affect disorders before, during and after childbirth be involved in causing depression and may be aggravated by disregarding health behavior. Health behavior education may be useful for the management of depression among pregnant women. This study aimed to evaluate the impact of behavioral health sleep education on the improvement of depression in pregnant women with sleep disorders.

**Materials and Methods:** This study is a randomized clinical trial on 96 pregnant women with sleep disorder (according to the Pittsburgh Sleep Quality Questionnaire).Tools for data collection included demographic questionnaire and Beck's Depression Inventory Questionnaire through which sampling was done in an easy and accessible, after sampling was randomly (simple) divided into control and case groups. The case group, health behavior sleep education were presented During a four-hour session in weeks 22, 23, 24 and 25 then followed up by fill out the Beck's Depression Inventory Questionnaire in the first meeting on the one month after intervention in week 29 of pregnancy and the second follow-up session two months later at 33 weeks by the subjects. The control group received no intervention, only routine prenatal care was provided to them. The results were assessed by Chi-square tests, independent t test and Fischer by SPSS version 18.

**Results:** A statistically significant change was reported in the depression of pregnant women with sleep disorders in the intervention group (case) in comparison to the control group [29 week ($p \leq 0.024$), 33 week ($p \leq 0.026$)].

**Conclusion:** Behavioral health sleep education improves the depression in pregnant women who are experiencing insomnia. Findings from this study add support to the reported effectiveness of behavioral health sleep education in the prenatal care and clinical management of insomnia in pregnancy.

***Keywords:*** Behavioral Health Sleep Education, Depression, Pregnant Women, Sleep Disorder

## $P_{nm}$-25: Nursing Information Systems: Issues and Challenges

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**Background:** The nursing process is often considered as core of the nursing care delivery and guides the care documentation. Currently, with rapid advance in Information and Communication Technology (ICT) this process can be supported electronically. Applying information systems improves care health processes. Nursing Information Systems (NISs) deal with nursing process.
**Materials and Methods:** Evaluation of some Canadian hospitals that use NIS technology versus traditional hospitals
**Results:** NIS also influences different aspects of nursing process. This paper presents a survey of NIS including concept, benefits and barriers, NIS Models, and major challenges including professional, organizational, technical, and communicational problems. However, the actual use of nursing information systems (NISs) in Iran is limited to a few sites. Therefore, it must be analyzed the problems that exist in the development and use of NIS and to look for solution to solve them.
**Conclusion:** Applying information systems improves care health processes. Nursing Information Systems (NISs) deal with nursing process. NIS also influences different aspects of nursing process. This paper presents a survey of NIS including concept, benefits and barriers, NIS Models, and major challenges including professional, organizational, technical, and communicational problems. However, the actual use of nursing information systems (NISs) in Iran is limited to a few sites. Therefore, it must be analyzed the problems that exist in the development and use of NIS and to look for solution to solve them. Eventually, patient care will benefit from professional involvement of nurses in the development and use of NISs.
***Keywords:*** ICT, Nursing Care, Nursing Information Systems

## $P_{nm}$-26: Prevalence of Candidiasis and Tricomoniasis Infections and Sexual Health Behaviors in Women Supported with Selected Health Centers of Tabriz

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**Background:** Vulvovaginal candidiasis, bacterial vaginosis and trichomoniasis include at least 90% of infectious vaginitis. Midwives have a primary role in vaginal health, not only in proper assessment and management, but also in prevention through patient education. The aim of this study was determining the prevalence of Candidia sis and Tricomoniasis infections and sexual Health behaviors in women supported with selected health centers of Tabriz.
**Materials and Methods:** This is a descriptive and analytical study, in which 1000 women who had the specifications of investigation units were selected by random sampling of 12 selected health centers of Tabriz. A questionnaire was used to obtain their personal and sexual Health behaviors information .Check list for clinical observations and culture methods (sabura agar and diamond) for diagnose vaginal infections was used. Data were analyzed by using SPSS 11.5, and frequency, mean and standard deviation, $X^2$, Fisher exact test, student t test were used to analyze.
**Results:** The prevalence of Candidia sis was 25.2% and Tricomoniasis was 9.2. The results about sexual Health behaviors questions showed that the majority of samples selected "Always" sentence and the minority of samples selected "Seldom" sentence, so majority of persons were in "Good" level of health (66.2%) and minority of persons were in "Weak" level of health (2.5%).
**Conclusion:** According to high prevalence of Candidia sis and Tricomoniasis infections and many infected women as asymptomatic carriers, it seems necessary to pay more attention to these infections, and more efforts should be done in order to begin prevention. Midwives and other health professionals can give more information about these infections and sexual health behaviors for improving their quality of life.
***Keywords:*** Prevalence, Tricomoniasis, Candidia Sis, Sexual Health Behaviors

## $P_{nm}$-27: Anti-Mullerian Hormone As A Predictor of Ovarian Response to Life Style Modification

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**Background:** Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility in women of reproductive age, affecting 7% of this population. Although not entirely understood, the etiology of PCOS is closely linked to obesity and abdominal adiposity that are considered to worsen the clinical presentation, particularly menstrual irregularities and hyperandrogenism. Lifestyle modification programs focusing on weight loss have been shown to be important for improving reproductive function in obese women with PCOS. But some studies have demonstrated that reproductive responsiveness to weight loss (shown by improved menstrual cyclicity or ovulation) is different among PCOS women and is related to the levels of anti mulerian hormone. The aim of this review article is to study whether baseline and/or change in AMH levels with weight loss predict improvements in reproductive function in overweight and obese women with PCOS.

**Materials and Methods:** This review article prepared by studying of articles obtained from Google, pub med sites with key words such as weight loss / anti-Mullerian hormone, reproductive function , menstrual cyclicity.

**Results:** Popova etal. (2012) examined thirty overweight unovulatory women with PCOS (age 25.7 ± 5.9 y, body mass index (BMI) 32.3 + 5.3 kg/m$^2$) followed a 6-month weight loss program. Mean reductions in weight by 9.0 ± 5.9 kg (p<0.001) and BMI by 3.3 ± 2.1 kg/m2 (p<0.001) occurred for the subjects over the study duration. Of 30 subjects, 15 (50%) responded to the intervention with improvements in menstrual cyclicity (responders). Compared to non-responders, responders had lower baseline anti-mullerian hormone (AMH) levels (6.3 ± 3.3 vs. 9.8 ± 4.2 ng/ml; p=0.015.The value of AMH less than 6.5 ng/ml can predict improvement of menses (sensitivity 73%, specificity 67%) by weight loss in overweight women with PCOS. Thomson etal. (2009) studied fifty-two overweight and obese women with PCOS and reproductive impairment (age 29.8 ± 0.8 years, BMI 36.5 ± 0.7 kg/m$^2$) followed a 20-week weight loss program. Participants who responded with improvements in reproductive function (n=26) had lower baseline AMH levels (23.5 ± 3.7 vs. 32.5 ± 2.9 pmol/l; p = 0.03) and experienced greater weight loss (211.7 ± 1.2 vs. 26.4 ± 0.9 kg; p = 0.001) compared with those who did not respond (n = 26). Logistic regression analysis showed that weight loss and baseline AMH were independently related to improvements in reproductive function (p = 0.002 and p = 0.013, respectively). AMH levels did not change with weight loss in both responders and non-responders. This study shows women with lower AMH levels experienced greater improvements in reproductive function. Moran etal. (2007) studied 26 overweight women with PCOS, (age 32.9 - 5.8 Y, weight 98.9 - 20.8 kg, BMI 36.1 - 7.0 kg/m) followed an 8w weight loss and 6-month weight maintenance program of 26 subjects, 15 (57.7%) responded to the intervention with improvements in menstrual cyclicity (responders). Compared to non-responders, responders had lower AMH levels at baseline (23.6 _12.0 vs. 37.9 - 17.8 pmol/liter; p = 0.021). Only responders had reductions in fasting insulin (6.1 - 5.9 mU/liter; p = 0.001) and homeostasis model assessment (1.3 - 5.9; p= 0.002) with acute weight loss (wk 0-8). Pretreatment AMH is a potential clinical predictor of menstrual improvements with weight loss in PCOS. Franasiak etal. (2012) examined Seventy-one women with PCOS participated in a randomized, double-blind, sham-controlled clinical trial of acupuncture. Three longitudinal AMH samples over the 5-month protocol were compared with objective ovulation parameters =. AMH was inversely correlated with ovulation and menstrual cycle frequencies in both arms combined (p < 0.001). There was no difference between the true and sham acupuncture arms in the change in AMH longitudinally.

**Conclusion:** These results suggest that AMH measurement can be useful in the pretreatment identification of women with PCOS who will benefit from lifestyle intervention by menstrual improvements.

***Keywords:*** Weight Loss, Anti-Mullerian Hormone, Reproductive Function

## $P_{nm}$-28: Longitudinal Changes of Maternal Anthropometric Indices: A Cohort Study in Women during Pregnancy and Postpartum

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**Background:** Evaluation of fat distribution is a way to assessment of physical health. Waist to Hip Ratio and waist circumference are the simplest anthropometric measurements to be used to determine the obesity and related disorders in pregnancy. Today a close consideration is given to WHR and WC assessment in order to evaluate physical health in epidemiological studies.

**Materials and Methods:** One thousand pregnant women with singleton pregnancies receiving prenatal care in the Health Medical Centers of Kashan University of Medical Sciences participated in this study and followed prospectively after delivery. Waist and hip circumference were measured at first antenatal visit (≤ 12 weeks of gestational age), 28-32 weeks of pregnancy and in post-partum (6 weeks after delivery). All result analyzed by using SPSS.

**Results:** Results indicated that the mean, median and standard deviation of women WC in 12 weeks of gestational age were 80, 79, 10.31 cm; in 28-32 week were 92.6, 92, 11.5 cm and in postpartum were 83.6, 83, 10.87 cm respectively. Also the mean, median and standard deviation of women WHR in 12 weeks of gestational age were 0.79, 0.78, 0.61; in 28-32 weeks were 0.85, 0.84, 0.82 and in postpartum were 0.80, 0.80, 0.67 respectively.

**Conclusion:** Although changes of anthropometric indices were acceptable in pregnancy and decrease of these indices were observed 6 week after delivery to state before 12 weeks of gestation. Therefore, physical activity and exercise recommended accelerating this reduction of fat distribution.

***Keywords:*** Anthropometric Indices, Waist Circumference, Waist to Hip Ratio, Pregnancy, Postpartum

## $P_{nm}$-29: Effect of Psychotherapy with Acceptance and Commitment Therapy Approach on Reduction of Infertility Stress and Increase of Infertile Couples' Intimacy

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**Background:** According to studies, about 50-80 million people all over the world are experiencing some kind of infertility, and this phenomenon leads that in countries

where fertility rate is high and fertility is of special importance for couples and their families in cultural and social terms, emergence of mental, social, and family problems of infertile people get more severe. Furthermore, stress of infertility, specially its social type, is among the most common and noticeable factor in system of family and society in these couples. Thus, attention of many experts of infertility field has been paid to interaction with psychologists and psychotherapists, and in the third wave of cognitive therapies, treatment of Acceptance and Commitment Therapy (ACT) with changes in four process of flexibility is significantly related to change in depression, anxiety associated with pain, physical and mental disability in expected directions and finally increase in these processes are related to performance improvement. The aim of the present research is the investigation of effect of ACT-approach group therapy on reduction of infertility stress and increase of infertile couples' intimacy so as to achieve acceleration of medical treatments of infertile couples with this method.

**Materials and Methods:** Among 110 infertile females (1 to 8 ages), aged 22 to 45, education diploma to PhD, 30 people were randomly selected with available sampling method and were put into two groups (N=15) of experiment and control. Pre-test and Post-test were administered using questionnaires of infertility stress (surrounding, social, sexual, relational, needing presence of child) and questionnaire of Bagarouzi's intimacy (emotional, intellectual, sexual, physical, spiritual, esthetic, and social-recreational intimacy), routine experiments of spermogram and sonography of ovulation and pursuing medical treatments. Moreover, psychotherapy by ACT approach was performed during 8 sessions (1 personal session, 2 sessions of couple therapy, and 3 sessions of group therapy).

**Results:** According to results of this research, this method of psychotherapy led to reduction in stress of infertile couple especially social stress and, increase of partners' intimacy. In addition, during 6 months follow-up after treatment, greater number of experimental group experienced pregnancy.

**Conclusion:** According to research results, this kind of psychotherapy can be used for reaching 6 certain treatment processes infertile couples are in dire need of, including:conscious acceptance, contact with the present moment, cognitive defusion, self as context, identifying values, and committed action in joint life, whereby leading to reduction in mental pressures associated with infertility and finally to more rapid treatment and reduction in mental-economic costs in these families.

***Keywords:*** ACT Treatment, Infertility Stress, Partners' Intimacy

## $P_{nm}$-30: Effect of Group Therapy and Couple Therapy with CBT Approach in Reducing Pre-Pregnancy Trauma to Lactation in Parents

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**Background:** Parents, in their joint life, with different developmental background, personality style, various experiences and family background as well as with the expectations that are the results of their families' impacts or previous relationships, come to experience new things. These experiences have an impact on their daily behavior patterns, cognitions, quality of dealing with problems and conflicts and on their affective approaches in changing matrimony interactional patterns. This process is continued until the child's behavioral and affective disorders, as deferment in diagnosing children's problems leads to the fact too that the child will be greatly overshadowed by a negative situation and that there will be unsolvable physical and intellectual problems in his or her rehabilitation or treatment process. This longitudinal study which lasted for two years investigated with the aim of primary prevention, the effect of group training, and combined couple therapy on sexual intimacy of partners during and before pregnancy, lactation, and also the effect of this type of treatment on parents-child stress during lactation.

**Materials and Methods:** This study was carried out among 150 women, who had come to counseling centers and clinics of obstetrics and gynecology for before-pregnancy-health, in 2009, among whom 24 couples were selected randomly and placed in two groups of control and experiment and the pre and post-test method was used. The experimental group underwent 3, 9, 4 sessions of group training and 2, 3, 3 sessions of couple therapy before and durin gpregnancy plus lactation, respectively, for 9 months. The Bagarouzi's Questionnaire of partners' intimacy (emotional, psychological, sexual, physical, spiritual, esthetic, social-recreational intimacy) in all three levels and stress questionnaire were administered.

**Results:** According to statistical results (in which t test was adopted), there is a significant difference between intimacy of partners undergoing treatment and stress of parent-child with the control group.

**Conclusion:** Based on the results of this research, one of the most important factors in preventing and solving children's future crises can be timely education before pregnancy, during pregnancy and lactation of parents in order that we can promote the mental health of family and safe environment for deeper and more influential and even more rapid physical-psychological development of child in future generation.

***Keywords:*** Group Therapy, Couple Therapy, Partners' Intimacy, Parent's Stress, Child

## $P_{nm}$-31: Nurses and Midwives: Do They Have a Role in Promoting The Well-Being of Infertile Couples? A Review of The Literature

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**Background:** People seeking fertility treatment are exposed to numerous stressors and many professionals are involved in their care. Most patients undergoing health interventions feel stress at some point in the process, but for infertile patients, this is pronounced. Patients who feel stressed are not experiencing well-being, so the role of the fertility nurse/midwife in alleviating stress and providing emotional support is important. This literature review focuses on the role of nurses and midwives in the promotion of patients' well-being.

**Materials and Methods:** A literature search was undertaken using the following databases: Sciencedirect, Cochrane library and CINHAL. The search was limited to the period from the late 2008s to 2013.

**Results:** There is an abundance of reports on the specific management of patients during their fertility journey; Concluded that infertility and failure to conceive through medical interventions could impact on individual well-being and emotional distress. Women undergoing fertility treatment have identified that psychological support is a necessity during all aspects of their treatment cycle, but particularly prior to their pregnancy test result where they often felt isolated, having received intense contact up to and including embryo transfer. Acknowledged the emotional trauma that fertility patients may experience, supporting the suggestion that midwives should be required to be aware of their patients' psychological well-being. However, midwives required adequate knowledge of reproductive medicine.

**Conclusion:** It is not unreasonable that nurses and midwives receive training in acquiring skills in the provision of such support to patients which, arguably, is consistent with the general requirement that their skills and knowledge are updated to ensure safe and effective practice, but this would have resource implications.

***Keywords:*** Infertility, Emotional Distress, Well-Being

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- **Executive Manager:** Faani N, M. Sc
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## Semen Analysis

- **Scientific Manager:** Salman Yazdi R, Ph.D.
- **Executive Manager:** Sabbaghian M, Ph.D.
- **Date:** September 2, 2013

## Advanced Vitrification Techniques: What to Vitrify and When to Vitrify?

- **Scientific Managers:** Conaghan J, Ph.D, Spach J, B.Sc, Karimian L, M.Sc
- **Executive Managers:** Eftekhari Yazdi P, Ph.D, Hadi M, B.Sc
- **Date:** September 2, 2013

## New Perspectives in COS and Embryology

- **Scientific Managers:** Humaidan P, M.D., Meseguer M, Ph.D., Ashrafi M, M.D.
- **Executive Manager:** Jahangiri N, M.Sc
- **Date:** September 3, 2013

## Sonographic and Color Doppler in Infertility

- **Scientific Managers:** Kurjak A, M.D., Ahmadi F, M.D.
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**International Journal of Fertility and Sterility (IJFS)**

**Guide for Authors**

**Aims and Scope:** The ***"International Journal of Fertility and Sterility (IJFS)"*** 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. IJFS has been certified by Ministry of Culture and Islamic Guidance in 2007 and was accredited as a scientific and research journal by HBI (Health and Biomedical Information) Journal Accreditation Commission in 2008. **This journal is a member of the Committee on Publication Ethics (COPE).**
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**Luveris®**
LUTROPIN ALFA

**Composition:** Active substance: lutropin alfa 75 IU. Other ingredients: polysorbate 20, sucrose, sodium dihydrogen phosphate monohydrate, disodium phosphate dihydrate, concentrated phosphoric acid, sodium hydroxide and nitrogen. The solvent is water for injections. **Properties:** Luveris is a medicine containing lutropin alfa, a recombinant luteinizing hormone (LH) which is essentially similar to the hormone found naturally in humans, but made by means of biotechnology. It belongs to the family of hormones called gonadotrophins, which are involved in the normal control of reproduction. Luveris is presented as powder and solvent for solution for injection. It is supplied in packs containing 1, 3 or 10 vials/powder together with the same number of solvent vials. Each vial of powder contains 75 IU of lutropin alfa and each vial of solvent contains 1 ml of water for injections. **Indications:** Luveris is recommended for the treatment of women who have been shown to produce very low levels of some of the hormones involved in the normal reproductive cycle. The medicine is used together with another hormone called FSH (follicle stimulating hormone), to bring about the development of follicles, which are in the ovary (the structure containing the eggs (ova)). It is followed by treatment with a single dose of human chorionic gonadotropin (hCG), which leads to the release of an egg from the follicle (ovulation). **Dosage and Administration:** Luveris is usually taken every day for up to three weeks simultaneously with injections of FSH. A commonly used dose starts with 75 IU (1 vial) of Luveris together with 75 IU or 150 IU of FSH. The dose of FSH may be increased, according to the patient's response, by preferably 37.5 – 75 IU at 7 – 14 days intervals. The treatment may be extended up to 5 weeks. When the desired response has been obtained, a single injection of hCG is given 24—48 hours after the last injection of Luveris and FSH. The patient is recommended to have an intercourse on the day of, and the day following, administration of hCG. Alternatively, intrauterine insemination (IUI) may be performed. If an excessive response is obtained, treatment should be stopped and hCG withheld (see Side Effects). For the following cycle, a lower dose of FSH will be prescribed. Luveris is intended for subcutaneous use only. Each vial is for single use only. **Side Effects:** The most frequent adverse events reported have been: headache, abdominal pain, breast pain, nausea, convulsions (symptoms), and ovarian cysts. Local reactions at the injection site (pain, redness, itching, swelling and bruising) have been observed in some cases. Treatment with FSH and LH followed by hCG can lead to ovarian hyperstimulation syndrome (OHSS), when the ovaries over-react to the treatment and large ovarian cysts develop. First of OHSS are pain in the lower abdominal region, possibly in combination with nausea, vomiting and weight gain. In the patient experiences pain (serious abdominal pain together with these symptoms, the injection should be stopped and the doctor consulted. A careful examination will establish whether the ovaries have responded excessively to the treatment. If so the FSH and LH treatment will be discontinued and hCG treatment will be abandoned to prevent the development of more severe OHSS. Rarely abnormal blood clots in veins have been seen to occur with similar medicines, so this could possibly occur with Luveris/FSH/hCG therapy. Torsion of the ovary and bleeding into the abdomen have not been reported with Luveris, however, there have been rare cases reported following treatment with human menopausal gonadotrophin (hMG), a unpurified preparation containing LH. Ectopic pregnancy may occur especially in women with a history of prior tubal disease. The doctor should be informed if any other side effects were noticed. **Storage:** Keep out of the reach of children. Do not store above 25OC **Packaging:** 1 vial of powder (75 IU) + 1 x 1 ml solvent (Water for injection); 3 vial of powder (75 IU) + 3 x 1 ml solvent (Water for injection); 10 vial of powder (75 IU) + 10 x 1 ml solvent (Water for injection). For further information, kindly contact: Merck Serono Middle & Near East, P.O. Box 221110, Dubai, United Arab Emirates, Phone: +971 4 375 2700, Fax: +971 4 429 1700, www.merckmiddleeast.com

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